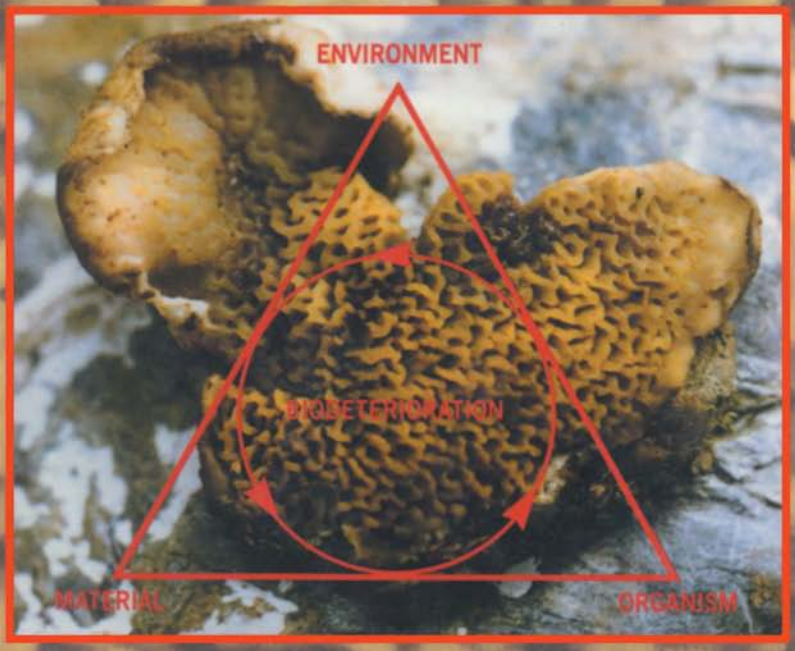
BUILDING MYCOLOGY

Management of decay and health in buildings



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Building Mycology

Management of decay and health in buildings

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Dedicated to my grandmother, parents, Pala, Rhianna and Dylan.

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Foreword

Biodeterioration of various kinds is a major cause of building decay. An estimate of the cost of repairing damage due to fungal decay in the United Kingdom alone is in excess of £400 million, and the decay organisms and the environmental conditions supporting them are a hazard to health as are many of the remedial chemical treatments used. The problem is not a new one and it was certainly familiar to ancestors of the most ancient times who well understood the importance of dry atmospheres and ventilation as controls and the danger of mycotoxins in food. Much of this understanding is inherent in traditional building techniques.

Building mycology is defined as that branch of mycology which deals with the study of fungi associated with buildings and their environments, which has a direct or indirect effect on the performance of building materials and structure and the health of occupants. These aspects of building performance and health interactions require the multidisciplinary understanding which gave rise to the annual Building Pathology conferences organized by Hutton & Rostron, Oscar Faber Applied Research and BRE and the compilation of this book.

Vernacular construction using natural, often organic, materials has survived for hundreds of years, despite somewhat cursory attention, and it has often only succumbed to gross neglect or destruction on economic grounds. Often it appears that the introduction of new materials and adaptation of buildings to new uses or lifestyles have been instrumental in causing decay; particularly when these involve the sealing of surfaces, the introduction of membranes, increased heating and reduced ventilation. These changes also lead to ill health of the occupants and similar conditions in modern buildings seem to be implicated in illness.

Water in all its forms pervades the earth and permeates our buildings. It cannot be defeated. Moisture and some organic source of carbon and nitrogen are essential for fungal growth. Defining the limits of the former and care in the use of the latter is basic to the understanding of fungal development, and these will be found to be common factors in the aspects of building mycology dealt with in the following chapters.

Fungi are an important part of the living world normally perceived in their more dramatic forms such as the fruit bodies of dry rot in buildings and ergotism in populations consuming affected grain. Fungi are pervasive in the world and in all those circumstances favouring their growth they can be of vital concern to the physical and economic welfare of people. Nowhere is this more important than in buildings occupied for work and storage, and in particular in homes. Moulds and fungi damage construction materials, often beyond repair; taint food, spoil clothing, books, prints and works of art; affect

furniture and equipment. Spores produced by moulds and fungi may be associated with asthma and respiratory illness, and mycotoxins excreted by them can cause a variety of medical conditions, including death, if ingested. These problems have always been known, and precautions have been taken in food storage, building construction and housekeeping to control the consequences. Essential amongst these precautions are the management of the moisture content of materials, ventilation of construction and living spaces, and routine cleanliness. More recently reliance has been placed on the use of impervious materials, air-conditioning and chemical treatments in buildings; and packing and preservatives for foodstuffs. These techniques have brought their own problems, not least of which is over- confidence that the danger has been eliminated, for example the common claim that a chemical treatment has 'eradicated' *Serpula lacrymans*. This is a timber-consuming fungus from the forest floor of the Himalayas, only capable of surviving indoors in Europe and probably introduced in the 16th or 17th century by ships trading with, and refitted in, India. As scientific knowledge has become deeper and more specialized, the professions more diverse and the experience of urban populations more remote from the mechanisms of nature it is important that a new unified view is developed of how we and our buildings relate to the environment and the living world of which people are a part. Such a broad understanding is necessary if buildings are to be durable, the contents undamaged, the occupants healthy and the environment unpolluted by the production and use of remedies of a toxic nature. To this end this book is addressed to legislators, building owners, professional advisers, and building occupiers; particularly those involved in building design, maintenance, inspection, restoration, conservation, building services, environmental investigations, financial management, operational research, public health and loss prevention.

The first six chapters of the book take a broad approach to the subject of fungi in buildings, the circumstances favouring their development; their identification, extent and economic importance; the forms fungal material take, the effect on the indoor environment and the damage caused to finishes. Three further chapters deal with inspection, monitoring and control of timber decay; detection and biocontrol and the use of chemical controls. The book concludes with four chapters by contributors from India, Canada, Denmark and Spain dealing with specific experience in different parts of the world.

Geoffrey Hutton, Senior Partner, Hutton & Rostron

Preface

Building mycology is defined as that branch of mycology which deals with the study of fungi in and around the building environment. This has both direct and indirect effects on the health of building materials, structures and occupants. The estimated cost of repairing damage caused by fungal infestation in buildings in the United Kingdom is £400 million a year.

Building mycology covers a diverse subject area which has not been adequately dealt with so far. This book covers the effects of fungi and the damage they can cause to building structures, contents, decorations and foodstuffs as well as to the health of the occupants. It also draws attention to a range of control strategies. The book comprises the invited contributions of national and international experts in their field of research to create an authoritative survey of current knowledge about the state of mycology in buildings.

It is clear from the foreword by Geoffrey Hutton that the building industry has learned that building problems need a multidisciplinary scientific approach.

The importance of understanding building environments and their interactions with materials, structures and occupants is dealt with in Chapter 1, which also draws attention to a range of ecological niches and microclimates provided by building structures for the growth and proliferation of fungi. Chapter 2 aims to give a basic understanding of the fungal problems encountered in buildings, including slime moulds, plaster fungi and infections on external masonry surfaces. Detailed descriptions of the physiology and morphology of fungi and decay mechanisms are given in Chapter 4. Much emphasis is placed on indoor aerobiology and building-related health problems in Chapters 5 and 11. Chapter 6 gives an account of the composition of interior finishes and the fungal flora to which they are exposed. Management of fungal infestation in buildings and three different approaches towards fungal control, i.e. environmental, biological and chemical control, are described in Chapters 7, 8 and 9. This provides a useful source of information on such matters as non-destructive inspection techniques and the monitoring of the building environment.

The last section of the book explores the experience in various countries of problems with fungi, both in terms of the decay of structures and health implications. The final chapters (10–13) should be seen as independent contributions describing the problems experienced in different countries. For example, Chapter 10 covers the biodeterioration of wall paintings in India, Chapter 11 focuses on the health implications of fungi in North American environments, Chapter 12 on the decay of structures in Denmark and Chapter 13 covers the effects of fungi on stone monuments in Spain.

This book draws attention to the importance of understanding the interrelationships of building structures and materials with their environments and occupants. It is intended to promote the multidisciplinary scientific study of building performance and to broaden awareness of mycological problems in buildings in order to improve design and management.

Jagjit Singh

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The built environment of fungi and the development

1

Jagjit Singh

INTRODUCTION

Building mycology deals with the study of fungi in and around the built environment which have direct and indirect effects on the health of the building fabric, its materials, structures, environments and occupants (Singh, 1991). Building structures and environments provide specialized microclimates and ecological niches in their ecosystem for the settlement, growth and proliferation of a variety of fungal organisms. These fungi can cause damage to the building structures, decorations and contents and can raise concerns for the indoor air quality and the health of the building's occupants (Cartwright and Findlay, 1958; Richardson, 1980; Allsopp and Seal, 1986; Singh and Walker, 1994).

The impact of building mycology on the biodeterioration of building structures, contents, materials and decorations is humankind's commonest problem and it can be traced back to biblical times or earlier. The estimated cost of repairing damage caused by fungi in buildings in the United Kingdom is £400 million a year. These fungi not only have a serious impact on the maintenance and repair of the national housing stock but also cause great concern regarding the conservation of ancient and historic buildings and buildings of special architectural and cultural merit including their contents and decorations. The most destructive fungal species which cause the decay of structural timber in buildings are *Serpula lacrymans* (dry rot) and *Coniophora puteana* (wet rot), but a number of other wet rot, soft rot and surface rot fungi and moulds also cause biodeterioration in buildings. Among the many insects found in buildings, beetles are the most dangerous, causing serious damage to timbers. *Xestobium rufovillosum* (deathwatch beetle) and *Pentarthrum huttoni*

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(wood-boring weevil) are extremely destructive insects and are found to be associated with fungal decay.

People spend long periods indoors, and both at home and at work the air that they breathe is mostly indoor air. Health and comfort problems associated with the built

environment have come to constitute a major problem in recent years (Berglund *et al.* 1988; Curwell *et al.* 1990). The main reason for the neglect of indoor air quality issues is lack of awareness, because the effects are mostly chronic and long-term, and are not directly and immediately life-threatening (Environment Committee, 1991). The biological factors causing building-related sickness may include mould, fungi, bacteria, viruses, protozoa, pollens, house dust mites, insect pests, algae, pigeons and rodents (Wathes, 1989; Burge, 1990; Burge *et al.*, 1990; Gravesen, 1990; Miller, 1990; Singh, 1991). For some people, ill health in the built environment may be triggered by non-biological factors such as chemicals and other indoor air pollutants, or by emotional stress, fatigue and changes in the weather. These factors burden allergy-prone people further if they are suffering from allergic reactions to biological contaminants. This combination is known as 'the allergic load'. Environmental, design, use, management and construction factors can affect the incidence of allergic components; for example, geographical location, time of year, time of day, altitude, weather conditions, flora and fauna, shape and configuration, materials and structures, design of ventilation systems, thermal insulation, tightness, air change and energy (Singh, 1993a). At present, there are no government guidelines or codes of practice on indoor air quality which specifically identify exposure limits for an extended list of pollutants based on possible total exposure (ambient, home and work place) (Environment Committee, 1991).

Orthodox remedial measures to control fungal infestations in buildings, including the use of chemicals, cause concern to health authorities and environmentalists and if used incorrectly can lead to resistance in target organisms. Remedial treatment used carelessly can result in the loss of irreplaceable decorative finishes and of building fabric of historic architectural importance. In most orthodox remedial treatments the fundamental biology of the organism is not understood. For these reasons, fundamental scientific study of these organisms together with multidisciplinary understanding of building construction and environments is desirable. These investigations can provide more effective, economical and less destructive environmental control strategies.

BUILDING ENVIRONMENTS

Buildings can be likened to living organisms. The useful life of a building depends on its internal and external environments, both in terms of longevity of materials and as an appropriate habitat for its occupants. Buildings provide spatial environmental ecosystems, ecological niches and a range of microclimates in their built environment for the development of fungi and must be understood as a whole. Ecological niches and microclimates in the built environment in which fungi live and interact have many dimensions. It is of fundamental importance to measure as many of the relevant variables as possible and to characterize the role of fungi in spatial ecosystems constructed by humans.

The main requirements for fungal growth in buildings are: a source of infection (spores, etc.), favourable temperatures (0–25°C), nutrients, oxygen and water. Fungal spores are ubiquitous, although number and types differ with time of day, weather or other factors. Fungi differ in their temperature tolerance, some species still growing slowly below 0°C and others tolerating up to 60°C. The nutrient requirements of fungi

are satisfied in the built enviromnent by dust and organic deposits and air movements can provide sufficient oxygen. Only water availability limits fungal growth in buildings. Water availability in buildings depends on its source and movement, the occurrence of moisture reservoirs, and sinks, heating insulation, ventilation, external conditions, orientation, the building materials and the occupants. The interrelationship between building structures and materials and their environments and with the living organisms within them are very complex. They must be analyzed through a multidisciplinary scientific study of buildings in order to improve the role of the building environment from a total health perspective.

Buildings separate their occupants from hostile external environments and create a better internal environment for them. Therefore buildings can be likened to human skin (a second skin) or an extension of our bodies as the third skin; that is, the body is the first skin and clothes are the second skin, forming a physical barrier to separate the inside from the outside (Schimmelschmidt, 1990; Walker, 1990)

The building shell needs to be adaptive, flexible and reactive in order to maintain a relatively constant internal environment in circumstances of regular or cyclical changing external conditions and the varying activities of the occupants. Changing internal environments and their effect on the fabric and contents of the building (both fully-controlled ‘museum’ environments and simpler and more intermittently-operated systems) must be understood holistically. These changes can be permanent radical change, for example the installation of a new heating system; shifting equilibrium, a progressive change from one state to another; spatial variations, non-uniform environments which result from diverse occupancy, both in space and time; cyclic fluctuations, the control strategies of most heating and ventilation systems; seasonal variations; and violent changes. The building envelope must function in close correspondence with the processes and biorhythms of the body—regulation of moisture, breathing and heat balance, for example. These issues have led us to recognize the need for the use of ecologically sound materials to design a breathing fabric which balances the sources of moisture with its reservoirs and sinks in the built environment.

Building defects and failures often lead to the development of fungi and (biological) decay problems. Building investigators have often failed to diagnose decay problems in buildings correctly because they have not approached the buildings as a dynamic complex system. Currently, decay problems are dealt with by using drastic measures, rather than by a comprehensive multidisciplinary approach. The causes of decay in materials and structures and the effects of decay on the health of the occupants are influenced by the internal building environment which has a varied microclimate depending upon the building’s structure and construction. The environment of a material is complex and dynamic. All organisms live in a biological equilibrium called biological balance, and for an organism to succeed it must be in balance with the environment within fine limits (Singh, 1986). Similarly, the internal building environment is a complicated interacting system involving water movement, air movement and the transfer of heat. In these ways a building modifies the external environmental conditions to create an internal environment. The internal building environment is specific to each building and depends upon its design, construction, materials, thermal mass, buffer effect and insulation, upon the standard of maintenance, the way in which it is used, as well as on its

acoustics, lighting, heating and ventilation. An imprudent alteration of the internal environment may upset a very delicate, long established equilibrium.

The environmental factors that cause building failures and favour decay in buildings are temperature, water, humidity and lack of ventilation. Internal temperatures within buildings are affected by occupant's use of heating and ventilation systems, the thermal insulation of the building and its capacity to store heat. The moisture levels found within buildings are affected by a combination of many factors, the generation of water by the occupants within the building, ventilation rates, air temperatures, building materials and other components and the capacity of furnishings and internal surfaces to absorb moisture. Studies of moisture profiles in walls have shown that occasional condensation is unlikely to lead to the development of moulding that will damage the structure or the materials. Studies towards the understanding of the built environment and of building biology are vital in improving design and performance of buildings.

Building mycology needs to encompass an understanding of the complex interactions between nutrients, organisms and the environment which lead to decay. Detailed knowledge is therefore required of the microenvironments which can be encountered in buildings, the nutrients available, and the nature of the organisms inhabiting it. This may include the detailed study of organisms' morphology, ecology, taxonomy, developmental versatility, variability and physiological functioning. These issues have been addressed in Chapters 2–4 and 12–15. The data acquired and knowledge gained from these observations allow the development of new approaches to the prevention and treatment of fungal decay, and also to novel methods for the management of decay by manipulating the environment. These methods are less destructive and more environmentally acceptable than conventional approaches (see Chapters 8–11 and 14). The understanding of building mycology and its health implications are dealt with in Chapters 5, 6 and 13. Multidisciplinary research is necessary to develop an integrated understanding of built environments.

MOISTURE IN BUILDINGS

SIGNIFICANCE OF WATER IN BUILDINGS

Water critically affects the development of fungi in buildings. The significance of water content may be judged by analysing the building in terms of water sources, reservoirs and water sinks (Figures 1.1, 1.2 and 1.3) (Hutton *et al.* 1991).

Moisture sources

Water can be introduced into the building fabric in several different ways, for example, from high ambient humidities, external weather conditions, building defects and vandalism. Moisture is generated inside the building fabric by the activities of the occupants such as cooking, washing, metabolism and heating (in properties with unflued paraffin or gas heaters). It is not possible to entirely prevent moisture entering a building and attempts to block the movement of moisture through a building structure using impermeable materials are often ineffective. They may also be counter-productive as they

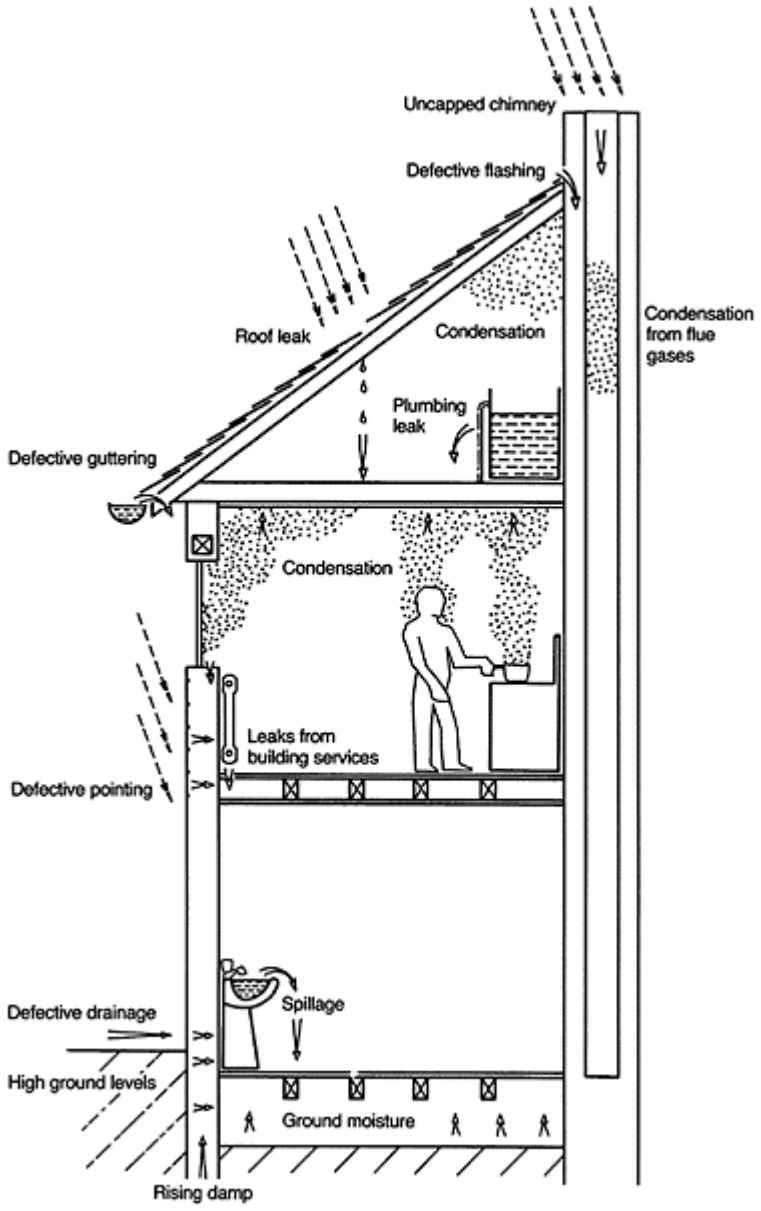

Figure 1.1 Examples of moisture sources and building defects.

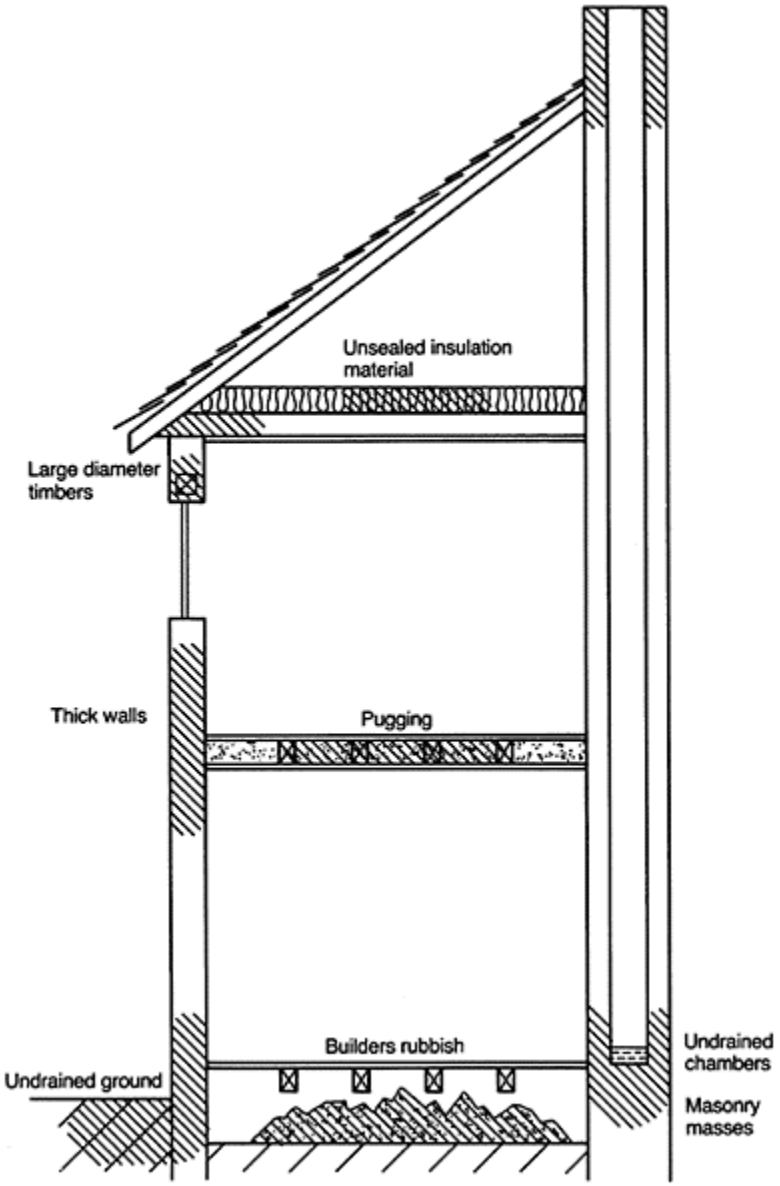

Figure 1.2 Examples of moisture reservoirs.

can prevent moisture being dissipated resulting in high humidities and condensation and fungal decay in adjacent materials. The more effective and robust approach is that used in traditional buildings in which porous materials are preferred and every moisture source is

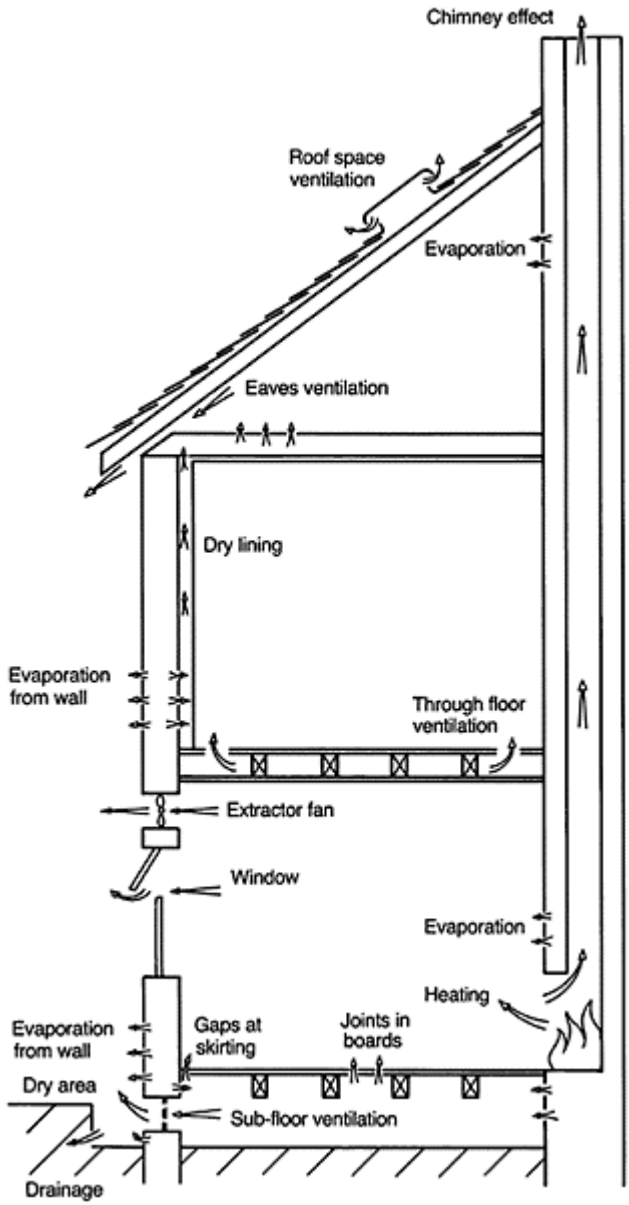

Figure 1.3 Examples of moisture sinks.

balanced by a moisture sink. Thus ground water may penetrate masonry but evaporates before it reaches timber structures. Similarly water vapour introduced by occupational activities, is removed by ventilation through windows and chimneys. Failure to balance a

moisture source with an appropriate sink may result in moisture moving into vulnerable materi- als and eventually causing moulding, decay and health problems.

Moisture reservoirs

Moisture reservoirs occur when a moisture source has not been balanced by a sink and water has accumulated in a porous material. Most building materials are hygroscopic and have the ability to absorb or release moisture into the air, referred to as the ‘sponge effect’. Buildings often become waterlogged, thereby ‘saturating’ the sponge effect, and leading to the development of fungi. Typical examples of this are to be found when thick masonry walls have been soaked by persistent leaks or when chimney breasts have been filled with rainwater from uncapped chimneys. Such reservoirs may take years to dry out, even when the source has been dealt with. As a result they can act as a source of moisture for the development of fungi over a long period. A special case of this phenomenon occurs when large quantities of water have been used in dousing a fire.

Moisture sinks

Moisture moves from its source through moisture reservoirs to a final destination in the form of a moisture sink. The movement of moisture through the building should not pose any threat of decay to the vulnerable materials. However, continued wetness will lead to moulding and decay.

In practice, therefore, areas of decay are associated with building defects, which result in an increased moisture source, a blocked or inappropriate moisture sink or a moisture reservoir. The appropriate building measures are then required to correct that defect.

A common example of a building defect is the bridging of a dampproof course by raised ground levels. This will allow the penetration of water to provide a source in an internal wall and may result in moulding or growth of decay fungi in the adjacent floor space. Lowering the ground level will cut off this source and will also provide a moisture sink by allowing evaporation from the exposed wall. The sub-floor level may also be controlled by increasing other available moisture sinks. This is often achieved by cleaning existing airbricks or inserting additional sub-floor ventilation. In general, it is important to increase evaporation surfaces and to avoid their obstruction during refurbishment.

Another common example of a moisture source would be a blocked and overflowing parapet gutter. This could wet gutter soles, joist ends and wall plates and other structures in an expanding cone extending from the leak down through the building. Preventing this moisture source may require such measures as increasing the capacity of downpipes, re-lining gutters and fitting thermostatically controlled trace heating tape to increase the free flow of snow melt water.

Failure in a roof finish, gutter or coping will usually result in water penetration into the masonry wall beneath, which then acts as a moisture reservoir. Any timber in contact with this reservoir will act as a wick carrying water from the masonry leading to a risk of fungal infestation. Such timber must therefore be isolated from the masonry using such measures as dpc (damp-proof course) membranes or joist hangers with an air gap. It will also be necessary to ensure that the timbers are adequately ventilated to allow absorbed

water to evaporate off. Closed cavities or water-impermeable layers over timbers at risk must therefore be carefully identified and rectified using knowledge of historic methods of construction. Bricked-in lintels and sealed up emulsion-painted sash windows are typical examples of structures at risk from fungal decay.

Having prevented the flow of water into moisture reservoirs and pro tected building materials at risk, it is next necessary to provide safe ‘sinks’ for the moisture to ensure that the reservoir is dried out over a period. Sometimes, the reservoir can be removed entirely, for example by removing and replacing damp pugging, but often it is only necessary to promote ventilation from around a wicking surface on the reservoir to the outside. This will avoid condensation and the development of mould fungi with health implications. Dry lining systems can be useful for this purpose as can the good old-fashioned chimney. Raising the temperature will promote the process of wicking and evaporation. General house heating can also help but care must be taken to ensure that water vapour is not just being moved to other parts of the building by a sequence of evaporation and condensation down a temperature gradient. Heating can be most useful if it can be applied to the reservoir material itself.

Sometimes dehumidifiers can be used in the short to medium term but care is needed. They often require special ‘tenting’ and monitoring so that moisture is removed only from the appropriate material and not from the general atmosphere. They also require high air temperatures and high relative humidities (RHs) to extract moisture efficiently.

MOISTURE PROBLEMS AND BUILDING PRACTICE

Occasional condensation does not necessarily lead to damage of the structure or the materials. It is possible and even likely that many buildings that seem to perform quite satisfactorily do experience limited condensation at one time or another. Serious moisture damage in buildings is usually the result of a combination of unfortunate circumstances and design flaws. It is often a failure of the building as a system, not of any individual building material. Although moisture damage can often be attributed to water leaking into the building envelope, at other times the causes are complex and difficult to determine. Many design solutions and remedial measures to control moisture in buildings attempt to control vapour diffusion through the installation of vapour barriers.

The susceptibility of building materials to fungal growth changes with their moisture content, which equilibrates with the relative humidity of the air. However, the equilibrium moisture contents of different materials exposed to air of the same relative humidity can differ widely. To formulate realistic performance criteria, we need to determine to what degree moisture can be allowed to accumulate. The availability of water to fungi in a given building material depends on the material’s equilibrium relative humidity (ERH) or water activity (a_w). Below 65% ERH ($0.65a_w$) there is no growth. As ERH (a_w) increases more species of fungi are able to grow and growth becomes more rapid. At 100% ERH ($1.00a_w$) water is freely available. Thus, at a given moisture content, the availability of the water to fungi may be different for different materials and constructions. For these reasons it is difficult to design a standard solution which will overcome all the problems of moisture and fungal growth. Once moisture criteria are established, reliable and practical design methods can be developed for building

envelopes that meet these criteria. These design methods must be based on verified mathematical models, just as with building energy design methods.

The operation and performance of a building and its components depends not only on design, construction, and materials, but also on the environmental conditions inside the building and their management. The design and orientation of the building, especially of the exterior envelope, determines to a large extent its tolerance for extreme interior conditions. The direction of the prevailing wind can be important. For example, buildings on the west coast of the United Kingdom suffer very severely from wind-driven rain. If the wind blows through the building from wet areas to dry areas, it will encourage fungal growth. A well-insulated building with good control of air and vapour flows decreases heat loss from the building allowing increased air temperatures. This also brings the temperature of internal wall surfaces closer to air temperature and thus significantly controls condensation and fungal growth.

MOISTURE CONTENT OF AIR: RELATIVE HUMIDITY AND VAPOUR PRESSURE

Relative humidity (RH) is a measure of the actual moisture content of air compared with the saturated moisture content at that temperature. Air moisture content or vapour pressure (VP) is a temperature-independent measure of the total quantity of moisture (water) in the air. In Britain RH averages between 80 and 90% all year round. If no water is added to the atmosphere, increasing temperature decreases relative humidity. Relative humidity above 65% allows fungal growth and development. Relative humidity below 50% encourages the drying out of organic materials and they become brittle, shrink and crack. The optimum RH in dwellings is about 50–65%. In Britain, indoor temperatures need to be raised only about 5°C above those outside all year round to achieve this. In summer, the sun can provide this 5°C increase in temperature.

Temperatures inside and outside buildings tend to equalize through the transfer of heat. Similarly, water vapour pressures tend to equalize through the transfer of water molecules. Air containing a large amount of water vapour has a higher vapour pressure than drier air, so moisture from the wetter air readily diffuses into drier air. Moisture generated in one area of the house will disperse to others or escape to the outside in different ways. Moisture can move through structures itself by gravity, capillary and osmotic effects or by air circulation caused by wind, draughts and convection currents. In winter, as warm internal air holds more water vapour than cold external air, the vapour pressure, being higher inside a building that outside, will cause water to move outwards through permeable walls in vapour form. In summer, the internal and external environments are much more in equilibrium.

INSULATION AND CONDENSATION

The amount of water vapour which the air can hold is limited and depends on temperature. When this limit is reached, air is said to be saturated. The hotter the air the more water vapour it can hold. Thereafter extra water vapour will be deposited in the form of condensation. Condensation can be caused by an increase in the moisture content of the air or a fall in its temperature.

Insulation decreases the rate of heat loss from the building and thus allows higher air temperatures. Insulation does not of itself increase air temperature unless there is some heat source inside the building, but, compared to an uninsulated building, the same heat input will, in the steady state, support a higher temperature differential with outside air. Thermal insulation also keeps the temperatures of internal wall surfaces closer to indoor air temperatures. To prevent condensation in buildings, thermal insulation plus some form of heating to the fabric, is required because insulation alone has little effect on condensation.

Interstitial condensation occurs within the thickness of a wall, in flat roof voids and in ceiling voids. The most common escape route for water vapour is diffusion through porous building fabric. Since there is a temperature gradient within the thickness of the fabric, the temperature of the inner surface of the building fabric is close to that of the room while the temperature of the outer surface is close to that outside. Water vapour moves down the temperature gradient in the wall; it may event-ually reach a position where the temperature is at dewpoint (the temperature at which air becomes saturated and water condenses). This interstitial condensation can lead in turn to the development of fungi which can potentially threaten the structural integrity of the building fabric and the health of the occupants.

CONDENSATION AND VENTILATION

Moisture generated by condensation in a static environment, particularly localized condensation of stagnant air, can provide suitable conditions for fungal growth. This may develop as mould on walls and ceilings or as dry rot in structural timbers. Internal air movements generated by the activity of occupants, for example by opening doors and windows, decrease the danger of stagnant air. Impervious coverings, like rubber backed carpets and linoleum (particularly on the ground floor if there is no damp-proof membrane and no sub-floor ventilation) increase the risk of fungal decay.

Permanent ventilation of the structural floor void, to prevent the air becoming stagnant, can be achieved by installation of ventilators in the floor surface, particularly along external elevations. Air ducts with grilles by a fireplace help extract air from the sub-floor and also decrease room draughts. Grilles under radiators are also worth considering as they assist sub-floor air circulation by drawing air upwards and warming it. Ducting may need to be introduced into sub-floors to ensure air movement, assisted by wind pressure differentials, from one side of the building to another.

Fungi are often most severe on the inner surface of the external northern or eastern walls, where wallpaper often becomes detached because of the dissolution of the glue. Paint flaking and salt efflorescence are also commonly observed on damp walls. Built-in cupboards against external walls create pockets of stagnant air due to insufficient ventilation, encouraging the development of fungi on the walls and also on their contents, for example, clothes, leather goods and other organic materials. Ventilation of these cupboards by installation of permanent ventilators in the top and bottom of the cupboard door, can minimize the development of fungi. Moisture vapour builds up if there is poor ventilation in recesses and external corners where there is most heat loss. Natural ventilation is one of the simplest ways of avoiding condensation and ensuring safe storage for the contents of buildings. In addition, moisture-laden air can be removed from

bathrooms, kitchens, basements and storage areas with extractor fans, ensuring that these do not discharge into the building envelope but are ducted to the outside.

VENTILATION

Any sign of fungal growth in the built environment indicates the presence of moisture and gives warning that some form of ventilation may be required.

STACK VENTILATION

The removal of air from the room via a vertical duct or chimney stack and its replacement by cooler air entering at low level or by natural means is referred to as stack ventilation and is greater in winter than summer. It takes place even in the absence of a fire. Stack ventilation decreases the risk of condensation, provided the flues are lined with special impervious lining materials to prevent condensate from reaching porous building materials. Traditional open fires are rare these days and chimneys are often blocked. These are now replaced by specially designed systems with better thermal insulation and by heating systems which use the principle of stack ventilation. However chimney stacks (flues) can introduce dampness into the building fabric if they are not capped, if the flashing is in poor condition, or through condensation of water vapour. For stack ventilation to be efficient, the vertical outlets should be placed as high as possible on roofs and walls, while inlets should be as low as possible. Cracks around doors and windows at low level often provide sufficient infiltration in winter.

NATURAL VENTILATION

Natural ventilation provides the gentlest form of air movement throughout the building fabric and is one of the best ways of avoiding condensation and the development of fungi. It can be provided by airbricks, air ventilators, open windows, roof ventilators, ventilators in the structural floor voids and dry lining systems. The extent of natural ventilation is affected by size of opening, wind speed and direction, cross ventilation and buoyancy. External weather conditions, particularly wind speed and direction, will affect air change rates within a building, especially when windows and doors are open.

The natural forces which affect ventilation rates are much greater in tall buildings. These have increased exposure at the upper levels, so that stack pressure differences can be relatively large because of the height of some internal volumes. Natural ventilation can generally be improved by using stack pressure forces, although these forces are less effective in summer.

The greatest amount of general air movement is produced when the air flow has to change its direction within a room. The change of dir-ection produces more turbulent mixing than when the flow pattern is straight through, thus the whole room benefits from increased air movement rather than only a small part of it. There is a tendency for air to rise externally against the side of a building. This upward movement is called buoyancy and it will have an effect on the air change rate of the building depending on its own rate of rise and type of window openings. Sash windows that open at the top and bottom will

produce greater ventilation rates due to buoyancy than windows with only a single opening (Garratt and Nowak, 1991).

The activity of occupants in terms of closing and opening of internal cupboards and doors and walking on suspended timber floors produces a diaphragm effect, introducing air movement into the built environment and helping to avoid condensation.

MECHANICAL AND ARTIFICIAL VENTILATION

For the extraction of moisture-laiden air from bathrooms and kitchens the use of extractor fans is desirable. They have an advantage over open windows, particularly when the wind is non-existent or in the wrong direction, for example, into the kitchen. Extractor fans create a pressure drop in the room, which decreases the escape of moisture into the rest of the house. Moisture-laiden air from the bathroom and kitchen can also be extracted vertically to a point above the roof through stack pipes. Airflow is proportional to the cross-section of the pipes which are usually of 100–150 mm diameter. This form of ventilation is called passive stack ventilation. To avoid condensation in the stack pipes, they should be well insulated when passing through roof spaces (which are generally cold) and should terminate above the roof in a position high enough to gain maximum draw and to avoid downdraft. Fan-assisted stack ventilation creates a slightly pressurized system which reinforces the stack effect. The stack used in this way acts as the extract for the whole house and removes air from the wet areas (kitchen and bathroom) to be replaced by drier air from the rest of the house.

Air-conditioning is useful in museums to control the environment within the precise limits required for certain objects such as paintings on wood. It is also beneficial for historic buildings situated in an unfavourable environment of dust, dirt and sulfur dioxide, as it reduces the damage caused by these agents to furnishings and contents. The control of humidity prevents wall panelling from shrinking and cracking if central heating is installed. It furthermore assists in dealing with the fluctuating environment created by large numbers of tourists (Feilden, 1970).

ENVIRONMENTAL ASSESSMENT

ASSESSMENT OF FUNGAL ACTIVITY

Fungal growth is present in 10% of the housing stock in the United Kingdom (Environment Committee, 1991). The prerequisites for the development of fungi are described above under ‘Building environments’. Materials such as wallpaper, painted or plastic-coated surfaces, insulating materials, textiles for interior work, floor coverings, wood and wood materials are potential food sources, and non-nutrient materials such as plaster and brickwork can support growth because they contain trace amounts of contaminating organic matter. Mould fungi in general have very wide temperature and humidity tolerances, although relative humidities exceeding 70% and temperatures in the range of 15–20°C are generally required (Garratt and Nowak, 1991). Many fungi, however, will grow happily up to 30°C and down to 10°C, or even below freezing.

There are several different techniques for the quantitative and qualitative enumeration of the fungi in the built environment. The methods range from simple visual investigations and source sampling to complicated analytical methods of air sampling. The details of different techniques are dealt with in Chapters 5 and 11. There are environmental health centres in the United Kingdom, USA and elsewhere which have developed methods to diagnose and treat the specific health-related problems associated with mould fungi in buldings (Monro, 1990). Unfortunately, there are no government guidelines available for quantifying risk levels for airborne fungi. However the BRE environmental assessment of new homes, BREEAM/New Homes Version 3/91, covers indoor issues with an emphasis on ventilation control systems, volatile organic pollutants, wood preservatives and non-gaseous indoor pollutants (BREEAM, 1991). The BREEAM assessment also covers issues such as global atmospheric pollution, local issues and the use of resources.

Assessment of decay fungi

There are several methods for assessing decay fungi in buildings. A visual investigation involves actually looking for fungal growth in potential risk areas, such as built-in timbers against external elevations, down-pipes or parapet gutters. Fungal growth can be observed in different morphological forms, as mycelial growth, rhizomorphs or strands, and as sporophores and in the appearance of damaged materials. These morphological characteristics are sometimes diagnostic, permitting identification of the fungi involved. For example, formation of rhizomorphs on masonry or brickwork is an indication of dry rot fungus. A key to the identification of fungal growth in buildings based on the characteristics of fruit-bodies, strands and mycelium was developed by Bravery *et al.* (1987). The details are described in Chapter 3.

Observation of the appearance of decayed materials can also allow identification of the fungi involved. For example, wood thoroughly rotted with *Serpula lacrymans* is light in weight, crumbles under the fingers, is dull brown in colour and shows a typical cuboidal cracking contrasting with that of any other species. Detection of decay can be based on colour and textural changes, distortion, loss of strength and loss of weight. The symptoms of decay fungi and their characteristics are discussed in Chapter 3. The detection of decay in timber can be done by coring, boring, drilling and the use of mechanical probes, or by non-destructive inspection using trained animals, fibrescopes or remote visual inspection with a borescope, or by measurement of electrical conductivity and resistance. High technology equipment can be used for detecting decay and quantifying its extent. For example, collimated photon scattering equipment has been used to determine decay in structural timbers. For further information on these techniques see Chapters 7 and 12.

Laboratory examination with optical and electron microscopy, and culture characteristics' are routinely employed to determine the types of fungi involved in decay. Other methods include biochemical and genetic fingerprinting (Palfreyman, 1991a, 1991b).

There have been (controversial) reports on the cause of sudden infant death syndrome (SIDS) with particular reference to *Scopulariopsis brevicaulis* and the production of toxic gas (Richardson, 1990; Kelley *et al.*, 1992). A number of potentially pathogenic or

allergenic fungi (Risk Group 2; Advisory Committee on Dangerous Pathogens, 1990) including *Aspergillus fumigatus, A. flavus, A. niger, A. terreus, Fusarium oxysporum, Rhizopus microsporus* and *Absidia corymbifera* have been isolated from cot mattresses (Kelley *et al.*, 1992). Several species of known toxin or active metabolite producing fungi were also isolated from the cot mattress studies. These include *Penicillium brevicompactum, P. chrysogenum, P. citrinum, Chaetomium fumicola, C. globosum, C. murorum* and *C. senegalense.*

Assessment of indoor airborne fungi

A range of methods have been developed for trapping fungal spores in the built environment for qualitative and quantitative analysis. For example spores have been collected on microscope slides coated with a sticky substance, for example petroleum jelly, or on Petri dishes containing nutrient media. However, suction traps, for example Andersen sampler, are a more reliable method through the use of suction traps (impactors). Recently, biological systems and bioassays have been developed for monitoring indoor air quality (Baird *et al.*, 1991).

A visual examination of the source, for example fungi growing on the edges of bath tubs, sinks, wallpaper and on items stored in damp cupboards and basements, and their symptoms can allow identification of the fungi involved. Mouldy odours can be assumed to represent fungal contamination. These can be assessed by using (animals for example) trained dogs or by complicated biochemical analysis.

Centralized air-conditioning systems in the built environment usually contain deposits of dust, for example in central humidifiers, which act as reservoirs of fungal growth. An alternative reservoir might be stuffed mattresses in long-term storage areas. Source sampling of these reservoirs allow confirmation of contaminants and identification of the specific fungi that might have been involved.

The commonest areas in the built environment which allow fungal growth and development are related to moisture sources and reservoirs. These reservoirs can be assessed for fungal contamination. They include humidifiers, dehumidifiers, wet carpeting, tiles, walls, wallpaper, sinks, bathtubs, window sills and basements. Damp organic materials in the indoor air, including stored food, wood chips, bird droppings, skin dropping of pets, etc., provide food sources for fungal growth and proliferation. In a number of cases house dust mite has been known to be associated with mould fungi (Burgess, 1993).

INVESTIGATION OF ENVIRONMENTAL CONDITIONS

The investigation of external and internal environmental conditions should be made using appropriate instruments. These may include the use of monitoring systems, including a full weather station. There are a variety of instruments which can be used to measure environmental parameters in the built environment. These instruments range from simple hand-held capacitance and moisture meters to computational fluid dynamics using tracer gases and infra-red photoacoustic detectors. Temperature measurements can be made with thermometers or with thermocouples and a data logger. The detailed description of

inspection and monitoring of environmental conditions within the building fabric is discussed in Chapter 7.

RISK ASSESSMENT

Data acquired from physical and biological measurements in the building can be interpreted to identify the causes and effects of the problem. Observation of the occupants' activities and of the building design, materials, finish and maintenance may lead to improved risk assessment. Environmental reactions and ill health associated with buildings are so variable that it is difficult to establish whether symptoms are caused by a specific factor. Large concentrations of fungal spores in a building, particularly when known to cause serious health effects, for example *Aspergillus flavus, A. parasiticus* and *Stachybotrys* spp., etc. should be considered a potential hazard.

The measurement of moisture, relative humidity, microventilation and salt content can lead to the assessment of decay activity. The data acquired from these observations combined with the level and extent of fungal activity and knowledge of building design and construction can be used to monitor and predict the risk of structural decay.

SUMMARY

The growth and development of fungi, causing microbial biodeterioration of building materials and of the contents of both modern and historic buildings is attributed to changes in the built environment and has led to an increased incidence of health problems (Berglund *et al.,* 1988; Raw, 1990; Singh, 1993). The built environment provides ecological niches for the development of fungi, according to a number of factors, including design and construction, shape and configuration, materials and structures, use occupancy and management. The main environmental parameters favouring the decay of materials and contents are water, humidity, increased temperature and the lack of ventilation. Building defects result in the ingress of moisture, associated with nutrient materials or detritus enabling moulds, fungi, insects and other biological organisms to develop.

Building mycology affects the health of occupants. The factors involved include the growth of fungi, moulds, bacteria, mites and other allergens. Symptoms include fatigue, headaches, skin irritation, and mucous membrane trouble. The medical field recognizes the following allergic diseases: asthma, allergic rhinitis, serous otitis media, bronchopulmonary aspergillosis and hypersensitivity pneumonitis. The effects of building mycology on the health of building structures, contents and decorations are well documented. Among the influencing factors are the biodeterioration of timber caused by fungi (dry rot and wet rot fungi).

The damage caused by fungi is very familiar as is the destruction caused in attempts to eradicate them, including the use of chemicals, which not only cause concern to health authorities, wildlife interests and environmentalists but can also result in resistance in the target organisms. For these reasons, fundamental scientific study of these organisms together with a multidisciplinary scientific knowledge of building construction and the indoor environment is required. These investigations can provide better understanding of

building-related sickness together with more effective, more economical, and less destructive environmental control strategies.

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Introduction, ecology, taxonomy and economic importance of fungi in buildings

2

Jagjit Singh

INTRODUCTION

Fungi form a distinct group of organisms within the plant kingdom which is now regarded as a separate kingdom of the living world, with the same status as the plant and animal kingdoms. A fairly diverse range of organisms is included within the fungal kingdom. Every living being on earth, animals (including humans) and plants are harmed or benefited directly or indirectly by fungi. Fungi are notorious in buildings as they have been known to cause damage to structural timbers and interior decorative substrate, and cause concern about the indoor air quality. They are also known to cause damage to building contents and furnishings, for example carpets, furniture, leather goods, museum objects, books, paintings and clothes.

Most house owners are familiar with the word fungus in terms of mushrooms, toadstools, mould and mildew. Fungi with mushroomshaped fruit bodies mostly form a group known as the Basidiomycetes. A few classified as Ascomycetes are commonly called ‘mushroom’ and ‘toadstool’. Toadstools are commonly regarded as inedible or poisonous and mushrooms as edible, but there is no clear difference between ‘mushrooms’ and ‘toadstools’.

WHAT IS A FUNGUS?

Fungi (L. *fungus*, mushroom) are nucleated organisms which lack green colouring matter or chlorophyll, and therefore cannot photosynthesize

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their food (Martin, 1955). They are heterotrophic, obtaining nutrition in diverse ways: from decaying animals or vegetable matter or products derived from animals and plants such as foodstuffs, artifacts and clothes. This is called **saprophytism** and the fungi are

hence referred to as **saprophytes** or **saprobes**. Nutrition from the tissues of a living plant or animal is referred to as **parasitism** so that the fungi are **parasites**.

Some fungi live symbiotically with the roots of higher plants, replacing root-hairs and absorbing food material from the decomposed organic substances present in the soil. This type of association of a fungus with the roots of higher plants is called a **Mycorrhiza** (fungus root) (Harley, 1969). Most mycorrhizal fungi are mushrooms or toadstools and without them many of our woods and forests would not exist. Mycorrhizal fungi are increasingly cultivated, particularly high quality truffles.

Thus, fungi are achlorophyllous and heterotrophic (saprophytic, parasitic, symbiotic or hyperparasitic). They are also eukaryotic and sporebearing, their cells being surrounded by a well-defined cell wall made up of chitin and fungal cellulose, along with many other complex organic molecules. Fungi usually obtain food by absorption, except a few lower groups which take in food by ingestion. The study of fungi is known as **mycology** (Gr. *mykes*, mushroom or fungus; *logos*, discourse).

NUMERICAL ESTIMATES OF FUNGI

Fungi are highly diverse and versatile organisms adapted to all kinds of environments. They are ubiquitous on earth, in water, in moist soil, in compost, or in decomposing organic matter as saprophytes. Manufactured artifacts provide specialized microclimates and ecological niches in spatial ecosystems for the growth and establishment of fungi, leading to their exploitation of the built environment. They may also occur in and on plants, humans and animals as parasites, symbionts or saprophytes. They thus play an essential part in the economy of nature. Out of about two million kinds of living organisms on the earth, the fungi may constitute up to 250000 species. Approximately 100000 are now known. The estimated number of fungal species recorded in Britain is about 10000 of which 2500–3000 can be classified as mushrooms and toadstools.

WHY SHOULD WE STUDY FUNGI?

The importance of fungi to humans, directly or indirectly, is immense (Garrett, 1956, 1981; Christensen, 1965; Burnett, 1976). They attack buildings, timbers, stored goods, clothing, animals and even their own bodies, through allergy and diseases. They also attack objects, specimens, books and paintings in controlled environments, and for example, damage optical instruments, leather, paint, kerosene, tapestry and paintings in museums, galleries and historic houses. Some mushrooms and toadstools are directly poisonous and others produce poisonous metabolites.

Many fungi have, however, proved useful. Many mushrooms and toadstools are widely used as food and a range of valuable medicines, antibiotics, enzymes and other drugs are fungal products. Saprophytic fungi, together with bacteria, docompose complex plant and animal materials into simple compounds which can be absorbed easily by green plants. In the absence of decomposition, future generations of green plants would die through lack of nutrients. Brewing, wine making, the manufacture of alcohols, bread and cheeses, the fermentations that produce organic acids and antibiotics or other drugs and products all rely on the metabolic activity of yeasts and filamentous fungi. Fungi are also

used in microbiology, biochemistry, genetics and other scientific research. Thus, knowledge about fungi is important for the layperson and professional alike.

SPORE, HYPHA AND MYCELIUM

Fungi propagate and survive through spores, which have a similar function to the seeds of higher plants. The number of spores produced by each fruit body is immense, for example a typical field mushroom produces about 16 billion spores, released at the rate of about 100 million an hour. Gregory *et al.* (1953) recorded almost 80000 basidiospores per cubic metre in the basement of a house containing active fruit bodies of dry rot fungus. Spores are dispersed by wind and water and if these spores happen to land in a suitable built environment they germinate and initiate new infections in buildings. The size, shape, colour and ornamentation including the chemical reactions of the spores, are consistent and play an important role in the description of each species.

The commonest spores in the indoor environments of buildings are *Alternaria* spp., *Aspergillus* spp., yeasts, *Trichoderma* spp. and *Penicillium* spp. The sexual spores when formed within a sac are called **ascospores,** for example Ascomycotina fungi. Sexual spores in Basidiomycotina generally develop from the end of a club-shaped structure, called the **basidium**. These spores are generally called **basidiospores,** for example the spores of *Serpula lacrymans* (dry rot) and wet rot fungi.

Under favourable environmental conditions, spores deposited on a suitable substrate germinate to produce one or more tube-like outgrowths, called **germ tubes**. Each germ tube elongates to form long, thread-like, fine-branched, tubular filaments called **hyphae**. Each hypha (Gr. *hyphe*, web) divides, by transverse walls or **septa** (L. *septum*, partition) into uninucleate or multinucleate cells. Such hyphae are called septate and are generally found in fungi belonging to Ascomycotina, Basidiomycotina and Deuteromycotina. The simpler filamentous fungi classified as the Phycomycetes do not develop septa. These non-sepate or aseptate hyphae with nuclei in a common matrix are referred to as **coenocytic** (Gr. *kolnos*, common; *kytos*, a hollow vessel).

The mass of hyphae constituting the thallus of a fungus is called the **mycelium** (Gr. *mykes*, mushroom or fungus). The **thallus** is differentiated into a vegetative part which absorbs nutrients and a reproductive part which forms reproductive structures. Such thalli are called **eucarpic** (Gr. *Eu*, good; *karpos*, fruit). Some thalli do not show any differentiation, and following a phase of vegetative growth, the entire thallus modifies into one or more reproductive structures. These thalli are called **holocarpic** (Gr. *holos*, entirely). When a mycelium contains genetically identical nuclei, it is referred to as **homokaryotic** (Gr. *homo*, the same, *karyon*, nut, nucleus), and when the nuclei are of different genetic constitution it is referred to as **heterokaryotic** (Gr. *heteros*, other). Hyphae may aggregate to form **rhizomorphs**—mycelial cords or strands which transport water and nutrients to the growing tip, while in plant pathogens **appresoria** and **haustria** may be formed to draw nourishment from the host. Rhizomorph (Gr. *rhiza*, root; *morphe*, shape) formation is seen typically in *Serpula lacrymans* (dry rot) fungus and *Armillaria mellea* (honey fungus).

In many Basidiomycetes a small hyphal outgrowth develops into a short branch just behind the septum. This outgrowth becomes curved

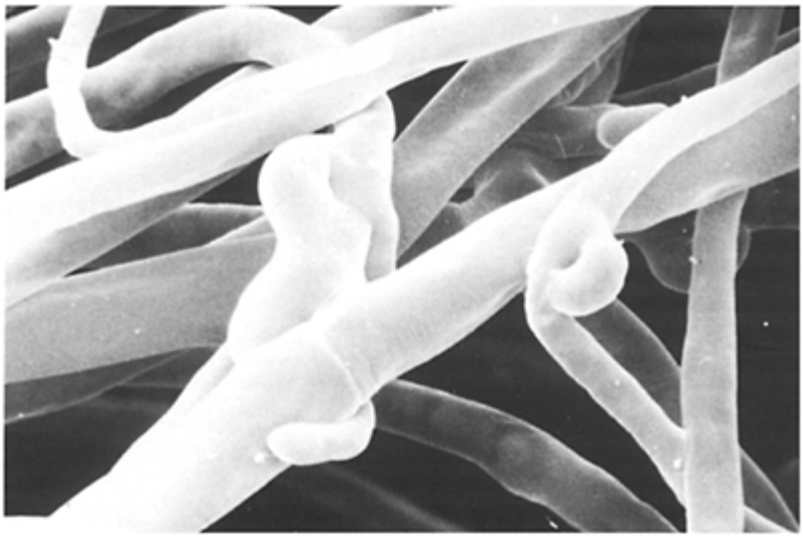

Figure 2.1 Clamp connection in basidiomycetes. (Reproduced with permission of Ingo NUSS.)

and its tip comes in contact and fuses with the adjacent cell allowing passage of nuclei from one cell to the next. This is referred to as a **clamp connection** (Figure 2.1).

HISTORY OF FUNGI

Our knowledge of the history of fungi is not as well documented as that of the plant and animal kingdoms, since the nature and structure of larger fungi have allowed little fossil evidence to survive. It is thus difficult to estimate the age of the fungal kingdom. The fungal fossil record extends back to the Devonian and Pre-Cambian eras (Martin, 1968).

The earliest written records of plant pathogenic fungi come from 1200 BC in the ancient vedas of India and in references to the blasting and mildews of wheat in the bible. The effects of poisonous fungi were referred to in an epigram written by Euripides in about 450 BC.

Fungi causing diseases in humans and other animals were almost completely neglected or ignored in human pathology until the last decade of the 19th century. However they are now recognized as potentially serious health hazards, and hence the expansion in the field of medical mycology. The exploitation of moulds to human advantage goes back to 3000 BC. The discovery of the nature of fermentation laid the foundations for a more scientific approach to the industrial exploitation of fungi. Industrial exploitation of fungi has led to many investigations of the role of fungi in the commercial production of enzymes, manufacture of cheese, food spoilage, and the rotting of textile fibres. The 20th century has seen major developments in knowledge of the cytology, sex, genetics and

physiological specialization of fungi and in the mycological aspects of medicine, industry, and plant pathology.

For detailed accounts on the history of fungi the reader should consult Martin (1968), Tiffney and Barghoorn (1974), Pirozynski (1976) and Pirozynski and Hawksworth (1988).

ECOLOGY

Ecology is concerned with the study of interrelationships between living organisms and their environment. This interaction between organisms and the surrounding environment is expressed as an ecological complex or ecosystem. Fungi play a dominant role in the decomposition and degradation cycle in nature. They do so by the secretion of various types of enzymes, such as cellulases, hemicellulases, proteases, pectinases and lignolytic enzymes. The decomposition of organic material due to fungal activity may be beneficial to people in some circumstances and detrimental in others. There are fungi that will utilize almost any non-living organic substrate, and a few that will invade plant and animal (including human) tissue.

Some fungi occupy very specialized ecological niches. *Serpula lacrymans,* for example, only occurs in buildings in Europe. *Cryptococcus neoformans* can be abundant in dry, shaded (indoor) pigeon droppings, surviving with little competition in this alkaline, high-salt environment. Exposure to sunlight or to soil containing amoebae causes rapid elimination of this fungus. *Histoplasma capsulatum* also utilizes bird droppings (primarily those of starlings) but also requires water and soil. Some insects only attack timber in buildings if it is partially digested by fungi.

The decomposition activities of fungi in the outdoor environment and the activities of decay fungi in the indoor environment produce a range of spores. For example, agricultural practices can introduce billions of fungal spores into the air. Similarly the deterioration of dairy products in the indoor environment is caused by colonization by the spores of *Geotrichum candidum,* and species of *Penicillium*, *Alternaria* and *Cladosporium.* If sufficient water is available, feeds and grains are commonly infested during storage by a range of fungi, including the species of *Aspergillus*, *Penicillium, Phoma, Cladosporium, Fusarium, Curvularia* and *Mucor.*

The majority of fungal spores in the indoor environments are derived from outdoor sources. However, when a spore with broad nutrient requirements (able to use a wide variety of substrates) encounters damp organic material indoors, it is able to germinate and grow, produce metabolites, volatile compounds, and new spores, resulting in further indoor contamination. It is important to remember that fungi will grow on any substrate, indoors or out, that contains reduced carbon compounds and other nutrients and is damp.

The environmental conditions in the built environment that are especially conducive to the growth of fungi are high relative humidity (leading to condensation and absorption of water by hygroscopic materials), moisture accumulated in appliances, leaks and floods. Below 65% equilibrium relative humidity (ERH) (0.65 a_w), there is no growth (p.11). In general, fungi prefer dampness rather than standing water, although some (e.g. *Fusarium, Phialophora,* yeasts) will grow in humidifier reservoirs. The formation of moisture

pockets with no ventilation, through interstitial condensation and the occurrence of cold bridges, helps fungi to grow and proliferate in the built environment.

The environment in which any organism lives comprises physical, chemical and biological elements which will affect the establishment, growth and development of an organism. The organism in turn will exploit and modify the environmental factors. Interaction is, by definition, a two-way process. There are five essentials for the growth of fungi on any substrate: (i) sources of inoculum infection, (ii) a suitable substrate, (iii) adequate water, (iv) adequate oxygen and (v) favourable temperature. The physical factors of most importance are those of temperature, water availability or humidity, O_2 and CO_2 levels, pH and perhaps pressure, light and other forms of radiation.

The main factors in the chemical environment relate to nutrition and inhibition. An organism requires both **micro**nutrients (trace elements, growth factors, vitamins) in small quantities and **macro**nutrients (a carbon source for energy and assimilation, the 'food' in the case of saprophytic fungi) for growth. Chemical substances may also be present which inhibit or modify growth. These compounds may be inherent in the material, may have been deliberately added to cause such an effect (as in the case of preservatives), or result from the metabolism of the organisms.

The physical and chemical tolerances and requirements of any organism are, of course, part of its physiological make-up, and there are few aspects of fungal ecology which do not go hand-in-hand with physio logical studies. Monographs on different fungal groups and critical lists of fungi from different substrates and habitats form a basis for future work on fungal ecology.

The environment in which a fungus grows is exceedingly complex. The biological factors in the environment include competition of all kinds from other organisms which may be present and also the various modifications to the physical and chemical environment which are induced by fungi as they develop. Organisms compete for nutrients and space, some organisms are pathogens of others, and the waste products and secretions of organisms may build up, perhaps changing the pH or directly inhibiting growth by toxic action. Organisms create and maintain their own microclimate. They may disrupt the substrate, and thus are able to modify not only the chemical but also the physical environment.

The environment is, therefore, seen to be both complex and dynamic, and for an organism to succeed it must be in balance, within fine limits, with the environment. If this balance can be disturbed to render it more hostile to the organism, less growth and therefore less damage will occur. This is the underlying reason why the mycologist needs to think in ecological terms, and why ecology is of primary importance and direct relevance to building mycology.

ECONOMIC IMPORTANCE OF FUNGI

Fungi are ubiquitous and almost all of us encounter them in the course of our daily lives. They may deteriorate cellulosic materials such as wood or wood-based products, textiles, paper and insulating materials. Fungi can also cause deterioration of non-cellulosic materials, such as plastic, glass, electrical equipment, fuel, paints, paint films, leather and glues, etc. Fungi often grow in jams, jellies, pickles or bread and may spoil stored fruits,

vegetables and grains. Contamination of food by the fungi not only decreases the food value, but may render them unfit for consumption through the production of mycotoxins (Webster, 1980).

Fungi play an important ecological role in decomposition. They have been decaying plants and animal bodies for the last two billion years and liberating various elements such as hydrogen, carbon, oxygen, nitrogen, phosphorus, potassium, sulfur, iron, calcium, magnesium and zinc. These would for ever be locked up in their bodies without the activity of fungi and bacteria, that liberate carbon dioxide into the atmosphere, to be used again in photosynthesis by green plants. The complex and multiple enzyme systems of fungi enable them to function as scavengers in the destruction of celluloses, hemicelluloses, lignins, pectins and various carbohydrates and nitrogenous substances which accumulate on the earth's surface with the death of autotrophic plants and heterotrophic animals and insects. Their ability to break down complex organic substrates of every kind is an essential activity in the recycling of organic materials.

People may suffer from fungal diseases such as histoplasmosis, coccidioidomycosis, aspergilloisis, ringworm or athlete's foot or from fungal allergies. Most fungal infections are of the skin but the respiratory tract, lungs, bones, viscera, intestine, liver, kidney, nasal sinuses, corneal tissue of eye, etc., may also be affected (sometimes severely). Some species of *Rhizopus* and *Mucor* fungi infect lungs, brain and gastric tissues (Webster, 1980), while *Neurospora* and *Fusarium* infect corneal tissue, and *Histoplasma* infects the lungs, spleen, liver, kidney and nervous system and also the lymphatic system. *Aspergillus* commonly infects the lungs and nasal sinuses. Some fungi are skin pathogens or dermatophytes of humans and other animals (Beneke and Rogers, 1970; Wilson and Plunkett, 1974; Cambell and Stewart, 1980).

Fungi collected for food include truffles, morels, mushrooms, puff balls, species of *Clavaria* and non-woody polypores. Many wild fungi are edible and have an excellent flavour but some of them are deadly poisonous. It is, therefore, important that their identity should be beyond doubt before they are consumed.

CLASSIFICATION AND TAXONOMY

While no practising building mycologist can hope to become an expert on all groups of fungi, some basic understanding of their systematic arrangement is necessary. Various schemes of classification have been proposed by Martin (1961), Ainsworth (1966, 1971), Kendrick (1971), Ainsworth *et al.* (1973), Sparrow (1973), Alexopoulous and Mims (1979), and Hawksworth *et al.* (1983). For general purposes, living fungi can be divided into **Myxomycota** (wall-less fungi, e.g. slime moulds) and **Eumycota** (the true walled fungi) (Webster, 1980). Slime moulds are the common Myxomycetes found in buildings. The division Eumycota is divided into five sub-divisions: Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina; the first four of these are of significance to buildings and are described below. The Mastigomycotina are water moulds and are described in detail by Sparrow (1973).

ZYGOMYCOTINA

The vegetative state is typically mycelial, without septa and asexual reproduction is by non-motile spores formed in a sporangium. The Zygomycotina comprises about 200 species, among which are a number of common moulds of considerable economic importance. The most common examples are the *Mucor* and *Rhizopus* species. The zygomycetes are common as saprophytes in soil and do not have the ability to degrade cellulose.

ASCOMYCOTINA

Sexual spores are produced in asci and are called ascospores. In most species each ascus contains eight spores. Asexual reproduction may be by conidia, which show great diversity of form and arrangement, or by oidia. The subdivision comprises about 29000 species and includes both saprophytes and parasites, ranging from microscopic species to large fleshy fungi, some of which are edible. The most common examples in buildings are species of *Peziza, Chaetomium* and yeasts. A number of important human diseases are caused by fungi in this subdivision, most notably the dermatophytes such as *Arthroderma* and *Nannizzia* spp.

BASIDIOMYCOTINA

The sexual spores are basidiospores, borne exogenously on special organs, known as basidia (Figure 2.2). Typically each basidium bears four spores but sometimes two or six. In the higher Basidiomycotina, the mushrooms and toadstools, the basidia are massed on the gills or pores of the fleshy sporophores and there are specialized arrangements for ensuring the widespread distribution of the spores. This subgroup includes about 16000 species. The most common species responsible for rotting wood and similar materials are *Serpula lacrymans* (dry rot) and wet rot fungi.

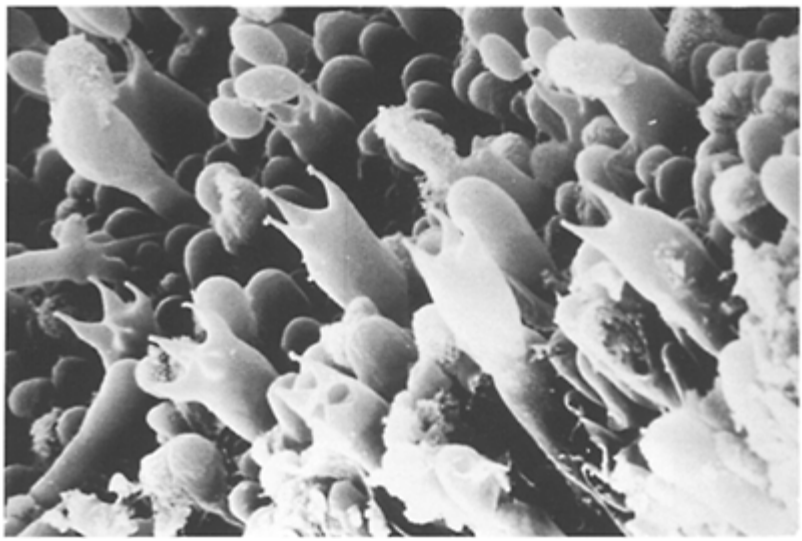

Figure 2.2 Basidiospores borne on basidium. (Reproduced with permission of Ingo NUSS.)

DEUTEROMYCOTINA

This is the second largest subdivision of the fungi and contains over 17000 species. It comprises fungi which produce neither ascospores nor basidiospores but reproduce solely by means of **conidia.** Most of the Deuteromycotina are conidial states of Ascomycotina but the ascomycete stage has never been found. The members of this group invade virtually every possible type of habitat and substrate causing biodeterioration of many materials. They form blue-stained patches on the surface of wood, cause infections of the skin, and produce a range of toxins in foods, feeds and grains in storage. The most common examples are species of *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Geotrichum, Penicillium* and *Fusarium.*

SUMMARY

Fungi are highly diverse and versatile organisms adapted to all kinds of environments. Every living being on earth is harmed or benefited directly or indirectly by fungi. Some fungi occupy very specialized ecological niches within our buildings, for example *Serpula lacrymans* only occurs in buildings in Europe. A range of fungi in and around our buildings, can cause damage to building structures, materials, and their contents, and also affect the health of occupants.

The species frequently responsible for rotting wood and similar materials, for example *Serpula lacrymans* and wet rot fungi, belong to the subdivision Basidiomycotina. The

most common fungal species causing infections of the skin and producing a range of toxins in foods, feeds and grains in storage belong to the subdivision Deuteromycotina.

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Nature and extent of deterioration in buildings due to fungi

3

Jagjit Singh

INTRODUCTION

Building structures, decorations and contents (including foodstuffs), are affected by fungal infestations, which may adversely affect the health of the occupants. Deterioration is a widespread and very familiar phenomenon which affects most organic materials and even some inorganic ones. Most buildings are made from materials that will deteriorate, decay and eventually disintegrate. The rates of decay of the parts of a building are not uniform. The structural elements may have a long life, operating at low stress, and even if damaged or decayed may have sufficient redundancy to continue to give an adequate performance. Factors which accelerate the rate of deterioration are: excessive light, pollution, insects, fungi and other biological agents, the wrong humidity and bad maintenance management. The materials used in buildings are subject to different types of chemical, physical and biological attack; however, the most important factor is the environment created by the building itself, associated services, and the uses to which the building is put. Thorough investigations of the building ecology, including the microenvironments in and around the material, are necessary to minimize the use of potentially hazardous chemicals. Although a home is the largest single investment most families make, this investment is too often inadequately cared for and periodic inspections or preventive maintenance are seldom performed (Verral and Amburgey, 1977).

People have always had to contend with biodeterioration and its record can be traced back to biblical times or probably earlier. Hueck (1968) defined biodeterioration of materials as ‘any undesirable change in the properties of material of economic importance brought about by the

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activities of living organisms’. The concept of biodeterioration is separated from biodegradation which can be considered as the harnessing by humans of the natural decay capabilities of organisms to convert a waste material into a more acceptable and

manageable form or to produce a useful end-product from waste materials (Singh and Faull, 1986).

THE EXTENT OF BIODETERIORATION

Biodeterioration covers a wide range of subjects, and this chapter will deal mainly with the microbiological and ecological aspects of decay and mould in buildings. Given appropriate conditions timber, paint and other organic materials in buildings will be degraded by biological agents such as insects, fungi, bacteria and lichens.

Materials subject to decay and deterioration are innumerable, for example agricultural products and food materials, and materials and artifacts that people have, usually at some considerable expense, designed and arranged to resist microbial attack: wood, fabrics, and building decorations and contents. Biodeterioration may involve a range of different mechanisms. In mechanical biodeterioration, the material is damaged by the physical forces exerted by the organisms. These include, for example, the disruption of floorboards and skirting boards by fungal fruit bodies. In chemical deterioration, the material is attacked by chemicals and enzymes produced by the organisms (chemical assimilatory deterioration) (e.g. oxidation, reduction, the production of organic acids). The fungi use the material as a food source, as in the breakdown of cellulose in building timber, cotton, textiles and rayon and the moulding of buildings (exterior and interior), paints, books, wallpaper, shoes and mattresses. Chemical dissimilatory deterioration includes processes in which the material is damaged by excretion products liberated from the organism, for example the production of secondary metabolites, toxins, pigments, and corrosive waste products. The damage caused to stone through the production of acids by lichens is another example of chemical dissimilatory deterioration. The implications to health of these organisms are dealt with in Chapters 5 and 11. In ‘fouling’, the function of the material is impaired by the presence of the organism, for example, fungal mycelium may bridge gaps in electrical equipment. The estimated cost of repairing damage caused by decay of timber in buildings in the United Kingdom amounts to about £400 million per annum (Singh, 1993).

There is a voluminous literature on the biodeterioration of materials. The following references will provide the reader with both general background and specific information on microbial biodeterioration of materials: *Introduction to Biodeterioration* by Allsopp and Seal (1986); Proceedings of the various *International Biodeterioration* Symposia.

The microbial biodeterioration of building materials has been described in publications from the British Wood Preserving Association, Rentokil Library and the Building Research Establishment, and include *Recognising wood rot and insect damage in buildings; Remedial treatment of buildings* by Richardson (1980) and *Decay of timber and its protection* by Cartwright and Findlay (1958).

FUNGAL DAMAGE IN BUILDINGS

A wide range of materials are subject to microbiological deterioration, which can be caused by a broad spectrum of microorganisms, including fungi and bacteria. Other

biological agents involved in the biodeterioration of building materials include insects, lichens, algae, mosses, rodents, mammals, birds and higher plants (Hicken, 1963a, 1971, 1975; Harris, 1971; Mourier *et al.*, 1977; Meehan, 1984).

NATURE AND TYPE OF FUNGIINVOLVED

Fungi which cause decay of timber in buildings mostly belong to the class Basidiomycetes (p. 30). Decay fungi are capable of enzymatically degrading complex cellulosic materials, such as wood, into simple digestible products. The decay of wood cells by these fungi results in losses in the weight and strength of the wood. Stain and mould fungi, however, are less able to use complex plant material as food and cause little loss in wood strength. They feed on starches and sugars stored in parenchyma cells of the sapwood, and in colonizing these cells, increase the porosity of the wood.

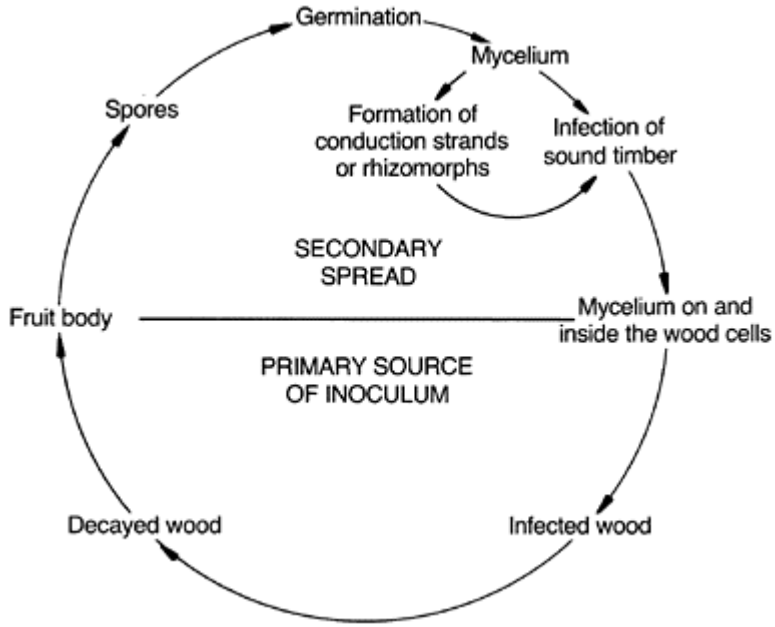


Figure 3.1 Life cycle of a decay fungus.

The typical decay cycle of a fungus begins with colonizing hyphae penetrating and ramifying wood cells and causing decay. When conditions are suitable this mycelium proceeds to form fruit bodies or sporophores on the surface in various forms and shapes. The life cycle of a typical decay fungus is illustrated in Figure 3.1. The appearance of fruit bodies usually indicates a fairly advanced stage of wood decay. Thousands of microscopic spores are produced in the fruit bodies which, when mature, are released into the air. Under favourable climatic conditions spores germinate and cause new infections.

Secondary spread is mainly by the germination of spores and the formation of conducting strands.

Common moulds found infecting building materials such as textiles, paper, painted surfaces, masonry, brickwork, concrete paving, etc., belong to the class Hyphomycetes of the subdivision Deuteromycotina. These fungi do not produce any fructifications, but instead produce condiophores openly on any part of the mycelium.

Many types of fungi can be found in buildings, only some of whichcause wood rot. There are two main types of wood-rotting fungi found in buildings—wet rot and dry rot. The identification of fungal infestations in buildings is not always easy, but various morphological characteristics can aid their identification: fruit bodies, strands, mycelium and the condition and symptoms of the wood. The following description of the most common wood-rotting fungi will help the reader in identification. Correct identification is essential if the right treatment is to be given.

Dry rot

The term dry rot in Britain refers to the decay of timber caused by *Serpula lacrymans* the true dry rot fungus (formerly known as *Merulius lacrymans*). The dry rot fungus mostly attacks soft wood and often causes extensive damage. The term dry rot is rather misleading, as moist conditions are required to initiate the growth (a minimum moisture content in timbers of about 20%), and optimum growth occurs at about 30–40%. Spore germination requires a favourable microclimate at the wood surface, i.e. a wood moisture content of 30% with little ventilation at the wood surface. The fungus has the ability to grow through plaster, brickwork and masonry and even to extend over a distance of several metres from its food source to attack sound timber using specialized hyphal strands (rhizomorphs) (Figure 3.2) (p. 25). Rhizomorphs may be up to 6 mm in diameter; they are relatively brittle when dry. These adaptations to the building environment make it a cause of rapid decay and is perhaps the most difficult decay fungus to eradicate. Conditions of static dampness are particularly favourable to *S. lacrymans;* however unlike wet rot fungi, they are able to tolerate fluctuating conditions (Butler, 1958). Active growth is indicated by silky white sheets or cottonwool like white cushions with patches of lemon yellow or lilac tinges where exposed to light, perhaps covered with tears or water drops in unventilated conditions. This exudation of water is the way the fungus responds to the atmospheric relative humidity and is the explanation for the latin name *lacrymans* (weeping). Mycelial strands are white to grey and often subsequently green through the development of superficial saprophytic mould growth.

Sporophores or fruit bodies generally develop under unfavourable conditions of temperature, humidity, exhaustion of nutrients and environmental stress. Sporophores are tough, fleshy, pancake or bracket-shaped, varying from a few centimetres to a metre or more across (Figure 3.3).

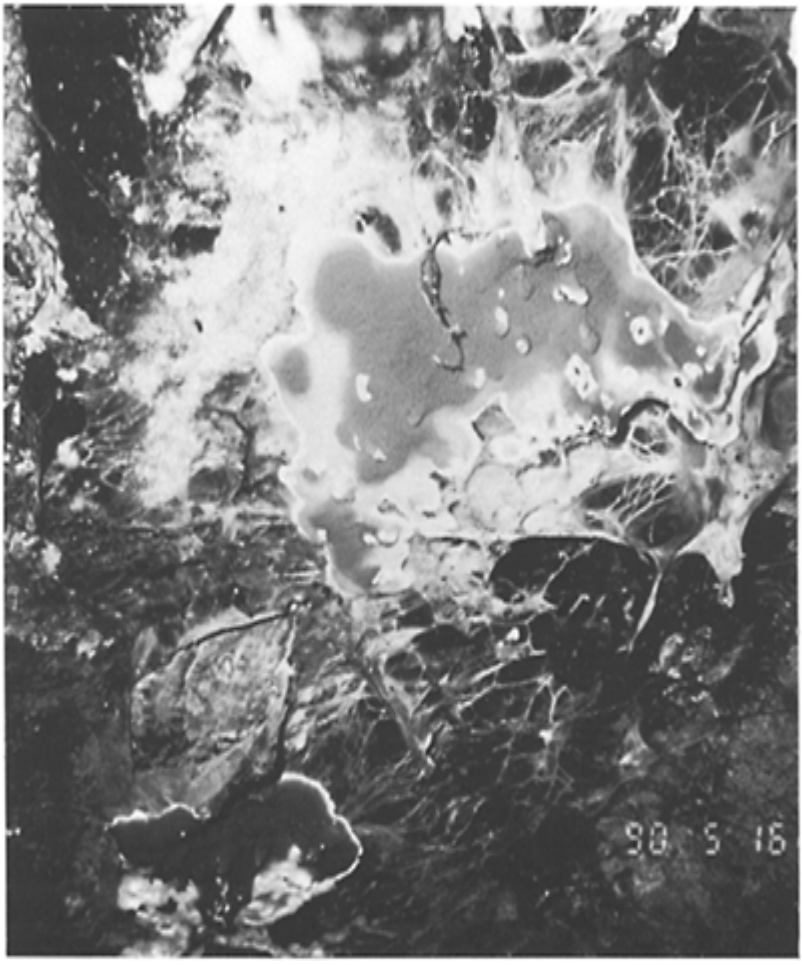


Figure 3.2 Dry rot *(S. lacrymans)* strands (rhizomorphs).

Figure 3.3 Dry rot fruit bodies.

The centre is yellow-ochre when young, darkening to rusty red when mature due to spore production. The fruit body is covered with shallow pores or folds and the margin is white and grey. The appearance of the fruit body together with a distinctive 'mushroom' odour, may be the first indications of an outbreak of dry rot, as fungal growth in buildings is generally concealed.

Wood thoroughly rotted with dry rot fungus, *S. lacrymans*, is light in weight, crumbles under the fingers, is a dull brown colour and has lost its fresh resinous smell. It has a typical cubical cracking along and across the grain (Figure 3.4). The dry rot is also called brown rot, a term relating to the manner in which it destroys the cellulose but leaves the lignin largely unaltered so that the wood acquires a distinctive brown colour and the structural strength is almost entirely lost.

Serpula lacrymans does not occur in the wild in Europe and has only been found growing in the Indian Himalayas. The wild *S. lacrymans*

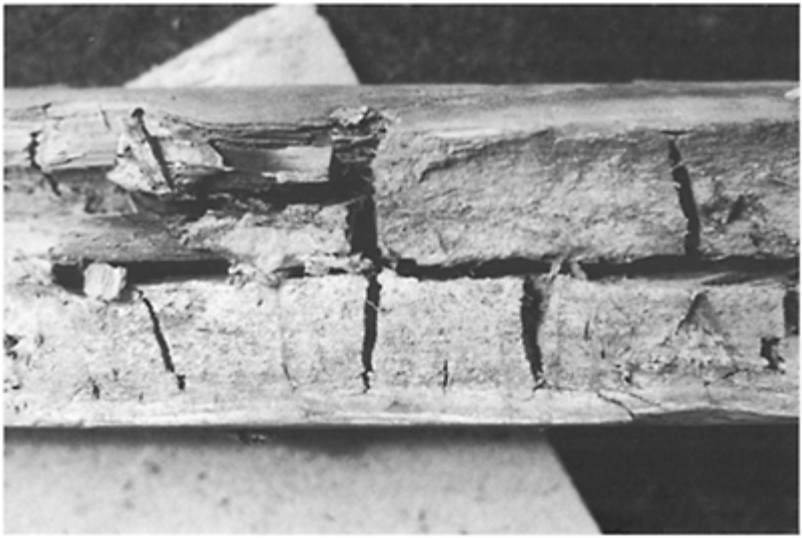


Figure 3.4 Dry-rot-decayed wood—cuboidal cracking.

fruiting body (Figure 3.5) was collected near Narkanda, India in July 1992 (Singh *et al.*, 1992, 1993; Bech-Anderson *et al.*, 1993a, 1993b). This fungus was originally isolated from the Indian Himalayas in 1947 and 1954 by Bagchee (1954).

Figure 3.5 Dry rot fruit body discovered in the Himalayas.

In Denmark, a number of other fungi related to the dry rot fungus occur in houses. These include *Serpula himantioides* (thin-fleshed dry rot fungus), *Leucogyrophana pinastri* (spiny dry rot fungus), *L. pulverulenta* (small dry rot fungus), *L. mollusca* (soft dry rot fungus) and *L. mollis* (Bech-Anderson, 1992).

Wet rot

Wet rot may be caused by several Basidiomycetes of which the most important are *Coniophora puteana* (cerebella), *C. marmorata, Phellinus contiguus, Donkioporia expansa, Pleurotus ostreatus, Asterostroma* spp., *Paxillus panuoides* and Poria fungi including *Amyloporia xantha, Poria placenta, Antrodia serialis* and *A. sinuosa*. Wet rot is also called white rot as it destroys both cellulose and lignin, leaving the colour of the wood largely unaltered but producing a soft felty or spongy texture.

Cellar rot fungus

Coniophora puteana and *C. marmorata* are the most common cause of wet rot in buildings which have become soaked by water leakage, e.g. soil moisture or plumbing leaks. The spores are ubiquitous and germinate readily, so that these fungi are likely to occur whenever suitable conditions arise. The hyphae are initially white, then yellow to brownish in colour, remaining off-white under impervious coverings. *C. puteana* forms rhizomorphs that are initially yellowish when young, becoming

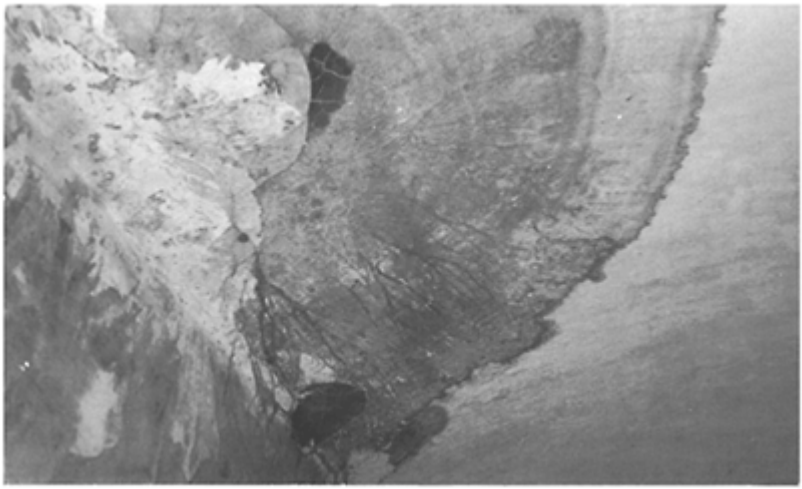


Figure 3.6 Wet rot *(Coniophora puteana)* mycelium and rhizomorphs turning black at maturity.

brown to black at maturity (Figure 3.6). They never extend from the infected wood.

Coniophora marmorata is often seen in connection with wood and calcium containing materials such as bricks, mortar or solid ground floor constructions. The spores of *C. marmorata* are shorter and wider than those of the cellar fungus. *C. marmorata* often contains yellow brown skeletals (cells with strongly enforced thickened walls) in the strandmycelium, unlike *C. puteana* which only possesses generative hyphae (Bech-Anderson, 1991/92). The sporophore of *C. puteana* rarely occurs in buildings and consists of an olive green to brown fruit body with a paler margin, thin skin and warty surface.

The fungus causes considerable shrinkage to wood, and cracking may occur, not unlike that caused by dry rot. The rotted wood is dark brown with dominant longitudinal cracks and infrequent crossgrain cracks. Freshly colonized wood usually shows a yellow coloration.

Poria fungi

These fungi generally attack softwood in buildings. They require a higher moisture content than dry rot but are tolerant of occasional drying and are therefore normally associated with roof leaks.

This group includes *Amyloporia xantha, Antrodia serialis, A. sinuosa* and *Poria placenta,* commonly known as the white pore fungi or mine fungi. *Antrodia* can cause extensive damage in buildings. *Antrodia serialis* is commonly called Row Pore fungus, because of its elongate or coherent, effused fruit bodies that are formed along star shakes. The species is common in damp, cool cellars and mines but it also occurs in roofs, on fence posts and on fir and pine trees. Fruit bodies grown in the dark are cauliflower-like, sterile, whitish yellow and those grown in the light are resupinate to pileate, growing in rows. The decayed wood is brown with cross-cracks and often a layer of mycelium is found in the cracks.

The mycelium of this group of fungi form white or cream sheets or fern-like growths, which may discolour brown on contact with iron. The rhizomorphs may be up to 3 mm in diameter, seldom thicker than twine, white to cream in colour, remaining flexible when dry, and they do not extend from their source of wood. The sporophore is rare in buildings; it is a white, irregular lumpy sheet 1.5–12 mm thick, covered with distinct pores, sometimes with strands emerging from its margins.

Spore-bearing surfaces are white to pale yellow, occasionally with pink patches (*Poria placenta* only). The decay damage to wood is similar to that caused by *S. lacrymans,* but the cubing is somewhat smaller, less deep and lighter in colour. When decayed wood crumbles between the fingers it is not so powdery as that attacked by *S. lacrymans,* but slightly more fibrous and gritty.

Phellinus contiguus

This fungus attacks both softwoods and hardwoods and is commonly found on the external joinery of buildings. The mycelium may be found in wood cavities, or around the sporophores in the f orm of tawny brown tufts. Mycelium does not form rhizomorphs as in the case of *S. lacrymans*. The sporophore is occasionally found in buildings and is thick, tough, elongated, ochre to dark brown in colour and covered in minute pores. The

decayed wood shows no cuboidal cracking, unlike *S. lacrymans* and *C. puteana*, and does not powder in the same way as dry rot, but instead, decayed wood bleaches and eventually develops a stringy, fibrous appearance.

Donkioporia expansa

This fungus attacks hardwood, particularly oak, in older buildings and in churches. However, once the fungus colonizes hardwood in a building it may spread to the adjacent softwoods. *D. expansa* tends to occur in much wetter conditions than dry rot and is found particularly on wood where there has been persistent water leakage. The fungus mycelium is yellow to reddish brown, forms a thick felted growth, often shaped to the contours of the wood, and exudes drops of yellowish-brown liquid. The mycelium does not form strands. The sporophore is thin, leathery, plate or bracket-shaped, or thick, hard and woody. The hymenium is cinnamon-brown or fawn in colour with numerous minute pores, often comprising several layers. *D. expansa* can cause more extensive damage to oak than any other fungi found in buildings, often attacking the ends of beams embedded in damp walls. Damage may be confined to the interior of the beam and not noticed until the typically bracket-shaped fruit bodies appear. It is often associated with death-watch beetle attack. Decayed wood becomes bleached and is reduced to a lint-like consistency, leaving stringy white fibres. Decayed wood is easily crushed, but does not crack.

Pleurotus ostreatus: oyster fungus

Pleurotus ostreatus is commonly called oyster fungus and belongs to the family Hymenomycetes, order Agaricales. The mycelium is whitish, and forms a woolly mat; rhizomorphs are not formed. The sporophore is a white-gilled, grey-capped mushroom with an off-centre stalk. It is occasionally found in buildings, usually associated with the decay of panel products. Decayed wood-board lightens in colour; in particle boards, the chips tend to separate.

Asterostroma

This fungus usually attacks only softwoods in buildings, and is commonly found on joinery, e.g. skirting boards, and is often limited in extent. The mycelium is white; toning cream and buff sheets are not always present. Hyphal strands form occasionally with a rough appearance. These remain flexible when dry, and some can cross masonry over a long distance. The sporophore sheet is very thin and hardly distinguishable from mycelial sheets. Pores are not present.

Paxillus panuoides

This fungus prefers very damp conditions. *P. panuoides* causes decay similar to that caused by cellar rot, i.e. deep longitudinal fissures and some fine cross-cracks appear. In the early stages wood is stained vivid yellow due to the mycelium but in a later stage, the decayed wood becomes soft and cheesy The mycelium is fine, soft, hairy and a dull yellow with occasional tinges of violet. The hyphae develop into fine branching strands,

coloured as the mycelium; they do not darken with age. The sporophore has no distinct stalk, but is attached at a particular point, tending to curl around the edges and eventually becoming rather irregular in shape. The colour is dingy yellow, but darkens as the spores develop, fan or funnel-shaped. The texture of the sporophore is soft and fleshy. The gills are yellow and branch frequently. The spore print is ochraceous rust coloured.

Gloeophyllum spp.

Two species (*G. trabeum* and *G. sepiarium*) of this fungus have been reported in Danish buildings (Chapter 12). *Gloeophyllum* spp. can tolerate higher temperatures than other fungi. The optimum conditions for their growth in buildings are a temperature of 35°C with relative humidity of 30–50%.

Lentinus spp.

Lentinus lepideus and *L. tigrinus* have been recorded in buildings in Europe. The fungi mainly attack structural timber in contact with the ground such as fence posts, poles, paving blocks, etc., and can tolerate temperatures of between 0 and 40°C. The decayed wood forms brown cubes. The fruit body in dark conditions such as in flat roof voids is abortive and appears like a Stag's Horn; in light conditions it has a fleshy mushroom cap set upside down on an extended stalk.

Soft rot

Findlay and Savory (1950) reported a form of deterioration resembling brown rot which resulted in unusual softening of wood. Savory (1954) applied to it the term 'Soft rot'. Soft rot is as prevalent as decay, however it is less damaging and less detectable. Soft rot can be regarded as a superficial form of wet rot. It is more usually found in timber in contact with the ground. Duncan and Eslyn (1966), in a taxonomic study of the organisms causing soft rot, identified 69 species. Most of them could cause substantial degradation of sapwood. The most destructive was *Chaetomium globosum*. Soft rot fungi have a number of distinctive physiological and ecological characteristics; they differ from decay fungi in the way they modify wood chemically, resembling white rot fungi in causing a comparatively small increase in alkaline solubility, yet behaving like brown rot species in being able to use the wood lignin extensively (Savory and Pinion, 1958, Levy and Preston, 1965). Partial weakening of the lignin carbohydrate complex in cooling towers wetted by water containing chloride will increase susceptibility to soft rot. Soft rotting fungi may lack such efficient precellulolytic enzyme systems as the brown rotting species, however they are capable of enduring the microclimate of wood surfaces, i.e. they can tolerate higher temperatures, higher pH values, and can grow in conditions of restricted oxygen.

Hardwoods are more susceptible to soft rot than are softwoods. It is mostly outer wood that is severely damaged by soft rot. As revealed by probing with a knife, the conspicuously degraded wood may be comparatively shallow and the transition between it and the underlying firm wood may be quite abrupt. When wet, the wood may be so decomposed that it can be scraped from the surface with a finger nail. When dry, the

surface of the wood may appear as though it has been lightly charred, and there will be profuse fine cracking along, and fissuring both along and across, the grain (Savory, 1954). Soft rot is mainly associated with water-logged wood, but quays, jetties, mills and boathouses may be affected.

Moulds

The great majority of moulds which are found in buildings belong to the class Hyphomycetes of the subdivision Deuteromycotina. Common species are: *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma viride*, *Alternaria* spp., *Aureobasidium* spp. They live mainly on starched and free sugars stored in parenchyma or on surface deposits of detritus on masonry, brickwork, concrete, rendering, tiles and paving and on surfaces of damp wood, plaster, wallpaper or paint. Moulds commonly occur on surfaces as a superficial growth, causing patchy surface discoloration, usually green, grey, or black, but occasionally pink or yellow. Some are rusty red and may be mistaken for spores of *S. lacrymans*, while others produce a fluffy mass of white growth. Some moulds colonize wood whose moisture content is above 20%, but appreciable development requires moisture contents at or above the fibre saturation point (28–32%). Mould grows best at temperatures ranging from 20 to 30°C.

Moulds cause some loss in wood toughness (resistance to shock) but usually have only a negligible effect on other strength values. They discolour and seriously weaken such materials as paper, leather, cloth, and fibre-based products such as acoustic tiles and insulation. Moulds greatly increase the porosity of wood, and moulded wood wets much more easily, thus increasing the likelihood of decay and moistureinduced deformations. The presence of actively growing moulds serves as an indication that a moisture problem exists, which may or may not present a potential decay hazard (Chapter 5).

Slime moulds

Slime moulds belong to the division Myxomycota (p. 30). Myxomycetes are very common on fallen trunks and branches on the ground. In buildings they are usually found on inorganic substrates, such as masonry, brickwork, concrete, rendering, tiles, paving, and also on damp wood, usually exterior joinery. Fruit bodies are 1–20 mm in diameter, variable in appearance from dark green, brown or black to, occasionally, bright colours sometimes on stalks or with silvery skin or uniform coating. All produce masses of brown spores. Myxomycetes feed on bacteria within wood and become visible only when cells aggregate and form fruit bodies on the surface.

Plaster fungi

These fungi are likely to be found on damp brickwork or plaster in buildings. Common examples are *Coprinus* spp. (Inkcap), *Peziza* spp. (Elf cup), and *Pyronema domesticum*. These fungi feed on surface detritus or on organic material included in walls, e.g. bitumenized felt, damp-proof membranes, and on hair contained in old plasters.

Coprinus

Coprinus spp. belong to the class Hymenomycetes of the subdivision Basidiomycotina. The sporophore of *Coprinus* has rather a peculiar organization found only in this genus. Monokaryotic mycelium, without clamp connections, is branched and this produces a conidial stage in the form of erect branches, each bearing a slimy head of unicellular and

Building defects **(See chapter 1, section 3.1 and chapter 7, section 3.3)**

Crow steps leading to water penetration as a result of poor flashing.

Over-discharge and blocked drainage leading to vegetative growth.

Extensive growth of vegetation due to blocked guttering and downpipe.

Fungus **(See chapter 3, sections 3.1.1 and 3.1.2)**

Serpula lacrymans (dry rot) fruiting body and mycelium filling a void.

Coniophora marmorata (wet rot). Courtesy of Ingo NUSS.

Remedial methods **(See chapter 1, sections 3.1 and 3.3)**

uninucleate spores. In this vegetative non-fruiting (oidium) stage of the life cycle it may cause some decay to hardwood and sapwood, for instance, to split laths. Fruit bodies are formed on the dikaryotic (binucleate) mycelium with clamp connections. They are small white or cream ‘mushroom’ type structures with black gills on a thin stalk, often in clumps. Black spores are often deposited as a spore print when the fruit body matures before it shrivels and collapses.

Joist ends isolated from masonry on a DPC membrane.

Ventilation of the wall void (via wood panelling).

Ventilation of the floor void (floor ventilators).

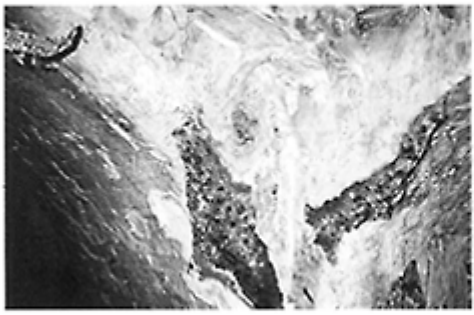
Ventilation of the roof void (roof ventilators).

Dry line area (French drain) to lower ground level against external wall.

Fungi in buildings **(See chapter 3, sections 3.1.1, 3.1.2 and 3.1.11)**

S. lacrymans (dry rot) mycelium and strands under stairs.

S. lacrymans (dry rot) mycelium in a void.



S. lacrymans (dry rot) fruiting body, mycelium and strands in the vault of a cellar.

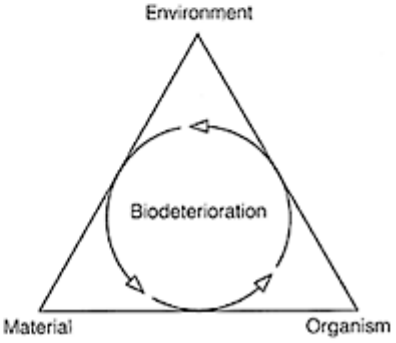
Lentinus lepideus (antler fungus or stag's horn fungus).

S. lacrymans (dry rot) fruiting body on burnt timber.

Mould growth on wall paper.

S. lacrymans (dry rot) fruiting body at the top of the window-frame with dry rot spores on the lower window-sill.

Decay cycle



Peziza spp.

This fungus, commonly called Elf cup, belongs to the class Discomycetes of the subdivision Ascomycotina. Fungus may be seen in damp plaster or on brick walls where they are saturated. The fruit body or ascocarp is small, pale brown or flesh coloured, cup-shaped without stalks and is up to 50 mm wide. It is pliant when fresh but breaks easily

with brittle fractures when stressed between the fingers. When dry it is hard. The most common species in buildings are *P. caerea* and *P. aurantia*. This fungus is a biological indicator of extreme damp within the building fabric.

Pyronema domesticum

The fruit bodies are small, bright orange, wrinkled, and the cup is jelly-like. The mycelium is profuse, pinkish but otherwise resembles that of *S. lacrymans*. This fungus is very common in buildings after fire damage.

Stain fungi

Staining of sapwood occurs on freshly felled wood with high moisture content. The damage persists after the wood dries, but is usually of no significance when found in wood in buildings. The stain fungi cannot grow in waterlogged wood or below about 20% moisture content. Stain can be identified in wood as black or blue-black streaks and patches in wood or coating; sometimes dark hyphae and fruit bodies are present (tiny nodular structures erupting through the coating and producing numerous dark spores). Staining can penetrate deeply and often cannot be removed by surface planing.

Stain fungi commonly colonize sapwood cells of moist wood, particularly the ray cells. For their early nourishment they depend on parenchymatous tissues as in the wood rays, and on sugars and starch in the cells. The virtual absence of blue stain fungi in heartwood seems to be attributable chiefly to a shortage of relatively easily metabolized carbohydrates rather than inhibitory heartwood activities. Blue stain in oak is caused by fungi such as *Ceratocystis, Scytalidium* and *Gliocladium* spp. Green stain in oak is usually caused by *Chlorociboria aeruginascens*.

Staining of wood under varnish (or invisibly under paint), usually blue or black, is often accompanied by black surface growths through the coating; it is sometimes accompanied by surface mould, but the stain itself develops under different conditions caused by moisture accumulations under coating systems.

Many stain fungi commonly found in buildings belong to the class Hypomycetes of the subdivision Deuteromycotina (e.g. *Aureobasidium pullulans* and *Sclerophoma pithyophila*). Some stain fungi, for example *Ceratocystis* spp., belong to the subdivision Ascomycotina.

Stains are troublesome owing to their objectionable appearance, disfigurement of the wood and especially of clear finishes; early failure of the coating surface may be brought about by rupture caused by the fruit bodies. Discoloration caused by Sap stain which has occurred in the log may still be detectable after drying and conversion of the timber, but this will affect only the aesthetic value of the wood. Damage of the coatings will occur only through the growth of blue stain in service.

Stone fungi

A number of fungi may be associated with stone. For example, *Botrytis* spp., *Mucor* spp., *Penicillium* spp. and *Trichoderma* spp. These fungi produce citric and oxalic acid which

result in solubilization of silicates and the weathering of stone (Richardson, 1978). The detailed mechanisms are given in Chapter 13.

Paint fungi

The discolouration of paint is caused by a number of fungi, such as *Alternaria alternata, Aspergillus flavus, A. versicolor, Aureobasidium pullulans, Penidllium expansum, P. purpurogenum, Cladosporium herbarum, Fusarium oxysporum, Paecilomyces variotii, Trichoderma viride, Ulocladium atrum* and *Phoma violacea*. These fungi can cause damage to interior decorations and are also a health risk to occupants (Chapter 5).

Fungi on glass, metal and sealants

A number of fungi colonize glass, metal adhesives and sealants and can cause discoloration and disfigurement. *Cladosporium resinae* is found growing on metal surfaces and some xerophilic fungi grow on glass. In kitchens and bathrooms, a large number of fungi are found extensively colonizing adhesives and sealants, for example *Aspergillus niger, Aureobasidium pullulans, Chaetomium globosum, Geotrichum* spp., *Penidllium luteum, Trichoderma viride* and *Streptomyces rubrireticuli. Penicillium variabile* has been found to be associated with PVC, plastic and rubber. Surface discoloration of polyesters is caused by *Aspergillus versicolor* and of polyurethanes by *Gliocladium roseum*.

Fungal damage to interior furnishings and contents

Carpets, furniture, leather, museum objects, books, painting, clothes, etc. are attacked by a range of different fungi. Damage to wool, fur and museum objects may be caused by *Trichophyton* spp. and Red Spot of leather by *Penicillium* and *Paecilomyces* spp. Damp carpets and clothing in mouldy homes are often infested by species of *Penicillium, Aspergillus, Cladosporium* and *Mucor*. Museum specimens, paintings and books in libraries with persistent damp are often infested with species of fungi such as *Penicillium, Aureobasidium, Fusarium, Aspergillus, Cladosporium* and *Trichoderma*.

Fungal damage to stored food

A number of fungi have been found to be associated with stored food and food products. The commonest species are *Aspergillus, Penicillium, Rhizopus, Fusarium, Myrothecium* and *Stachybotrys. Aspergillus flavus* and *Penicillium* spp. produce mycotoxins such as aflatoxin, patulin and ochratoxin which can have serious health implications (Chapter 5). *Fusarium, Stachybotrys* and *Myrothecium* produce trichothecene-type mycotoxins which have been known to damage the health of sensitive individuals.

ECOLOGICAL FACTORS INFLUENCING DECAY

When considering any form of biodeterioration three factors are concerned: the material, the environment and the organism. Ecology as an aspect of science is usually confined to a very close analysis of the interaction of organisms with one another and with their environment. The environment in which any organism lives will contribute physical, chemical and biological factors which will have a bearing on the settlement, growth and development of an organism.

The fundamental role of ecology in studies of biodeterioration is to predict the susceptibility of a given material to attack by various organisms, depending on prevailing environmental conditions. Macroenvironmental parameters such as average temperatures, humidities and hours of light have little significance in determining the activities of deteriogens. Instead, the details of microenvironments and their relationships to organisms must be examined.

The macroenvironment includes a whole series of microenvironments, on and within the materials, such as the micronutrient level of the materials, the microclimate of the materials, population density, frequency of distribution of propagules and relative frequency of biodeteriogens to other organisms. For example, with a piece of exposed structural timber in a particular situation, climatic information concerning average temperatures and humidities will have little meaning. It is difficult to establish the precise effect that insulation has on a piece of wood. It could change the surface and internal environment to such an extent as to have little superficial correlation with general air conditions; yet it is precisely these microclimates on and inside the timber which are of greatest significance in determining the organisms which will be present and able to grow. In such a situation, therefore, it is not surprising that it is possible to identify a complete temperature spectrum of fungal deteriogens, up to those able to live and break down wood at 45°C. Thus, within the substrate there may be populations of deteriogens, different members of which are able to grow as the temperature changes both diurnally and seasonally and with depth within the wood. To be able to identify a fungal deteriogen at one average temperature does not necessarily mean that it may be the only organism to be considered. However, water availability may help in identification.

Water accounts for between 80 and 90% of the weight of a microorganism and microbial metabolism requires an aqueous environment (Rose, 1981). Wood-rotting fungi are unable to colonize wood which has a moisture content below 20%. Although some moulds can develop below this range.

TEMPERATURE AND pH REQUIREMENTS

Fungi differ in their optimum temperature, but for most the range is between 20 and 30°C. The optimum temperature for dry rot growth in buildings is about 23°C. Maximum temperatures for continued growth are about 25°C and the fungus is rapidly killed above 40°C. For species of mould commonly found in buildings, e.g. *Penidllium* spp. the optimum temperature is 20–25°C, while *Aspergillus* spp. grow best at about 30°C (*A. fumigatus* grows at 55°C). All wood decaying fungi require oxygen, which they get

directly and indirectly from air. Cartwright and Findlay (1958) reported a lower limit of pH 2.0 for growth of decay fungi, but concluded that the optimum for most species lies between 4.5 and 5.5, which is within the normal range of wood.

ECOLOGICAL SUCCESSION

Most studies of the ecological successions and microbial associations during biodeterioration have employed debarked logs and branches, or crop residues. Little work has been done on biodeterioration of building materials.

When the material is exposed to the air a large number of spores from a range of species will settle on the surface and if it is moist they are likely to germinate. If the material is in contact with soil the surface will be colonized initially by both mycelium already actively growing in the soil, and by fungi growing from previously dormant spores (Cartwright and Findlay, 1958).

A number of different factors affect the pattern of colonization of substrates (Odum, 1953). Many substrates are first colonized by what are known as sugar fungi, capable of growing and sporulating rapidly on simple, soluble sugars and nitrogen sources. Later colonies are able to feed on more highly polymerized compounds such as hemicellulose and cellulose, secreting extracellular enzymes. The latter are frequently associated with secondary fungi which utilize some of the enzymatically produced sugars. Thus, it is often difficult to isolate cellulose or lignin decomposers on standard laboratory media containing simple sugars, as the medium favours faster-growing sugar fungi that overgrow the polymer decomposers before they have a chance to develop (Allsopp and Seal, 1986).

The availability of nutrients to potential colonizers will change as the attack progresses. Thus, one microorganism may have an initial selective advantage as far as available nutrients are concerned, but then may be replaced or joined by other species, giving rise to an ecological succession. Clubbe (1980) recognizes six such ecological niches or physiological groups: bacteria, primary moulds, stainers, soft rots, wood-rotting Basidiomycetes and secondary moulds. Carey (1980), in an extensive survey of exposed painted simulated joinery components, has shown that an ecological sequence of colonization by microorganisms starts with bacteria, moulds and stainers, and climaxes with Basidiomycetes. Several of these early colonizers have been shown to increase the absorbency of the timber from the joint in a manner similar to the saturation of the timber.

SUMMARY

Microbial biodeterioration of building materials and contents in both modern and historic buildings is attributed to changes in the built environment. The main environmental parameters favouring the decay of materials are water, humidity, temperature and lack of ventilation. The causes of decay in materials and structures are influenced by the internal building environment, which has a varied microclimate depending upon building structure. Preventive maintenance should in most cases forestall the need for major

interventions, and has been proved to decrease the cost of conservation. Since the internal environment of a building is such a delicate balance of influences, before undertaking any intervention it is advisable to study in detail the ecological factors such as temperatures and humidities at microenvironmental levels and the building's response and performance.

Correct identification of fungal material is important, as not all fungi are equally destructive. Some rots are present in timber when cut, or acquired in storage, and these may be present in heartwood or sapwood. Fungal material may also be dead or dormant, representing conditions now past.

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The physiology and decay in buildings morphology of fungal

4

Sarah Watkinson

INTRODUCTION

Fungi damage buildings by using timber as a food source, weakening and ultimately destroying it. The buildings of human beings have existed only in the last 20000 years while the earliest known fossils of Basidiomycete wood decay fungi are about 300 million years old (Meyen, 1987). Thus the fungi which damage buildings by rotting timber have evolved in the wild to use wood as a source of food, each species with its own preferred habitat in terms of physical and chemical variables, its own 'ecophysiology'. The preference reflects the conditions under which the fungus is best able to survive and compete with rival organisms, and is different for each species. If the conditions in the building reproduce those that the fungus has evolved to flourish in, the timbers will be liable to decay. This chapter describes some of the aspects of Basidiomycete and Ascomycete ecophysiology that make them able to grow in buildings.

NUTRITION IN FUNGI

WOOD AS THE NUTRIENT MATERIAL

Nutrient requirements of all fungi include an organic carbon source such as hexose or pentose sugars (which can be taken up from solution or derived from extracelluar solubilization of macromolecules such as cellulose), a source of nitrogen such as nitrate or ammonium ions, amino acids or proteins, and mineral salts, with potassium, magnesium, iron, phosphorus (normally as phosphate) and sulphur (normally as sulphate)

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being required in the largest amounts. Fungi feed osmotrophically, that is they absorb all these nutrients from the aqueous solution which surrounds them even when they are

growing on a solid substrate. Wood supplies all these nutrients in varying amounts, and can serve as the sole nutrient source for timber decay fungi. Carbon is abundant as cellulose, hemicellulose and pectins, and nitrogen is present in relatively small amounts as protein. Minerals are present in small amounts, being more abundant in fresh sapwood.

WOOD DECAY FUNGI HAVE ENZYMES TO SOLUBILIZE WOOD

Extracelluar lytic enzymes produced by the fungal hyphae depolymerize insoluble nutrients such as cellulose, lignin and protein. Analysis of the products of cellulase action (Montgomery, 1982) shows that fungi produce different enzymes which each break a different type of bond within the molecule, and which act synergistically on cellulose. Endoglucanases break the β(1→ 4) bonds that join the glucose residues in the cellulose chain, liberating a variety of oligomers. Exoglucanases break off either single glucose molecules or pairs of molecules, producing glucose or cellobiose respectively. The need for these enzymes is signalled to the fungus by the presence of cellulose. It continually secretes small amounts of cellobiase and responds to the presence of cellobiose, which is only liberated if cellulose is present, with increased cellulase synthesis. As well as this positive feedback, there is a negative feedback mechanism by which glucose in the vicinity of the fungus inhibits cellulase production. This is thought to prevent wasteful protein synthesis and secretion, and to avoid feeding competing organisms.

Endo- and exoglucanases act by binding to the cellulose molecule in the normal process of enzymic catalysis. They can break down soluble forms of cellulose *in vitro*. However cellulose is normally in the form of highly organized, crystalline micelles in which most of the cellulose chains are not accessible to enzymic binding. Initial attack on this structure requires contact with living mycelium and has been shown in the case of some brown rot Basidiomycetes to be the result of an enzymic process by which the fungus generates powerfully oxidizing free radicals which break the chemical bond of the chains and open up the structure of the micelle to hydrolases. This system requires iron in trace amounts, and oxygen. Some fungi, the white rot fungi, produce a ligninase enzyme system which breaks down lignin. This also has a free radical oxidization step as the first stage in the process (Kirk and Farrell, 1987). A variety of chemicals are released since lignin is a complex heteropolymer of phenylpropanoid subunits. Production of the ligninase system has been intensively studied in *Phanerochaete chrysosporium* where it is regulated in response not to the products and reactants of enzyme action like cellulases, but is switched on and off by nitrogen availability (Fenn and Kirk, 1981). White rot fungi in general have phenoloxidases which oxidize the liberated phenols to quinones. There is evidence that oxygen may be cycled between the quinones so produced, and the oxygen requiring initial step of cellulose breakdown, a likely adaption to oxygen shortage within a large volume of wood (Eriksson *et al.*, 1974). Most wood nitrogen is present as protein. Proteinase enzymes of several different types are secreted by wood decay fungi, those so far characterized being mainly aspartic proteinases active at low pH (North, 1982; Kalisz *et al.*, 1987). *Serpula lacrymans* has been shown to produce an extracelluar aspartic proteinase active only at or below pH 4, as well as an intracellular one active at neutral pH which appears to leak passively into the substratum as the mycelium ages.

ADAPTATION OF EXTRACELLUAR ENZYMES TO HABITAT

The kinds of extracelluar enzymes that a fungus secretes, and the way in which secretion responds to signals from the environment of the mycelium, differ from species to species depending on the niche of the species. Thus white rot fungi such as *Donkioporia expansa, Phellinus contiguus, Pleurotus ostreatus* and many Ascomycetes produce ligninases and phenoloxidases, while brown rots do not. *Phanerochaete chrysosporium* ligninase production is suppressed by nitrogen nutrients (Fenn and Kirk, 1981), while that of *Coprinus* (a Basidiomycete naturally growing on dung) is not. Among brown rot fungi some, such as *Serpula lacrymans* and *Coniophora puteana,* can break down pure unassociated cellulose, and so can decay paper, while others, also timber decay fungi, only attack cellulose associated with lignin, as it is in timber (Nilsson and Ginns, 1979). So variation in the mechanisms for hydrolytic enzyme secretion and regulation is one possible reason for the association of specific fungi with particular habitats.

ACTIVE AND SELECTIVE UPTAKE OF NUTRIENTS FROM SOLUTION

Soluble nutrients must be absorbed by the fungus through its cell walls, and through the plasmalemma which lines the wall. The cell wall gives structural rigidity to the hypha, consists mainly of chitin microfibrils and amorphous polysaccharide (Burnett, 1979), and may also in places contain hydrophobic material making it relatively impermeable to water. Dissolved substances are assumed to diffuse across the wall in permeable regions, and then are selectively taken up across the plasmalemma. Some nutrients such as ammonium ion diffuse passively across the plasmalemma into the cell in response to a concentration gradient which is maintained by metabolic uptake of the substance inside the cell. Other substances such as sugars and amino acids are actively accumulated in a process requiring metabolic energy from respiration, and sometimes the co-transport of various ions. Proteins in the plasmalemma mediate this active transport.

There is variation between fungi in the affinity of the uptake proteins for their substrates. The uptake systems of wood decay fungi have not been much investigated. It is likely that the cellular mechanisms are like those in other fungi such as *Aspergillus, Neurospora* and yeasts for which there is more information, but that regulation of uptake varies with habitat. *Neurospora* appears from kinetic studies of uptake in culture to have at least three different uptake systems for amino acids, according to whether they are basic, acidic or neutral (Ogilvie-Villa *et al.*, 1981). These accumulate amino acids into the mycelium against a concentration gradient, producing an internal pool of free amino acids which can be interconverted with protein. The internal free amino acid pool is a general feature of fungi. In *Serpula lacrymans* it increases at first when the fungus is well supplied with nitrogen nutrients and then decreases when protein is synthesized (Watkinson, 1984a). Under nitrogen starvation conditions, intracellular protein is broken down and the free amino acid pool, consisting predominantly of glutamic acid or glutamine, increases (Venables and Watkinson, 1989a). It is known from studies with yeast that amino acid uptake shows preferences for one amino acid over another, so that amino acids are taken up from a mixture in a definite order.

Sugar uptake has similar characteristics to amino acid uptake, being active, selective, and mediated by transport proteins in the plasmalemma which may be constitutive (always present) or inducible (present only when their substrate is available). Again, most of our knowledge of the process comes from a few fungi which are not wood decay Basidiomycetes. Charged ions are transported across the plasmalemma by ion pumps: protein-mediated systems requiring energy. Hyphae of *Neurospora* pump protons across the plasmalemma at the hyphal tip. Uptake of some nutrients, such as amino acids, is linked with ion transport, so that nutrient absorption may generate electrical fields around hyphae. Electrical fields around growing hyphal tips may have a role in maintaining polarity and hence direction of growth. Water also enters the cells by processes requiring metabolic energy, for example the active uptake of substances, which creates a water potential gradient osmotically, favouring uptake. The osmotic potential inside the mycelium can also be altered by macromolecules being interconverted with their monomeric components. The hydraulic pressure generated by water uptake drives the elongation of the hyphal tips at the margins of colonies, and the enlargement of fruiting bodies. It has also been suggested that it helps to supply the growing edge of a colony with nutrients, swept along in solution inside the hyphae (Jennings, 1984).

All these uptake systems can only function when there is water next to the hyphae, even if only a film between hypha and substratum. Hyphae do not take in substances by pinocytosis or phagocytosis. The need for a film of water, and also for metabolic energy provided by aerobic respiration, confines wood decay fungi to damp but aerated surfaces. Uptake systems have been shown to be related to habitat in some fungi. *Chaetomium,* a cellulolytic soil Ascomycete, takes up glucose preferentially to fructose after deriving both in equal amount by the activity of an extracelluar invertase on sucrose, which could reflect a need to remove the more generally utilized sugar first from competitors in the soil. Fungi living in nutrient-poor habitats and needing to scavenge soluble nutrients from dilute solutions have been shown to have uptake systems with a stronger affinity for their substrates than similar fungi which habitually grow in a nutrient-rich environment.

SOME MOULDS CAN GROW IN NUTRIENT-POOR ENVIRONMENTS

Some fungi obtain parts of their nutrients from the atmosphere. Moulds such as *Aureobasidium pullulans* can grow on inert, non-nutrient surfaces such as paintwork or stone. In nature an important habitat for such fungi is the surface of leaves. Nutrients are derived partly from leaf exudates but also from bacteria which can convert carbon dioxide and nitrogen from the air into nitrogen and carbon compounds that fungi can use. Some kinds of airborne particles may serve as food, for example pollen grains provide food for fungi on leaves of flowering plants. Soil fungi are adapted to live under carbon-poor conditions and the common soil Ascomycete *Fusarium oxysporum* can derive more than half its cell carbon from carbon dioxide when no other source is provided (Parkinson *et al.,* 1991). The atmosphere also contains gases such as oxides of nitrogen and sulphur which dissolve in water and provide a limited supply of these elements. Fungi adapted to oligotrophic environments which grow in buildings are the moulds which develop on damp surfaces. Individual colonies are small, and the mould causes damage not by structural decay like the big colonies of wood decay fungi, but by spoilage, production of

potentially allergenic spores and, occasionally, harmful metabolic products, and by the unpleasant appearance of their colonies which are often dark in colour (the so-called ‘sooty’ moulds).

GROWTH AND DEVELOPMENT IN FUNGI

The mode of development of fungi is closely related to habitat. The appearance of colonies like those of *Coniophora puteana* and *Serpula lacrymans* changes with the type of surface over which they are growing and other chemical and physical features of their environment. Fruit bodies form in characteristic places, for example those of *Serpula lacrymans* are commonly found in the angles between walls.

HYPHAE

The basic developmental unit of all filamentous fungi is the hypha, a cell which unlike those of plants and animals, polarizes all its growth at one end so as to form an elongating filament. Materials for growth are derived as described mainly from nutrients taken up from the surface over which it is growing, or transported from other parts of the mycelium. The cell wall is plastic and deformable at its extreme tip, but a few micrometres behind the tip the components of the wall become cross-linked and it becomes rigid, confining continuing extension to the tip which is driven by internal hydrostatic pressure. The hypha is thus able to exert pressure to grow through solid substrata like wood. As hyphae are of microscopic width they are also able to grow through very fine interstices in porous material. This ability enables mycelium of fungi such as *Serpula lacrymans* to grow through masonry. It depends, however, on the fungus being one which has the ability to mobilize nutrients within the hypha so as to supply the advancing tip with material for growth through non-nutrient environments. Not all fungi have this ability to translocate food. As the hypha elongates, it develops internal cross-walls which are normally perforated by holes so that the cytoplasm is potentially a continuum, though the holes may later become occluded. It also branches at intervals. Branch frequency depends on the species, so is genetically controlled. Observation of hyphae of *Coprinus* growing over a nutrient-free glass surface revealed that the fungus maintains a constant order of primary and secondary branches which show apical dominance with the main hypha leading and giving rise to primary and secondary branches. The angles at which branches of each order diverged was also constant. Branching behaviour is therefore at least partly genetically determined. However it is also variable with nutrient concentration in the substratum, for example colonies of a fungus in culture on rich media branch abundantly but under oligotrophic conditions the same fungus can produce long, very sparsely branched hyphae. The non-metabolizable amino acid, α-aminoisobutyric acid (AIB), that is actively taken up but then accumulated, unused, in the free amino acid pool (Watkinson, 1984b), increases branching frequency in *Serpula lacrymans* (Elliott and Watkinson, 1989), suggesting the hypothesis that it interferes with a mechanism the fungus may have for sensing its internal amino acid status and developing localized branches of mycelium on patches of rich substrate.

Alternatively, AIB may increase the internal osmotic pressure, leading to increased branching.

COLONIES

As the tip grows on, it leaves behind a growing mass of branching mycelium, the colony. Colonies of wood decaying Basidiomycetes can live for many years and continue to develop and expand, differentiating different types of hyphae and multihyphal structures such as fruiting bodies, mycelial strands and rhizomorphs. Colonies of Deuteromycete moulds, on the other hand, are usually ephemeral, with more limited differentiation and development. The growth of a colony makes the fungus visible as a macroscopic structure. The way in which the colony develops has been intensively studied in culture. Under standardized and controlled conditions with fungal colonies growing on uniform nutrient agar medium for short periods of several days, some characteristics of colony growth are reproducible enough to be described by mathematical equations, and it has been found that for any one fungus the rate of radial extension depends on the specific growth rate (rate of increase of mass per unit mass) and on the length of hypha which that fungus characteristically requires to provide the materials for tip growth (Bull and Trinci, 1977). The specific growth rate varies with temperature in a way typical of each species. Even hyphae in young colonies on agar jelly do not take up nutrients uniformly. Most uptake occurs close behind the tip, in the same region as the electrical activity generated by the ion pumps, and uptake decreases further back where the mycelium is older. Enzyme activity is also found to be localized using cultures on agar, for example *Serpula lacrymans* proteinase is more active in the region of older mycelium in the centre of colonies than at the edge near growing tips (Venables and Watkinson, 1989b).

Hyphae growing in a colony usually grow out from a point of origin where the starting culture or spores (inoculum) was first placed. The direction of growth of the hyphae is evidently regulated because at first the colony under uniform conditions is normally circular, all its component hyphae maintaining an outward orientation of growth, extending at a constant rate with even spacing between hyphae round the circumference. However the mechanisms of regulation are barely understood. For example, the apical dominance of branches seen in isolated hyphae cannot be maintained in a colony because if it were, the generally-observed even spacing of hyphal tips at the colony margin could not occur.

DIFFERENTIATION AND SECONDARY METABOLISM

While the tips continue to grow at the margin of a colony, the older mycelium left behind progresses to a further stage of development. The hyphae may start to differentiate. Changes in form include thickening or thinning of the walls, vacuolation, occlusion of septal pores, development of asexual spores such as conidia of moulds, and changes in the spatial relationships of new hyphae to each other and to older ones so that for example they aggregate into strands rather then diverge as they did at the margin. The initial circularity of the colony breaks down with the emergence of more complicated patterns of growth. Accompanying these morphological changes new substances are produced according to the type of fungus, the so-called ‘secondary metabolites’. These

are remarkably diverse though derived from common primary metabolites, for example many aromatic compounds are made by fungi from intermediates of the shikimic acid pathway (see the monograph by Turner and Aldridge (1983) for a catalogue of chemicals produced). The types of secondary metabolite are characteristic of the type of fungus. The colours, tastes and odours of fungi, as well as their diverse and potent pharmacological effects are due to these compounds.

Secondary metabolism is intensively investigated in relation to industrial processes such as antibiotic production and the search for new drugs, using techniques involving artificial standardized culture methods, but relatively little is known about the regulation of the production of secondary metabolites in relation to colony development. The secondary metabolites of *S. lacrymans* include substances which are specific enough to enable dogs to identify the fungus by smell (Chapter 7).

MULTIHYPHAL STRUCTURES

Mycelial strands and fruiting bodies

The most conspicuous multicellular structures that develop from mycelium are the fruit bodies of Ascomycetes and Basidiomycetes. Aspects of development are reviewed by Moore (1994). A completely new programme of development is switched on, with the organized production of a mycelial aggregate fuelled with food reserves such as glycogen, followed by growth of hyphae in a coordinated manner to produce the structure, for example the vertical stalk, horizontal cap and vertical radiating gills of a toadstool. At the same time the cell nuclei in hyphae at the surface of the gills, or other spore-bearing surface, undergo a sexual fusion akin to that of gametes, immediately followed by meiotic division to produce spore nuclei. The spores are produced continuously over long periods. The wood decay fungi that produce large mycelial colonies in buildings belong to two types, Ascomycetes and Basidiomycetes, with different ways of liberating spores and hence different types of fruit body. The colonies look similar, except that Basidiomycetes may sometimes be diagnosed by the presence of ‘clamp connections’ on the hyphae, visible with a microscope. However, when fruit bodies are produced the fungus may easily be identified. Ascomycetes liberate their spores by the explosive discharge of a microscopic sac, the ascus. The fruit body has a surface covered with asci and exposed to the air from which the spores are shot upwards and outwards, so it typically takes the form of an upward facing cup, or a cushion or crust of mycelium. Fruit bodies of the genus *Peziza* are brown cups several centimetres in diameter that are found growing out from damp plastered walls. The fungus is widespread in natural habitats. *Daldinia concentrica* is common in nature on dead ash branches, indicating a tolerance of hot and dry conditions. It can survive in seasoned timber. It has been reported in buildings fruiting on hardwood floor blocks possibly because it was already present in the timber when it was used in construction. The fruit bodies are black balls of hardened mycelium several centimetres in diameter, and the asci are produced inside microscopic holes all over the surface, being discharged when the fruit body is damp. Like other Ascomycetes, it can also produce asexual spores directly from the mycelium (‘conidia’). The mycelium produces a white rot, as Ascomycete wood decay fungi commonly do.

Basidiomycete fruit bodies are recognizable by their umbrella-like form, the spore-bearing surface being always on the underneath of a toadstool (in buildings, *Lentinus lepideus, Paxillus panuoides, Pleurotus ostreatus*), bracket (some fruit bodies of *Serpula lacrymans*) or less well-defined cushion (*Poria, Fibroporia* and *Coniophora* species and sometimes *S. lacrymans*). A microscopic structure, the basidium, flicks spores away from the surface a few micrometres and then they are liberated from the fruit body by falling out of the tubes or gills on its underside. As the fruit body develops, its orientation is precisely regulated in relation to the surface it emerges from and also to the direction of gravitational force, so that spores may drop vertically out of tubes or gills. Light direction, wind, gravity and concentration gradients of volatile substances have all been shown to affect the orientation and development of fruit bodies (Moore, 1991).

Fruit bodies of wood decay fungi are provided with food by the mycelium growing in the wood, from which they develop and to which they remain attached. The mycelium absorbs nutrients from the wood as described above and translocates them to the fruit body which uses them to make spores. Calculation of the amount of nitrogen in fruit bodies and in the wood from which they grow has shown that the fungus can concentrate the nitrogen into one fruit body from a colony in the tree measuring several metres (Merrill and Cowling, 1966). Spores can be produced at the rate of millions per day, and become airborne in eddies of air as they are very small (about 1 µm in diameter). Because they are produced by a sexual process they are not all genetically the same, as most mould spores from one colony would be, but each is different in the same way as siblings of one family are, due to different mixing of the same parental genes.

Mycelial strands and cords

As well as fruit bodies, the mycelium of Ascomycetes and Basidiomycete mycelium of many species can develop longitudinal aggregations that are clearly visible and may, as the colony ages, remain as the only fungal structure to be seen. These are the **cords** or **mycelial strands**. (They are also sometimes called rhizomorphs though this term is more precisely used to refer exclusively to aggregates of hyphae that develop from organized multicellular apices like roots.) Depending on the growth conditions cords may give rise to unaggregated, diffuse mycelium again, and so are not set irreversibly on a programme of development like a fruit body. Those of *Serpula lacrymans* form behind the margin of colonies, the leading hyphae at the margin becoming invested further back in the colony by their branches, which instead of radiating away from their parent hyphae stick to and coil around them. Later there is more differentiation of hyphae within the strand, which ends up as a cord several millimetres across with a thickened outer rind reinforced with a ring of thick-walled longitudinal fibres (called **skeletal hyphae**) and a central channel containing wide thin-walled hyphae without cross-walls, which look like conduits for fluid to flow through. There are also some hyphae which appear less changed and probably contain living material.

These strands are found connecting advancing edges of the large colonies with other regions where wood is being decayed. They survive and remain when unaggregated parts of the mycelium have died away. They can be found crossing many feet of intervening non-nutrient surface such as plaster or masonry, and must obviously act as supply lines when they are the only connection between a food or water supply and a growing

mycelium. When they are cut, mycelial advance can continue for as long as the reserves already present in the strands allow (Bravery and Grant, 1985). The strands of *S. lacrymans* have been intensively investigated because the ability of this fungi to spread within buildings is at least partly due to them. However *Coniophora puteana* and several other wood decay fungi of buildings form strands without having the invasive ability of dry rot.

DEVELOPMENT OF MULTICELLULAR STRUCTURES IN RESPONSE TO ENVIRONMENTAL CUES

Mycelium of Basidiomycete and Ascomycetes is developmentally very plastic. Unlike plants and animals these fungi can live as microscopic separate hyphae or assume a variety of macroscopic structures. Parts of a colony may break away to form new colonies, and colonies may fuse and join resources. These changes are due to inbuilt capacities to respond in definite ways—such as increased or decreased growth, or switching on or off development programmes—to particular circumstances such as changes in internal, external or physical variables. Some of these cues may be very precise, for example multicellular development in a *Penicillium* species is switched on in direct proportion to glutamate concentration, and other nutrient amino acids have no effect (Watkinson, 1977, 1981). *S. lacrymans* forms mycelial strands on media over a critical C/N ratio, and on inorganic rather than organic nitrogen sources (Watkinson, 1971, 1975). While many cues to developmental switching are nutritional changes, other types of cue such as light intensity and spectral composition, non-nutrient and toxic chemicals, and other physical stimuli also trigger multicellular development (Watkinson, 1979).

Evolution of fungi has probably resulted in selection for the types of developmental response which optimize foraging strategies. Experiments to identify the effect of particular environmental variables on hyphal development have necessarily been done under standardized laboratory conditions to isolate and study one variable at a time. However, results from experiments using homogeneous nutrient-rich culture media (supplied as solutions), in the absence of competing organisms and under constant favourable physical conditions, obviously do not apply directly to the way in which colonies respond to their real environment. Fungi have evolved to live under fluctuating physical and chemical conditions in spatially non-uniform environments and in the presence of all the other organisms in the ecosystem. Food for wood decay fungi often occurs in nature as discrete spatially separated units. Moreover, their environment is subject to the whole gamut of climatic variations, and they coexist and interact with other fungi, plants and animals, particularly insects and nematodes. Understanding how the mycelium survives in the presence of the multitude of opportunities and threats surrounding it will need integration of physiological and ecological, descriptive and analytical approaches, and recognition of the complexity of the system.

PHYSIOLOGICAL IMPORTANCE OF DEVELOPMENTAL CHANGES

THE ECOLOGICAL ROLE OF MYCELIAL STRANDS AND CORDS

It is interesting to compare the developmental behaviour of wood decaying fungi in buildings with that of related fungi living in comparable natural environments. Many species of Basidiomycetes which live on fallen dead wood and plant litter have mycelium that is conspicuous because of the thick cords which it forms. Of the fungi causing wood decay in buildings listed by the Building Research Establishment, a majority are cord formers. Study of wild cord formers may tell us something about the adaptive significance of this kind of colony development and why it is so regular a feature of wood rotting fungi in buildings. The corded mycelium of wild Basidiomycetes can be revealed by shallow excavation around logs and tree stumps and forms networks across the soil which can extend many metres (Rayner and Boddy, 1988). A common example of a fungus with this growth habit is *Hypholoma fasciculare*, the sulphur tuft toadstool. Fruit bodies appear on dead tree stumps in the autumn. Excavation reveals a mycelium consisting of strands in the leaf litter.

The foraging patterns of such fungi have been investigated with cultures growing from wood blocks colonized previously with the mycelium, and then placed in soil trays to see how the mycelium develops. The presence of natural soil, rather than a sterile laboratory medium, stimulates the mycelium to aggregate into cords, and in most species investigated very little unaggregated mycelium appears. Cord formation appears to protect mycelium from antagonistic effects of the natural soil microbial population. However, separate hyphae are usually apparent at the ends of the cords. At first there is circular outgrowth of the colony from the foodbase. If a second, fresh wood block is placed in the soil some distance from the first as bait, the cord whose margin first encounters the bait is stimulated to further growth, becoming thicker, and at the same time mycelium in other parts of the colony starts to die back. This probably shows the fungus concentrating its limited resources to exploit the food source it has located. While cellulose provides abundant carbon, other nutrients, and water, may be in shorter supply and need to be recycled within the colony. A comparable phenomenon also occurs in laboratory cultures of *Serpula lacrymans* grown from one foodbase to another over a non-nutrient surface. Contact with the second foodbase stimulates the development of connecting strands and decreases outgrowth in other directions (Butler, 1958; Watkinson, 1971).

Thus, the fungus has developmental responses which operate to produce a supply line between foodbases, much as a human transport system might develop to connect two fertile and productive regions of land separated by a barren area. The analogy can be further pursued by examining the initial stages of outgrowth from the foodbase. In *Serpula lacrymans* this takes the form of single, unaggregated, exploratory hyphae. Experiment shows that the hyphae extend faster and more sparsely from a poor foodbase than from a rich one (the nitrogen source, sodium nitrate, was the nutrient varied in this case), a pattern of growth which allocates proportionately more of the limited resources

of the foodbase to reconnaissance when the need for food is more immediate. This experiment shows that the rate of extension of hyphae is regulated in response to the level of substances present inside them, as well as by the level of substances in the medium they are growing over.

If, instead of a usable food source like sodium nitrate, the foodbase is made with the non-metabolizable amino acid α-aminoisobutyric acid (AIB), which is actively taken up but accumulates unmetabolized in the intercellular free amino acid pool, the hyphae extending from the foodbase elongate much more slowly and branch abundantly in the region close to the foodbase (Elliott and Watkinson, 1989). This may be explained in terms of a hypothetical intrahyphal signal receptor which reacts to a high internal amino acid concentration by slowing extension at the hyphal tips and promoting branching. On a nitrogen-rich natural foodbase with a rising intrahyphal amino acid level, the mycelium would limit the searching activity of hyphal extension away from the foodbase and increase local growth to exploit the rich food source, a response which normally would help to optimize its foraging pattern. AIB, as an amino acid, could trigger this response, even though it is not a metabolizable nitrogen source like the protein amino acids encountered by the fungus. In other words, it is a foreign substance which gives a misleading signal. It provides a possible control method for limiting the damage done by fungi, for example controlling the rate of spread of *Serpula lacrymans* in buildings (Watkinson, 1991). There is some reason to suppose that AIB inhibits wood decay fungi in nature. It is a component of a peptide antibiotic produced by the soft rot mould *Paecilomyces variotii* which antagonizes wood decay Basidiomycetes, and AIB could probably be released from the peptide by proteinases secreted by wood decay fungi which would then take up the α-aminoisobutyric acid released.

HYPHAL DEVELOPMENT AND THE WOOD CELL WALL

The formation of multicellular structures such as strands and fruit bodies is highly regulated in response to the environment (Watkinson, 1979). This is also true at the unicellular, microscopic level, where the hyphae of wood decay fungi develop precisely in response to cues provided by their local environment, the interior of cells that make up wood. Wood is composed of masses of elongated plant cells whose walls have become thickened with cellulose and protein and impregnated with lignin as the tree grew. These compounds are not uniformly added to the walls. At first each living cell lays down cellulose and protein and adds lignin to the **middle lamella,** the layer between adjacent cells called **S1,** as it is the first secondary wall. The mainly cellulosic thickening that constitutes the thickest layer of the wall is the **S2** layer. Later, as the central lumen of the cell decreases in size with the thickening of the walls, a final internal lignified layer, **S3,** lines the internal surface of the wall. The final internal diameter varies from hundreds of micrometers (μm) in vessels to a few μm in tracheids. Adjacent tubular cells are connected by contiguous microscopic holes **(pits)**. Ultimately the cell dies, leaving the inert, layered wall. In sapwood, dead cells are interspersed with living cells which after felling still contain soluble substances such as sugars and amino acids. Heartwood consists entirely of dead cells and also contains secondary metabolites of the tree such as resins and tannins.

The dimensions of fungal hyphae are such as to allow them to pass through pits in the wood cell walls and to grow into the tubular spaces inside vessels and tracheids, lying on the internal surfaces of the wood cells. Thus they encounter a chemically and physically variable environment as they grow. Investigation of the microscopic appearance of wood decayed by white rot, brown rot and soft rot has shown that different species of fungi have different and characteristic ways of growing into and breaking down the cells (Rayner and Boddy, 1988, for a detailed review). Generally speaking, in white rots (fungi which can degrade lignin as well as other cell wall components) hyphae typically lie on the internal surface of the wood cell, and erode the cell wall in their immediate vicinity, cutting enzymically through lignified and cellulosic layers, to leave narrow channels (‘boreholes’) around the hyphae. Brown rots produce a more diffuse disintegration of cellulose cell walls throughout the affected wood, with a sparseness of hyphae that suggests that they autolyse and disappear with age.

The sequence of decay has been investigated (Doi and Nishimoto, 1985), by observing cellulose breakdown in the different layers of the wood cell with a polarizing microscope, which reveals where there has been loss of birefringence, and hence destruction of the long parallel molecules of cellulose. This technique, together with scanning electron microscopy, shows that the S2 is lost first, and later the S1. No boreholes were seen. Soft rot fungi bore inside the thickness of the wall, aligning their direction of growth with that of cellulose fibrils and producing a very characteristic branching pattern with division at the tips, resulting in T-junctions, where branches grow in opposite directions at an angle of 180° to each other. The tips of the boreholes are typically conical. Unlike brown and white rots, soft rots are found on the outer surfaces of timber only.

The gross effects of the different types of rot are different, reflecting the differences in the type of damage to the wood cells. White rot fungi remove the brown lignin, leaving the wood bleached. The wood is soft and light because the lignocellulosic structural components have been largely removed, and also fibrous rather then powdery in texture, probably because some cellulose remains, the rot being centred around boreholes. Brown rots are so called because the brown lignin remains. Strength is destroyed and the wood shrinks because of removal by free cellulases of cellulose from the S2 layer, the main part of the wood cell wall and also the main hydrophilic component. Soft rot fungi produce a superficial softening of the timber.

These differences in mode of attack on wood result from the different responses of hyphal development and enzyme secretion in relation to the wood cell surface and chemistry. The ability of mycelium to sense and respond to physical features of a surface is called **thigmotropism** and is seen in many fungi but very little studied or understood. Hyphae can sense microscopic features of a surface and develop accordingly. For example, some fungi which are pathogens of wheat leaves are triggered to develop specialized parasitic structures by physical features such as microscopic ridges, and artificial models of the leaf surface made of chemically inert materials mimic the effect. Similar thigmotrophic responses are probably operating when hyphae of wood decay fungi colonize wood. Hyphae of *S. lacrymans* inside tracheid cells branch opposite pits in the cell wall, and the branches then cross through the pit into the next cell, allowing lateral colonization across the grain of the wood. This fungus colonizes wood by branching abundantly over the surface, colonizing the nutrient-rich parenchyma of the sapwood and extending from this throughout the interior of the wood.

Apart from responding to the physical nature of the wood surface, hyphae must respond to the different chemicals which are arranged in a definite order in wood. They will be encountered in sequence by the hyphal tip as it grows into the wood, secreting enzymes and taking up solubilized nutrients as it goes. Presumably, the secretion of hydrolytic enzymes and ligninases are regulated in the same way as they have been shown to be *in vitro*. In this case a sequence of events at the hyphal tip might be secretion of cellulase, proteinase and other hydrolases in response to induction by their substrates present in the cell wall, followed by uptake of sugars and amino acids, potassium and phosphate, and extension of the hyphal tip. Excessive hydrolase production would be repressed by catabolite repression—the prevention of cellulase synthesis by glucose, for example. Ligninase, produced by white rot fungi and shown in one wood decay fungus to be elicited when nitrogen is exhausted and secondary metabolism begins, might be released only when primary growth had used up the extracellular nitrogen sources released by proteolysis of the cell wall protein. Solubilization of lignin might then unmask further layers of cellulose and protein. Diffuse destruction is probably produced by freely diffusible enzymes, while the boreholes of white rots and soft rots could be the result of the activity of enzymes which are not freely released but held attached to the hyphal wall, perhaps in a film of hydrophilic material such as wall polysaccharide.

Metabolism and development in the mycelium must both be regulated for successful colonization of the wood substrate, and the patterns of regulation are as diverse as the number of species that live on this food source.

BASIDIOMYCETE AND ASCOMYCETE FUNGI IN BUILDINGS

THE BUILDING AS A HABITAT

The colony of a wood decaying fungus is a finely adapted foraging system. Fungi that decay building timbers are applying the strategies they have evolved in the wild. Killing the fungus is one way to protect buildings. Another is to frustrate its strategies. To do this, we must know how the fungus forages in the wild, and what it is about our buildings that enable it to forage in them, as well.

Eleven species causing brown rot and five causing white rots are identified by the Building Research Establishment (Garston, Watford, UK) as common causes of decay in buildings in the United Kingdom (BRE, 1989a). The brown rot fungi are *Serpula lacrymans, Coniophora puteana, C.marmorata, Fibroporia vaillantii, Poria placenta, Amyloporia xantha, Dacrymyces stillatus, Lentinus lepideus, Coprinus* spp. (ozonium stage, a type of mycelium), *Paxillus panuoides* and *Ptychogaster rubescens*. The white rots are *Donkioporia expansa, Daldinia concentrica, Asterostroma* spp., *Phellinus contiguus* and *Pleurotus ostreatus*. All of these except *S. lacrymans* are known in the wild in Europe where they have characteristic habitats. *S. lacrymans* has been found growing wild in the Himalayas (Chapter 3). They are found on timber of living or dead trees, or both. Thus *Coniophora puteana* is found on fallen logs (Ellis and Ellis, 1990), *Daldinia concentrica* on dead branches still attached in the upper parts of ash trees, *Paxillus panuoides* on conifer debris (Phillips) and *Pleurotus ostreatus* on tree stumps. Some show preference for wood from particular types of tree, for example white rots are

more common on hardwoods than softwoods, or even for particular species of tree. They infect building timbers either because their spores of mycelium are already in the wood or because the wood becomes infected by spores from the air or invasive mycelium from an adjacent foodbase.

Recent work on the decay of wood in nature suggests that decay often originates from fungus already present in the wood which was quiescent while the tree was alive but initiates active decay as soon as it dies (Boddy and Rayner, 1984). Since some fungal spores remain viable for decades, it is possible that decay of timber in buildings may also start from dormant fungi already present, which start to attack the wood when conditions change so as to make growth possible. *Donkioporia expansa* is found fruiting on oak and sweet chestnut wood in nature, though it is not common (Ellis and Ellis, 1990) and in buildings it fruits on the ends of large oak beams embedded in walls (Building Research Establishment, 1989). Its rarity in nature, and its specificity for oak, suggest mycelium or spores already present in the wood when it is used in construction as the most likely source of infection.

Modern immunological techniques enable identification of fungi present only in microscopic amount and could probably be developed as a diagnostic method for testing construction timber for the presence of specific decay fungi (Chapter 8). Fungi which are not confined to one type of wood, and whose appearance in buildings is closely related to conditions in the building, are believed to infect the timber after it has already been incorporated in the building, and to arrive as spores, although the critical experiment to show this is possible—initiating rot in a building by application of spores—has not to my knowledge been done.

SERPULA LACRYMANS

Conditions for growth

Serpula lacrymans is an example of a fungus whose mode of attack is closely related to conditions in the building. It is the most damaging of wood-decaying organisms in Northern Europe and has been the subject of several monographs (Hartig and von Tuboef, 1902; Falck, 1912; Hennebert *et al.*, 1990; Jennings and Bravery, 1991). Its destructiveness comes from its ability to spread from its wood foodbase. Once established as a result of dampness in timber in one part of a building, it can spread from there to attack timbers which may be many metres distant and separated from the point of outbreak by less damp masonry (BRE, 1989b).

Carey *et al.* (1986) analysed 288 buildings with dry rot. Irrespective of timber type, the fungus preferred timbers used in particular places; thus, floor joists were affected in 73% of the buildings, floorboards in 69%, skirtings in 67% and window frames in 58%. Dry rot was the commonest type where dampness was due to rainwater, while other rots were usual when rising damp was the cause. Rainwater was found to produce spreading zones of damp masonry through which *S. lacrymans*, but not other fungi, can grow.

The physiological limits and optimal conditions have been measured by many investigators. Growth is optimal under cool conditions around 22°C and significantly less at 25°C. In spite of a long history of growth in buildings, more thermotolerant forms do not seem to have developed. Optimal temperatures for growth generally are well

conserved in fungi since well-defined optima are characteristic of most species. Humidity must be high for fungal growth. For growth of *S. lacrymans* to start, wood must be above 30% water by weight. Very wet wood (over 80% water) cannot be colonized except after the fungus has exported the water from the wood on to its outer aerial surface (BRE, 1989b). In this way *S. lacrymans* differs from the brown rot fungi *Paxillus panuoides* and *Coniophora puteana* which are found only on very wet timber. Drying to below 20% water stops growth of all fungi.

Temperature and humidity are the overriding factors in determining whether building timbers are decayed by *S. lacrymans*, but when these are favourable, other factors such as light and gaseous composition of the atmosphere also seem to affect the appearance and extent of mycelial growth in houses with dry rot. There has been no systematic investigation of these effects but as fungi respond to both factors by changes in development and metabolism they almost certainly occur, and may be important in determining the direction of spread of the mycelium by acting as cues during its foraging behaviour.

Development of *Serpula lacrymans* in relation to the environment

The spreading behaviour of *S. lacrymans*, seen as the key to its destructiveness, has been intensively investigated. The morphology, development and morphogenesis of the strands has been described (Falck, 1912; Butler, 1958; Jennings and Watkinson, 1982; Nuss *et al.*, 1991, among others), and their role in transport of water (Jennings, 1984) and supply of nutrients to the mycelial margin from the foodbase (Butler, 1958; Watkinson, 1971; Bravery and Grant, 1985). While investigation at the microscopic and physiological level has been intensive, it does not seem to have yielded an understanding of how the whole colony is organized to exploit its unusual environment.

A new approach to *S. lacrymans* is suggested by recent work and thinking by microbial ecologists working on the ecology of wood decay fungi, which broadly speaking treats the colony as an organism evolved to optimize foraging in a heterogeneous environment Boddy (1993). Such organisms must coordinate colonization, exploitation, exit from and growth towards their discrete food sources, separate pieces of wood. From the experiments on cord-forming colonies described above it is clear that the signals, whose nature is not understood, pass between different parts of the colony, for example eliciting lysis of one part of a colony when another part has found a new food source.

Is colony development responsive to a need for resource relocation?

One approach to investigating colony development as a foraging optimization system is to study the way in which the organism allocates scarce resources. This approach has been discussed by Dowding (1981). Since wood provides more carbon substrates than the other essential nutrients, it seems likely that these others, such as nitrogen, phosphate, and potassium limit growth; in other words, more growth would be possible if wood were supplemented with them. This is an oversimplification, as attempts to increase decay by addition of such ‘fertilizers’ have shown. The complexity of the relationship of the hypha to the wood surface means that it is not possible to apply the concept of limiting nutrients

to this system in the way that it is used in the theoretical analysis of growth in continuous culture. The mycelium is too heterogeneous. Adding more nitrogen may not increase decay because the fungus is so well adapted to extract and concentrate nitrogen from the wood and from mycelium into parts of the colony where it is needed (for fruit body development, mycelial extension over non-nutrient surfaces, cord thickening, enzyme secretion). The fungus has inherited a gift of good housekeeping from its forbears in the forest.

Experiments to determine activities rate-limited by nitrogen supply (Watkinson *et al.*, 1981) showed that some were enhanced by extra nitrogen and others were depressed. Nitrate solutions were added to cellulose filter paper so as to give a cellulosic medium with a higher N/C ratio than in wood. This resulted in increased cellulolysis of pure cellulose, and also more growth of biomass when fungus was grown on synthetic culture medium, showing that these activities could be affected by the rate at which the fungus can concentrate nitrogen. In a homogeneous culture medium, the nitrogen levels characteristic of wood limit these activities. However, the fungus grows well in wood, and adding a solution of sodium nitrate does not increase wood decay. The explanation probably lies in the heterogeneity of the natural wood-fungus system and the fine adaptation of the fungus to its wood substrate.

When the fungus is growing in wood, nitrogen compounds in solution must be supplied from within the hyphae to the growing tips where biomass is added, and to the regions of the colony secreting cellulase. These supplies are provided by the activities in the rest of the mycelium which is colonizing fresh wood, solubilizing protein, taking up amino acids and transporting this nitrogen, through cords if necessary, to the hyphal tip. In other words, although the gross concentration of nitrogen overall may be low, the fungus has the mechanisms for concentrating it where it is needed.

The essential role of nutrient transport systems in the mycelium has been demonstrated. Nitrogen sources supplied in one part of a colony of *S. lacrymans* enhance cellulolysis at another point, separated by a non-nutrient surface, showing that nitrogen can be brought to the spot by translocation through the hyphae (Watkinson *et al.*, 1981). Recently this has been shown to occur in buildings where nitrogen from the soil under a house was taken up into mycelium and translocated to the point of decay (Doi, 1991). As the rate of extension of mycelium from a foodbase over non-nutrient surfaces is greater when the foodbase is nitrogenpoor, the colony seems to regulate its development so as to allocate resources most efficiently. According to this hypothesis, the need to conserve nitrogen within the colony and allocate this scarce resource to essential activities might have been a selective pressure in the evolution of foraging strategies in *S. lacrymans*.

The fungus has responses that can be convincingly interpreted as nitrogen-conserving strategies, and if they are, it might be controlled if these strategies could be frustrated, for example by interfering with sensing or transport mechanisms. The use of non-metabolizable analogues of nutrient substances may give the fungus the false message that food levels in the mycelium are normal and lead to a fatally flawed allocation of limited resources.

An alternative model for foraging mycelium

While evolution for optimal resource allocation is one theory to explain why colonies develop in one direction rather than another, a completely different interpretation has recently been offered by Rayner (1991). He has suggested that during active growth a colony develops an internal hydraulic pressure which is canalized by the hyphae and results in extension of the colony margin wherever growth is greatest. Pressure would be confined by hyphal walls where hydrophobic materials such as lipids, phenolic polymers and hydrophobic proteins have been added to the wall, but would push out new hyphae where the wall is permeable and plastic. In this model, the colony will spread in directions decided by the whereabouts of active growth or wall thickening. So, localized growth itself initiates the signal for the mycelial contents to flow towards a newly colonized foodbase. There is no need then to postulate a sequence of sensing, signal transduction and transmission and response activation. The theory is appealing, as it is the most holistic so far of approaches to the question: what controls the direction in which fungal colonies spread? Building mycologists have a particular interest in answering this question. Recent developments in fungal ecology have provided an excellent theoretical framework within which to formulate new experimental approaches.

SUMMARY

A building will suffer from fungal growth when conditions develop in or on it which resemble the natural environmental niche in which a fungus has evolved to live. Fungal spores are ubiquitous and will colonize parts of a building where conditions become suitable. This chapter gives examples of ways in which parts of a building may come to provide suitable habitats for different types of fungi, and thereby suffer from fungal spoilage or structural decay. The targeting of remedial and preservative measures at a particular fungal problem may be assisted by a consideration of the biological characteristics of the causal species, for example its mode of dispersal and survival, nutrient requirements and methods of degrading materials in feeding, foraging strategy, and tolerance of variations in temperature, light and humidity. Some biological features of commonly occurring spoilage and decay fungi which might be relevant to their control are also discussed.

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Indoor aerobiology and health

5

John Lacey

INTRODUCTION

Microorganisms may enter buildings from outside but the most important sources are usually within the building. Organic dusts, rich in microorganisms, can originate from many different materials in buildings. They may come from wood-rotting and mould fungi in domestic buildings but from cereal grains and animal feeds in farm buildings. In buildings associated with other occupations, sources may include foods, fibre crops, timber and wood products, composts, biotechnological processes, cutting oils and even air-conditioning systems. Microorganisms may supply other components in organic dusts through their metabolites which may include endotoxins (lipopolysaccharides) from Gram-negative bacteria, enzymes from bacteria and fungi and mycotoxins and (1 → 3) β-D- glucans from fungi. These occur in a mixture with fragments of plants, starch grains, proteins and other components; mites, and their products, including fecal material; dander, urinary and serum proteins and fecal material from animals; soil; and, perhaps, pesticidal chemicals.

This chapter describes the numbers and types of microorganisms in buildings of different types, the factors that affect their occurrence and their effects on the occupants.

SOURCES OF AIRBORNE MICROORGANISMS IN BUILDINGS

OUTDOOR AIR

Microorganisms are almost always present in outdoor air but their numbers and types change with time of day, weather, season, geographical location and with the presence of local spore sources. In temperate

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climates, *Cladosporium* is the predominant spore type during dry days in summer with *Alternaria* the second most numerous (Lacey, 1981). At night, the predominant type is

Sporobolomyces, a pink yeast that produces ballistospores. This is most numerous when the area of green leaf tissue is greatest during summer but later in the year ascospores of fungi such as *Didymella* or *Leptosphaeria* may be more numerous, especially close to harvest in areas where cereal crops are extensive. In autumn and especially near extensive woodland, basidiospores may be numerous.

Rainfall can have a profound effect on the airborne microflora. The first drops disturb vegetation and have a tap and puff effect, blowing fungal spores off with the puff of air that is pushed in front of the spreading water film from a rainsplash. Prolonged rain washes most spores out of the air but then, after the rain ceases and the foliage starts to dry, ascospores are released in large numbers. Under these conditions, epidemic outbreaks of asthma have been reported which may be related to the abundance of ascospores, especially *Didymella* (Harries *et al.*, 1985).

In temperate countries, airborne fungal spores are most numerous in summer and are generally few in winter, but in tropical countries they may be numerous throughout the year although possibly slightly less so in dry seasons. The air spora in different regions of the world shows remarkable similarity. Although the species may differ, over the whole year, the same types predominate in most regions. Thus *Cladosporium* is generally the most numerous type although it may be predominant in only one season in some tropical areas. *Alternaria* is usually the second most abundant but it can become the most numerous in warm, dry savannah regions and in some seasons elsewhere. Ascospores and basidiospores may be the most numerous type in some forested subarctic regions (Lacey, 1981).

The numbers of airborne spores in outdoor air may occasionally reach one million/m^3 in particularly favourable conditions, but maximum numbers are more often 10000–100000/m^3. In temperate regions, the scale of the spore concentration is largely determined by the numbers of *Cladosporium* by day and of *Sporobolomyces* by night with other types providing relatively small contributions. *Didymella* is one of the few exceptions having been recorded in concentrations up to $10^6/m^3$ near ripening cereal fields. Numbers of airborne spores tend to be larger in temperate and tropical regions than at high latitudes and in deserts where, respectively, numbers are restricted by short, cool seasons and by high temperatures, dry conditions and sparse vegetation.

RESIDENTIAL BUILDINGS

Airborne spores in dwellings may enter from outdoors and during ventilation, especially in summer; or come from wood-rotting fungi; from moulds growing in condensate on walls and windows or on food scraps and other organic material in house dust or retained in crevices; from fuels for furnaces; or from humidifiers of air-conditioning systems. Unless there is a source of spores in the building, spore concentrations indoors, especially of species of *Cladosporium, Alternaria, Botrytis* and *Epicoccum,* are generally smaller than those out of doors (Solomon, 1975; Ebner *et al.,* 1992). However, in winter when ventilation is less and spore concentrations out of doors are much smaller than in summer, numbers indoors will exceed those outdoors and may be much larger if there is dampness and visible fungal growth; predominant types will be determined by their source. Prevalence of all respiratory symptoms has been reported to be greater in damp

and mouldy houses than in dry homes, although no specific cause has been identified (Dales *et al.*, 1991b).

The dry rot fungus, *Serpula lacrymans*, produces spores prolifically. Thus, the cellar of a house, with extensive fruiting bodies of this fungus, yielded nearly 80000 spores/m^3 air while the remainder of the house, with no fruiting bodies, yielded 16000–26000 spores/m^3 air (Gregory *et al.*, 1953). In a country mansion, there were 360000 spores/m^3 in the cellar and 1600/m^3 in a draughty corridor above the site of three small fruiting bodies. It was estimated that the fruiting bodies were producing 3000 spores/cm^2 per day. The related *Serpula (Leucogyrophana) pinastri* has been recorded as a cause of allergic alveolitis in Australia but the occurrence of its spores in air has not been confirmed (Garfield *et al.*, 1984; Stone *et al.*, 1989). *Sistotrema brinkmannii* is associated with decaying window joinery (Savory and Carey, 1979) and is therefore, perhaps, more widespread than *Serpula lacrymans* although it does not produce so many spores. However, it had never been reported in the air spora of dwellings before Hunter *et al.* (1988) found it in 56% of samples from 51% of Scottish homes, comprising 20% of all colony-forming units (cfu) on Andersen sampler plates.

Fungi require water for growth. In buildings this is chiefly provided by rising damp, water penetration, high relative humidities resulting from insufficient ventilation and by condensation on cold surfaces. Relative humidities greater than 65–70% will allow some xerophilic species to grow and as the humidity increases a greater range of species are able to start growth. With such high humidities, cold walls may cool the air at the surface to increase relative humidity locally, perhaps sufficiently to cause condensation. Nutrients may be provided by painted or papered surfaces, by cotton and other cellulose materials or by food scraps and other organic matter in house dust. There is general agreement that the predominant types in the air spora of dwellings in Europe and North America are species of *Cladosporium, Penicillium* and *Aspergillus* (Nilsby, 1949; Maunsell, 1952, 1954; Schaffer *et al.*, 1953; Van der Werff, 1958; Ackerman *et al.*, 1969; Oren and Baker, 1970; Gravesen, 1972, 1978; Solomon, 1975, 1976; Hirsch *et al.*, 1978; Kozak *et al.*, 1979, 1980a, 1980b; Hocking and Pitt, 1980; Beaumont *et al.*, 1984, 1985; Holmberg, 1984; Kodama and McGee, 1986; Hunter *et al.*, 1988; Verhoeff *et al.*, 1990b; Van Reenen-Hoekstra *et al.*, 1992) but many other genera have also been recorded, including abundant *Alternaria*, both in settle plates, which favour large spored types (Lumpkins *et al.*, 1973; Lumpkins and Corbit, 1976) and in Andersen sampler collections (Hirsch and Sosman, 1976). However in winter, *Alternaria* was less numerous (18% of plates colonized) than *Cladosporium, Penicillium* and *Aspergillus* (21–22%), but both *Alternaria* and *Cladosporium* were much less numerous in winter than in other seasons (Hirsch and Sosman, 1976). In New Zealand, unidentified Basidiomycetes formed a large component of the colony counts on settle plates (Sheridan *et al.*, 1983) while in Hawaii, air in air-conditioned homes had fewer *Cladosporium* and more *Aspergillus* and bacteria than outdoor air or that in naturally ventilated homes (Kodama and McGee, 1986). Total concentrations were often less than 200 cfu/m3 but sometimes exceeded 1000 cfu/m^3.

In studies of houses in London and Scotland during the winter months, using volumetric samplers to isolate airborne fungi, *Cladosporium, Penicillium* and *Ulocladium* spp., *Aspergillus versicolor, Phoma herbarum, Geomyces pannorum* and yeasts predominated, giving total concentrations of <12 to 450000 cfu/m^3 air (Hunter *et al.*, 1988). At this time of year, concentrations were generally larger than those out of doors

and *Penicillium* spp. formed a larger proportion of colonies indoors than out. At one house, *Penicillium* spp. formed 50% of colonies isolated in the living room and 63% in the bedroom but only 14% outside. *Penicillium* spp. were isolated from 96% of homes sampled in Scotland and from 90% of samples and, with *Sistotrema brinkmannii* and *Cladosporium* spp., comprised over 70% of the total cfu. Yeasts comprised 13% of cfu, *Aspergillus* spp., mostly *A. versicolor*, 7% and *Ulocladium* spp. 1%. Airborne spores were more numerous in those houses in which visible moulding was found on the walls. In the absence of visible moulding, 44% of counts were in the range <12–200 cfu/m^3 air; with moulding elsewhere in the house, most counts still fell in the same range but this accounted for only 31% of the total; while with mould present in the same room, all samples contained >400 cfu/m^3 and the modal class was 1001–2000 cfu/m^3.

In other studies, concentrations up to about 3100 cfu/m^3 (Miller *et al.*, 1988), 6000 cfu/m^3 (Maunsell, 1954; Solomon, 1975; Kozak *et al.*, 1979, 1980b), 13000 cfu/m^3 (Beaumont *et al.*, 1985), 15000 cfu/m^3 (Holmberg, 1984), 20000 cfu/m^3 (Solomon, 1976; Reynolds *et al.*, 1990) and >75000 cfu (Hyndman, 1990) and 48200 spores (assessed microscopically)/m^3 (Curtis and Hyndman, 1989) have been recorded using volumetric samplers. *Penicillium* spp. were generally present and sometimes predomin-ated in the air. In Kansas, USA, numbers of *Cladosporium*, *Alternaria*, *Aureobasidium* and *Epicoccum* in the air were greater where gas stoves were used for cooking than with electric cookers while *Penicillium* and *Aspergillus* spp. were more numerous in houses with poorly-ventilated crawl spaces with dirt floors than in those with occupied basements (Su *et al.*, 1992). *Fusarium* and other water-requiring fungi were numerous where houses had problems with dampness. Health risks in dwellings may not only relate to numbers of spores or cfu but possibly also to metabolites, especially endotoxins and mycotoxins. Spores of *Penicillium* spp. and *Stachybotrys atra* may carry mycotoxins that can affect the immune response of the lung (Croft *et al.*, 1986; Miller *et al.*, 1988) while the health effects of volatile metabolites produced by many fungi are unknown.

Most of the moulds found in dwellings have no specialized mechanisms for the liberation of their spores and are largely dependent on disturbance for the dispersal of large numbers. Thus, counts often reflected the level of activity in the home with high counts when construction or repairs were being undertaken or during cleaning activity, especially if surface mould on walls was disturbed, and low counts when the level of occupation was temporarily decreased but sometimes fluctuations could not be explained. Increases are sometimes attributable to a single species, e.g. *Penicillium spinulosum, P. brevicompactum, P. corylophilum.* Vacuum cleaning can cause up to a 17-fold increase in numbers immediately and leave numbers elevated for at least 1 hour afterwards while physical disturbance of surface mould can cause a 3300 times increase in airborne fungi within 0.3 m of the source (Hirsch and Sosman, 1976; Miller *et al.*, 1988; Hunter *et al.*, 1988; Reynolds *et al.*, 1990).

The contribution of fungi growing on walls and painted surfaces and of those growing on scraps and in dust have seldom been separated. Among the fungi isolated from walls in German apartments, *Cladosporium herbarum, Penicillium brevicompactum, Aspergillus versicolor* and *Penicillium glabrum (P. frequentans)* were most numerous (Erhorn and Gertis, 1990). *P. chrysogenum* and *P. aurantiogriseum (P. cyclopium)* could also be common in bathrooms and other wet rooms (Tiefenbrunner, 1990). Actively growing *Aureobasidium* was found on the frames of visibly mouldy insulated windows in

Finland but species of *Penicillium, Phoma, Trichoderma, Sporobolomyces* and *Cryptococcus* were also isolated; particularly in older wooden houses, *Penicillium* was numerous in the air (Käpyla, 1985). The predominant fungi colonizing painted surfaces include *Cladosporium sphaerospermum, C. cladosporioides, Penicillium* spp., *Aspergillus flavus, Acremonium* spp., *Gliomastix murorum, Phoma herbarum, P. nebulosa, Phialophora fastigiata, Alternaria alternata, Fusarium* spp., *Rhodotorula glutinis* and *Ulocladium atrum* (Springle, 1990). Homes in coastal California are subject to seasonal growth of moulds on windows, walls, ceilings, floors and furniture during the rainy season, November to March. These include species of *Penicillium, Alternaria, Cladosporium* and *Fusarium,* and their growth is often accompanied by odour problems (Macher, 1987). Food scraps and other organic materials are commonly colonized by *Aspergillus, Eurotium* and *Penicillium* spp., some of which are able to grow at low relative humidities and all of which produce large numbers of spores which can easily become airborne when disturbed. However, xerophilic fungi, often important components of the indoor air spora, have been inadequately studied because suitable low water activity media, e.g. DG18 (Hocking and Pitt, 1980; Samson, 1985; Miller *et al.,* 1988), have rarely been used. *Aspergillus versicolor* has been isolated from house dust (Davies, 1960) and was commonly present on exposed surfaces in Dutch houses (Van Reenen-Hoekstra, 1992). However, it was a minor component of the mycoflora in Canadian houses in which *Penicillium* spp., especially *P. viridicatum, P. fellutanum* and *P. decumbens,* predominated (Miller *et al.,* 1988). *Wallemia sebi* was common in the air of Japanese homes and was implicated in allergic reactions among the residents (Sakamoto *et al.,* 1989).

Stachybotrys atra, well known as a producer of macrocyclic trichothecene mycotoxins, has been isolated from ducts, insulation and structural materials of a Chicago house (Creasia *et al.,* 1987) and was frequent in the air of Scottish houses, numbering up to 1.8×10^4 spores/m^3 where it also occurred in surface mould (Hunter *et al.,* 1988). Contamination of a basement with sewage appeared to contaminate the environment with *Acremonium* spp. (Patterson *et al.,* 1981). Other sources of fungi, especially *Aspergillus* spp. including *A. fumigatus,* may be potted plants growing in compost of hydroculture (with porous clay granules), although few spores are said to get into the air from these sources (Staib *et al.,* 1978a; Staib and Rajendran, 1980; Samson, 1985). Exceptionally, slimy spored species, such as *Phoma violacea,* can become airborne in aerosols created by showers (Green *et al.,* 1972).

Bacteria may also be present in the air of dwellings and, indeed, may outnumber fungi. Counts of 120000, 12200 (including 154 actinomycetes) and 22000 bacteria/m^3 have been found, respectively, in the air of overcrowded houses, in modern Finnish homes associated with damp, mould or health problems and in Scottish houses (Carnelley *et al.,* 1887; Nevalainen *et al.,* 1988; Flannigan *et al.,* 1991). Counts in Finnish houses without problems were only up to 4500 bacteria/m^3 (no actinomycetes). Raising dust increased the proportion of bacteria to fungi (Carnelley *et al.,* 1887).

In Canada and Scandinavia, wood is cut into chips for use as fuel and these are often stored in basements. Moulding of chips can occur, sometimes with spontaneous heating, with the predominant species including *Aspergillus fumigatus, A. niger, Mucor* spp., *Rhizopus microsporus* var. *rhizopodiformis, Paecilomyces variotii, Penicillium* spp., *Talaromyces* spp., *Aureobasidium pullulans, Phanerochaete chrysosporium* and

Thermoactinomyces vulgaris (Van Assendelft *et al.*, 1973; Thörnqvist and Lundström, 1982; Pellikka and Kotimaa, 1983; Kotimaa, 1990b; Terho *et al.*, 1987; Miller *et al.*, 1988) most of which can readily become airborne and can then circulate throughout the building. Two samples of 1 kg wood chips in Finland yielded 3.4×10^6 and 4.5×10^6 fungal spores/m^3 when shaken, with *Penicillium* predominant (2.9×10^6 spores/m^3) in one and only slightly less numerous (1.2×10^6) than *Cladosporium* spp. (2.0×10^6) in the other (Van Assendelft *et al.*, 1985).

The effects of air-conditioning will depend on the nature of the system. Water may accumulate in some systems from condensation on cooling coils while humidified systems may contain reservoirs of stagnant water or surfaces wetted by condensed steam which may sometimes become heated when located close to the furnace. Nutrients may be provided by accumulated dust deposited from the recirculating air, allowing the growth of a range of organisms, including bacteria, fungi, protozoa, nematodes and even aquatic mites. Cold-mist vaporizers have been colonized, in one instance, with *Aspergillus niger*, *Cladosporium*, *Penicillium*, *Rhodotorula* and *Cryptococcus* spp. (Hodges *et al.*, 1974) and, in another, with *Rhodotorula* spp., *Sporobolomyces roseus*, *Penicillium*, *Aspergillus* and other fungi (Solomon, 1974). Their operation increased airborne cell/spore loads 100- to 240-fold, sometimes to >14000/m^3 air (Solomon, 1974). Similarly, although an evaporative air-cooler decreased spore concentrations indoors to levels comparable with those out of doors, through the introduction of large volumes of outdoor air, it also introduced microorganisms, including *Aspergillus* and *Penicillium* spp., into the air which were not present out of doors (Macher, 1987). Another humidifier was contaminated by an *Acremonium* sp. and there was circumstantial evidence of its aerosolization in the development of extrinsic allergic alveolitis in residents (Patterson *et al.*, 1981).

Central air-conditioning in Hawaiian homes decreased *Cladosporium*, compared to outdoor air where it predominated, but increased numbers of *Aspergillus*, even when total counts indoors were smaller than those outdoors (Kodama and McGee, 1986). Similar air-conditioning systems in Wisconsin, USA, also decreased *Cladosporium*, compared to matched non-air-conditioned buildings, but had no effect on numbers of *Penicillum* spp. (Hirsch *et al.*, 1978). *Cladosporium* appeared to come from sources outside the home and numbers were decreased in air-conditioned homes because windows were open for less time and spores were removed by filters in the system. By contrast, *Penicillium* and *Aspergillus* spp. probably came from indoor sources and filtration was inefficient in removing their spores from the recirculating air. The predominance of *Aspergillus* spp. in Hawaiian homes and *Penicillium* spp. in Wisconsin homes may reflect climatic differences since *Aspergillus* spp. is more abundant in warmer climates and *Penicillium* spp. in cooler ones. Similarly, mechanical supply and exhaust ventilation systems in Finnish apartments decreased numbers of fungal spores but had little effect on bacteria (Reponen *et al.*, 1990).

Leakage from humidification systems can result in mould growth on the flooring and in one instance to abundant *Penicillium chrysogenum* and *P. aurantiogriseum* which could be isolated from the air (Fergusson *et al.*, 1984). Thermophilic actinomycetes have sometimes been implicated in illness, suggesting their presence in the air, when humidifiers in domestic heating systems have been mounted close to the furnace (Sweet

et al., 1971; Barboriak *et al.*, 1972; Fergusson *et al.*, 1984). However, their presence in the air has not been confirmed.

NON-INDUSTRIAL WORKING ENVIRONMENTS

Many of the factors affecting the aerobiology of offices are the same as those affecting dwellings but often the scale is much larger. By contrast with dwellings, air in modern buildings is less likely to enter by natural ventilation, less timber is used in construction and condensation may be controlled by central air-conditioning systems. However, these systems can also rapidly disperse microorganisms or their products throughout the building and, as in houses, humidifiers can provide sources of contamination.

In the absence of air-conditioning, greater numbers of airborne micro-organisms, mostly species of *Penicillium, Cladosporium* and *Aspergillus*, occurred in the air of schools and offices where floors were carpeted, especially if these had been wetted frequently. Up to 6000 bacteria and large numbers of *Penicillium* spp. and *Trichothecium roseum* were present in the air and in carpet dust at a school where leaks from the roof had repeatedly wetted the carpet (Gravesen, 1987). Some species that were frequently isolated from carpets, e.g. *Fusarium*, could not be found in air while *Alternaria* spp. were more common in carpet dust than in the air (Gravesen *et al.*, 1986). In the United States, up to 7300 fungal cfu/m^3 were found during simulated play in a playroom damaged by mould after a water leak but only 330/m^3 when there was no activity. By contrast, there were only 80–630/m^3 in offices and the lobby in the same building. *Penicillium* spp. were predominant through the building but the species differed between the lobby and offices (Reynolds *et al.*, 1990). *Aspergillus versicolor* and *Penidllium aurantiogriseum* predominated in museums, where there were 10^6 viable and 10^8 total fungal spores/m^3 air (Kolmodin-Hedman *et al.*, 1986).

Much aerobiological interest has focused on the role of air-conditioning systems in humidifier fever, building-related illness, sick-building syndrome (SBS) and legionnaire's disease. Air-conditioning systems often contain drip trays and water reservoirs to collect condensate and to provide water for humidification and moist baffle plates that all provide environments for the abundant growth of fungi, bacteria (including actinomycetes), protozoa, nematodes and even aquatic mites (Morey *et al.*, 1986a; Macher and Girman, 1990). The bacteria may include *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter* sp., *Flavobacterium, Cytophaga* and *Acinetobacter* sp. and, sometimes, thermophilic actinomycetes but there is usually a mixture of fungi also present, that may include *Aureobasidium pullulans, Paecilomyces lilacinus, Fusarium* spp., *Phialophora hoffmannii*, unidentified *Phialophora* spp. and *Phoma* spp., all typical of wet environments (Banaszak *et al.*, 1974; Solomon, 1974; Pickering *et al.*, 1976; Rylander *et al.*, 1978; Storms, 1978; Hollick *et al.*, 1980; Gravesen and Jepsen, 1981; Austwick *et al.*, 1982; Bernstein *et al.*, 1983; Samson, 1985; Lacey, 1988; Cosentino *et al.*, 1990). These may be accompanied by *Penicillium* and *Acremonium* spp., perhaps growing in drier parts of the system on filter materials and on accumulated dust. Thus, the coarse filters from the air-conditioning system of a hospital yielded *Aspergillus versicolor, Eurotium* spp., *Penicillium brevicompactum, P. chrysogenum* and *Wallemia sebi*, as well as *Cladosporium herbarum* and *Aureobasidium pullulans*. It was considered that these moulds could grow through the filter material in high humidity and

produce spores that were dispersed in the air stream (Elixmann *et al.*, 1986, 1987). Even *A. fumigatus* has been found colonizing the airconditioning system of a factory producing synthetic fibres (Wolf, 1969).

The drain pans of main air-handling units and the peripheral fan coil units of an office building yielded up to 10^7 bacteria/ml water and an air filter 3×10^7 cfu fungi/g dust (Morey, 1984). When the fan coil unit was first turned on or the drain pan agitated, concentrations of airborne fungi increased to almost $10^7/m^3$ from $7000/m^3$ in undisturbed conditions. If the humidifier does not emit microorganisms to the air, humidification may perhaps halve numbers of airborne fungi and bacteria in air-conditioned rooms and most of the bacteria may come from the people in the room and fungi from pot plants. Even though the water in the recirculating tanks of the evaporative humidifiers was heavily contaminated (10^4–10^5 bacteria/ml, 10–10^2 fungi/ml), their operation did not increase the airborne microbial load in the air-conditioning system (Pitkanen *et al.*, 1987). However, other types of contaminated humidifier can release large numbers of spores into the air. Thus, air samples in a polyamide carpet yarn texturing plant yielded up to 100 cfu/m^3 of *Trichoderma* spp., *Cladosporium* spp., *Penicillium roquefortii, P. glabrum, P. corylophilum, P. brevicompactum, P. rugulosum* and *P. expansum* (Pal *et al.*, 1985) while sensitivity to *Paecilomyces lilacinus* from a humidifier in a print works has been found in exposed workers (Gravesen and Jepsen, 1981).

No clear differences in the air spora have been found between works where humidifier fever has occurred and those with no outbreak. Sources of antigenicity in water from humidifiers is often difficult to determine (Hodges *et al.*, 1974). Microbial concentrations have often been below 1000 cfu/m^3 in the air of premises where outbreaks have occurred and samples have yielded as many as 31 fungal taxa and 14 bacterial, including eight actinomycetes. These have included *Trichoderma viride, Aspergillus fumigatus* and *Rhizopus arrhizus* and species of *Penicillium, Rhodotorula, Cladosporium, Fusarium, Phoma, Acremonium, Phialophora, Rhizopus arrhizus* and *Aureobasidium* as the most frequent fungi (occurring in more than 25% of samples) and *Bacillus, Pseudomonas, Micrococcus* and *Alcaligenes* spp. as the most frequent bacteria. *Phoma, Phialophora* and *Fusarium* spp. have been observed growing in sludge in humidifier tanks but *Trichoderma viride* was the species isolated most frequently only from humidifier fever premises. Although exposed workers gave immunological responses to humidifier water none reacted to any microorganism (Austwick *et al.*, 1986). By contrast, in seven Belgian offices affected by humidifier fever, there were up to 2000 fungal cfu/m^3, 79% of which were *Aspergillus* spp. and only 7.7% *Penicillium* spp. and 3.3% *Cladosporium* spp. Of the *Aspergillus* spp., 57% comprised three species with *Eurotium* teleomorphs, 35% were *A.restrictus* and 6% *A. versicolor* (Nolard-Tintinger *et al.*, 1986). No fungi but 3000 *Flavobacterium* spp./m^3 were found in a Swedish outbreak (Rylander *et al.*, 1978). In other outbreaks, *Bacillus subtilis, Pseudomonas* and *Cytophaga* spp. have been identified as antigen sources and implicated in humidifier disease (Flaherty *et al.*, 1984). In one study, antibodies were most commonly found to *Acinetobacter calcoaceticus* but in similar numbers in affected and non-affected workers (Lewis *et al.*, 1990). Actinomycetes have rarely been numerous but a Welsh outbreak was characterized by up to 700 *Thermoactinomyces* spp. cfu/m^3 air. However, although numbers initially correlated with outbreaks of humidifier fever there was no correlation between immunological response and disease (Edwards, 1980). In other outbreaks,

Thermoactinomyces spp. and *Saccharopolyspora rectivirgula (Micropolyspora faeni)* have been implicated (Banaszak *et al.*, 1970; Fink *et al.*, 1971; Weiss *et al.*, 1971; Hoschek, 1972; Kurup *et al.*, 1980) but their presence in the air has not always been determined.

Amoebae have been shown to account for some of the antigenicity of humidifier water, but again did not correlate with symptoms of humidifier fever (Edwards, 1986; Finnegan *et al.*, 1987). Algae have also been implicated.

Endotoxins from Gram-negative bacteria have sometimes been implicated in humidifier fever (Rylander and Haglind, 1984; Polla *et al.*, 1988) and 0.1–0.4 μg endotoxin/m^3 air was found in a print works where humidifier fever occurred (Rylander and Haglind, 1984). However, only one sample yielded 0.8 ng endotoxin/m^3 air, the lower limit for detection, in a Finnish outbreak (Pitkanen *et al.*, 1987). *Legionella pneumophila* dispersed from air-conditioning systems has caused spectacular outbreaks of atypical pneumonia and febrile illness which have become known as, respectively, Legionnaires' disease and Pontiac fever. Outbreaks have been associated with cooling towers, hot-water systems, whirlpool spa baths, clinical humidifiers in respiratory equipment, a supermarket vegetable spray, a natural spa bath, a fountain and potting compost (Lee and West, 1991). *L. pneumophila* has been isolated in buildings where no disease has occurred and, in the United Kingdom, was isolated from hot and cold water supplies or cooling systems of about 60% of large buildings, with 56% of hot water systems, 13% of cold water systems and 44% of cooling towers yielding the organism. *L. pneumophila* dispersed from cooling towers and from evaporative condensers can become entrained within air-conditioning systems and dispersed from the outlets. It is widespread in the environment and may be found in streams, potable water supplies and in plumbing systems but there are few records of its occurrence in air. It survives but does not grow in water in the absence of other organisms but is able to grow in association with green and blue-green algae and with some other bacteria. An interaction between *L. pneumophila* and amoebae has been suggested to explain Pontiac fever. It was suggested that either the amoebae decreased populations of the bacterium to below a critical level (Nagington and Smith, 1980) or that workers became sensitized to the amoebae, responding with a febrile reaction which restricted *L. pneumophila* infection (Rowbotham, 1980). However, although *L. pneumophila* was shown to be present in conditioned air in Pontiac (Glick *et al.*, 1978) there are no records of airborne amoebae or their products. Temperatures of 35–40°C, which may occur in pipework and holding tanks, are favourable to growth of *L. pneumophila* (Ager and Tickner, 1983; Godish, 1989). Aerosol survival is best at 65% relative humidity and worst at 55% and there is evidence that aerosols of *L. pneumophila* have spread 0.5–3 km from their source.

Patients whose immune response has been affected as a result of HIV infection or other underlying disease or by drug or radiation therapy are susceptible to nosocomial infections. Consequently, opportunistic pathogens, such as *Aspergillus fumigatus, A. flavus* and *Nocardia asteroides* are undesirable in a hospital environment. Mean concentrations of *A. fumigatus* in the air of an American hospital ranged from 2.0 to 6.3/m^3, with maxima up to 123 cfu/m^3 (Solomon *et al.*, 1978). By contrast, concentrations in a London hospital were up to 1000 times larger than this during the winter peak period (Noble and Clayton, 1963). In another American hospital, *A. fumigatus* colonized pigeon droppings in the exhaust duct of an air-conditioning system

in which a malfunctioning fan caused a reversal of the airflow so that spore-bearing air was blown into the renal transplant unit which it served (Kyriakides *et al.*, 1976). *A. flavus* was found growing on the external louvres of one ventilation system and damage to the filters allowed spores to penetrate into the wards, increasing 6-fold the frequency with which the fungus was isolated from sputum (Sarubbi *et al.*, 1982). Filtration of the air supply decreased concentrations compared with natural ventilation but airconditioning units have also been found to disperse *N. asteroides* (Rose and Hirsch, 1979; Streifel *et al.*, 1989). Potted plants could be a source of some *A. fumigatus* spores (Staib *et al.*, 1978a, 1978b, 1978c; Staib and Rajendran, 1980; Summerbell *et al.*, 1989).

Laboratory animal units are potent sources of airborne allergens. These are mostly derived from the animals themselves, especially urinary pro teins, but some may also come from bedding materials (Burge *et al.*, 1979; Price and Longbottom, 1988). Up to 11.3 µg rabbit allergen/m^3 air was detected in personal samples from a worker and up to 4.9 µg/m3 in the general atmosphere. Equivalent samples in a rat room were 3.6 and 1.4 µg/m3, respectively, and in a mouse room 0.2 and 0.1 µg/m3. Most allergen was in particles >6 µm diameter. Most fungi in animal houses were *Penicillium* spp. which gave concentrations up to 6.9×10^3 cfu/m^3 air although total concentrations were usually $>10^3$ cfu/m^3.

INDUSTRIAL BUILDINGS

Many different industrial processes involve the handling of fresh or stored agricultural produce which can carry large and diverse populations of microorganisms, while air-conditioning systems contribute other airborne microorganisms as described above. The nature and composition of the air spora depends on the source of the airborne microorganisms, the way in which it has been stored and handled and the amount of ventilation.

In food processing, airborne microorganisms can originate both from components of the food and from its contaminants. *Agaricus bisporus* and *Boletus edulis* are both used in the manufacture of mushroom soup and their spores have been shown to escape into the air during its preparation (Symington *et al.*, 1981). Bacteria were the predominant microorganisms in a sugar beet factory, with *Leuconostoc mesenterioides, Bacillus* spp. *Pseudomonas* spp. and *Enterobacter agglomerans* most common (Forster *et al.*, 1989). Sugar beet protein was also released into the air, however, and was implicated in occupational asthma. Bacteria, especially *Staphylococcus* spp., were also numerous in the plucking, eviscerating and packing areas of poultry-processing plants (Patterson, 1973) but may be replaced by *Corynebacterium* spp. including *C. xerosis*, in duck-processing plants (Dutkiewicz *et al.*, 1982). In a pork-processing plant, airborne bacteria averaged 318–10 800 cfu/m^3 air and yeasts and moulds 271–635 cfu/m^3, with most of both groups where sausage emulsions were prepared. Coliforms were few (<180 cfu/m^3 air) (Kotula and Emswiler-Rose, 1988). Total bacterial counts ranged from 1.1×10^3 to 2.6×10^3 cfu/m^3 in different areas of fluid milk processing and ice cream plants (Ren and Frank, 1992a, 1992b).

By contrast, heavy exposure to fungal spores can occur if products have moulded during storage. For instance, *Citrus* fruits infected with *Penicillium digitatum* and *P. italicum* can release many airborne spores into the air of packing houses (Barkai-Golan,

1966; Ström and Blomquist, 1986). Similarly, coffee beans, prior to roasting, carry fungal spores which can be dispersed into the air, with up to 2.4×10^4 cfu/m^3 air, including *Aspergillus fumigatus, A. flavus, A. niger,* and the xerophilic *Eurotium* spp. and *Wallemia sebi,* in containers being unloaded at a warehouse (Thomas *et al.,* 1991). Within the warehouse, exhaust ventilation effectively decreased airborne spores in the general environment but workers emptying sacks were still heavily exposed.

Yeasts, and species of *Aspergillus, Penicillium* and *Mucor,* are common in the air of bakeries with flour and dried fruit forming important sources (Wallerstein *et al.,* 1980; Dutkiewicz, 1983). In a British bakery, up to 1.2×10^3 cfu/m^3 air, mostly *Penicillium* spp., were found where flour was weighed and up to 2.3×10^3 cfu/m^3 air, mostly *Wallemia sebi* and yeasts, where dry fruit was handled (Crook *et al.,* 1991b). *Penicillium* spp. are also important for the maturation of some cheeses and sausages and may grow abundantly on their surfaces (De Weck *et al.,* 1969; Gari *et al.,* 1983; Gerault *et al.,* 1984; Morin *et al.,* 1984). When such products are cleaned before sale, the spores are dispersed but concentrations of airborne spores have never been determined. Extensive mould growth can also occur in food and drink processing factories where steam often condenses on the walls (Heaton *et al.,* 1990). In a food and vegetable cannery there were up to 3.8×10^4 cfu/m^2 wall surface but only 2% were fungi. Large numbers of fungi were isolated from malting kilns.

New hazards are being introduced into buildings through biotechnological processes. Yeasts, such as *Candida tropicalis,* and perhaps fungi, such as *Fusarium graminearum,* may form aerosols from cultures grown for food protein resulting in occupational asthma (Cornillon *et al.,* 1975). Both occupational asthma and extrinsic allergic alveolitis caused by fungi associated with enzyme fermentations have also been described. However, the fungi involved are not always those being cultured but are sometimes contaminants. Thus, Hořejší *et al.* (1960) described alveolitis during citric acid production caused by spores of *Penicillium* spp. and *Aspergillus fumigatus* contaminating surface cultures of *A. niger,* while Topping *et al.* (1985) found occupational asthma caused by *A. niger* and detected airborne allergen in the factory. Sensitivity to fungal enzymes and antibiotics, perhaps through inadequate dust control, has also been described (Davies *et al.,* 1974; Forsbeck and Eckenvall, 1978; Pauwels *et al.,* 1978; Baur *et al.,* 1986; Butcher and Salvaggio, 1986; Losada *et al.,* 1986).

Processing of fibre crops can often lead to large concentrations of airborne microorganisms. Cotton is a classic example and the nature of its airborne dust has received much study on account of its association with byssinosis, an enigmatic occupational lung disease. Fungi, bacteria and actinomycetes have all been isolated from the air of cotton mills. However, although microbiological causes have been considered, no one organism could be implicated in byssinosis, and the likely cause appears to be inhalation of endotoxins. Organisms present in cotton mills include *Penicillium* and *Aspergillus* spp., especially *A.niger* and *Cladosporium, Fusarium, Mucor* and *Rhizopus* spp., while among the bacteria, *Bacillus* spp., including *B. pumilus* and *B. megaterium,* have often predominated although Gram-negative bacteria, especially *Enterobacter agglomerans* and *Pseudomonas syringae,* appear more closely related to disease (Rylander and Snella, 1976; Salkinoja-Salonen *et al.,* 1982). Actinomycetes have usually been few, mostly *Streptomyces* spp. and *Nocardiopsis dassonvillei,* but in a traditional Lancashire mill, *Saccharopolyspora rectivirgula,* the causative agent of farmer's lung

disease, and *Thermoactinomyces* spp. were abundant in the air, reflecting the poorer quality of the cotton (Lacey and Lacey, 1987). In a new mill in north east England, fungi numbered up to 4.0×10^5 and actinomycetes up to $1.2\times10^7/m^3$ air but, in the Lancashire mill were 1.2×10^6 and $9.8\times10^6/m^3$, respectively. Microorganisms were generally most common in the air during the early stages of processing.

Sugar cane bagasse, the squashed, chopped fibre left after sugar is extracted from sugar cane, is sometimes used to manufacture particle board, paper and other products. It forms a good substrate for microbial development since it is largely composed of cellulose and when it leaves the mill it contains up to 50% water and 5% sucrose. When stored in bales, the bagasse may heat to above 50°C for 2–3 months and develop a characteristically thermophilic microflora dominated by *A. fumigatus* and thermophilic fungi and actinomycetes (Lacey, 1974). However, although the pattern of moulding is similar to that of hay, the species involved are often different. For instance, *Thermoactinomyces vulgaris* occurs widely but *T. sacchari* is much more frequently isolated and occurs in larger numbers (Lacey, 1971a, 1974). Propionic acid treatment of the bagasse resulted in *Paedlomyces variotii* becoming predominant, and this released large numbers of spores into the air when the bagassse was used subsequently to make particle board (Lacey, 1974).

Timber can also form a source of exposure to fungal spores in power stations, sawmills and woodworking workshops. The types of fungi involved differ with the origin of the timber and the environment in which it has been maintained. Sawmills were one of the first work environments in which allergic alveolitis was associated with fungal colonization. *Cryptostroma corticale* is a pathogen of maple trees in North America which infects trees before they are felled and then continues to grow under the bark afterwards, producing abundant spores which are released when the bark is removed in the sawmill, giving up to 1.3×107 cfu/m3 air (Wenzel and Emanuel, 1967). *Aureobasidium pullulans* and *Graphium* spp., growing on redwood sawdust, have likewise been implicated in sequoiosis in the USA, even though their spores are slimy and seem unlikely to become airborne in large numbers (Cohen *et al.*, 1967). Both *Rhizopus microsporus* var. *rhizopodiformis* and *A. fumigatus* have been implicated in allergic alveolitis of woodtrimmers in Swedish sawmills (Belin, 1980, 1987; Land *et al.*, 1987). Some isolates of *A. fumigatus* produce tremorgenic mycotoxins and it has been suggested that these might have a role in the disease (Land *et al.*, 1987), although the toxin has not been demonstrated in the spores. When cut into chips and stored in bulk for use as fuel, heating and moulding of the chips can occur as described above. Endotoxin levels associated with timber handling range from <4 µg/m3 air in sawmills to 40 µg/m3 in a chipboard factory (Dutkiewicz, 1989).

The processing of cork involves immersing the slabs in vats of boiling water for several hours and then stacking them in small humid warehouses for several days. During this period, there is abundant mould growth with *Penicillium glabrum (P. frequentans), P. granulatum, Aphanocladium album, Monilia sitophila* and *Mucor plumbeus* predominant. These give rise to concentrations ranging from 2.6×10^7 spores/m^3 air in the small warehouses to 10^6–10^7 spores/m^3 within the factory, while up to 9×10^7 spores/m^3 air were present in the breathing zones of workers moving the mouldy cork (Avila and Lacey, 1974). About half the airborne spores were of *Penicillium* type but only *P. glabrum* was found in large numbers throughout the factory. Where cork had been

conditioned by steaming immediately before processing instead of by boiling and stacking, there were fewer spores in the working environment (Lacey, 1973).

AGRICULTURAL BUILDINGS

Typically, the storage microflora of hay and grain is dominated by species of *Eurotium* (members of the *Aspergillus glaucus* group), *Aspergillus* and *Penicillium*, but the predominant species depends on the conditions in which the material is stored and particularly its water content when placed in store and the subsequent degree of spontaneous heating. Species present before harvest only survive in hay or grain stored dry, with water contents of 12–13% (Gregory *et al.*, 1963; Festenstein *et al.*, 1965). Table 5.1 shows how the predominant species in grain are affected by water availability and temperature during storage but the succession in hay is very similar. However, *Penicillium* spp., *Aspergillus candidus* and, especially with maize, *A. flavus* are more abundant in grain but this is seldom stored wet enough to develop large actinomycete populations, except occasionally when it is kept on farms for animal feed (Lacey, 1971a). Where hay is dried for long periods on racks, as in Scandinavia, heating may be decreased together with the incidence of *S. rectivirgula*, although *Thermoactinomyces* spp. and *Eurotium* spp. remain abundant (Terho and Lacey, 1979; Kotimaa *et al.*, 1984). Straw yielded many more spores of thermophilic fungi and actinomycetes than hay and grain (Kotimaa, 1990a).

Table 5.1 Effects of water availability and spontaneous heating on colonization of cereal grains by fungi with *Aspergillus* and *Penicillium* anamorphs and by other microorganisms

a_w	Maximum temperature (°C)	Predominant fungi		
		Aspergillus	*Penicillium*	Other species
<0.60 species	Ambient			Survival of field species (e.g. *Cladosporium; Alternaria*)
0.75	Ambient	*A. restrictus*		
0.85	Ambient	*Eurotium* spp.		
0.87	Ambient		*P. brevicompactum*	
0.88	Ambient		*P. verrucosum*	
0.89	Ambient		*P. expansum* *P. granulatum* *P. griseofulvum* *P. rugulosum*	
0.90	25	*A. versicolor*	*P. aurantiogriseum* *P. citrinum* *P funiculosum* *P. hordei* *P. janthinellum* *P variabile*	*Streptomyces griseus*

0.92	30		*P. capsulatum*	
0.93	35		*P. plceum*	
0.95	50	*A candidus* *A. flavus* *A. niger* *A. ochraceus* *A. terreus* *Em. nidulans*	*P. purpurogenum* *P. rugulosum*	*Absidia corymbifera* *Streptomyces albus*
< 0.95	60	*A. fumigatus*	*Talaromyces* spp.	*Malbranchea cinnamomea* *Rhizomucor* spp. *Thermomyces* spp. Thermophilic actinomycetes

A., Aspergillus; Em., Emericella; P., Penicillium.

Storage of moist barley and of maize silage in sealed and unsealed silos leads to an accumulation of carbon dioxide in the intergranular atmosphere from respiration of the grain and its microflora that modifies the mycoflora depending on the degree of anaerobiosis achieved. Yeasts and *Penicillium roquefortii* are among the species most tolerant of high CO_2 concentrations but with increasing aeration are replaced by other species more typical of grain storage (Lacey, 1971b). Most storage species produce abundant spores that easily become airborne when the substrate is disturbed, giving concentrations up to 10^{10} spores/m^3 air of which, with heated hays and grains, up to 98% may come from actinomycetes (Lacey and Lacey, 1964; Zeitler, 1986). However, the spectrum of species in the air will generally reflect that in the substrate (Williams *et al.*, 1964; Dennis, 1973; Lacey, 1980). Mites are often attracted to stored hay and grain by fungal development and may contribute to the airborne allergen load on farms (Cuthbert *et al.*, 1979; Revsbeck and Andersen, 1987). Fungi both provide food for mites and contaminate their products in the dust. Both *Bacillus* spp. and Gram-negative bacteria may also be found in the air but the storage environment is usually too dry for their proliferation. However, Gram-negative bacteria are generally carried into store on the freshly harvested crop and may be dispersed during handling to give concentrations exceeding 1000 cfu/m^3 air (Dutkiewicz, 1978), while in granaries up to 1.3×10^6 bacteria/m^3 have been found, with *Enterobacter agglomerans (Erwinia herbicola)* predominant (Dutkiewicz *et al.*, 1974). Similar contamination may occur on farms, at country and terminal elevators, docks and feed mills. When grain is used for malting, poor germination and the conditions on the malt floor appear to favour *Aspergillus clavatus*. Turning the sprouting grain then releases abundant spores into the air, obscuring vision across the floor (Riddle *et al.*, 1968).

Airborne microorganisms in animal houses can come from a range of different sources, including both mouldy feed and bedding and the animals and their excreta. The microflora of feed and bedding is determined by water content and temperature, while the animals yield airborne skin scales, fur or feathers, which may all carry bacteria. Urine and fecal material may become aerosolized, carrying Gram-negative bacteria and endotoxins into the air (Clark *et al.*, 1983; Brouwer *et al.*, 1986; Travers *et al.*, 1988). Fungi from feed dominated the air spora in a British cowshed, with spores from species

of *Aspergillus*, *Mucor* and *Cladosporium* predominant; the thermophilic fungus *Thermomyces lanuginosus* and actinomycetes were also present, suggesting heating of the hay during storage (Baruah, 1961). Up to 1.6×10^7 spores/m^3 were found in the air but the sampling method used may have underestimated the numbers of bacteria and actinomycetes and, in three Danish calf houses, there were up to 10^5 bacteria and 1.3×10^4 fungal cfu/m^3 (Blom, 1984) while pig houses yielded up to 8×10^6 bacteria/m^3, mostly of Gram-positive types (*Staphylococcus lentus, S. hominis, Aerococcos viridans, Micrococcus lylae, Bacillus* spp., enterococci) associated with skin particles. Fungal and actinomycete spores were much less numerous in pig house air (up to 10^5/m^3) than bacteria (up to 1.25×10^6/m^3) and individual taxa were isolated from fewer than half the samples (Curtis *et al.*, 1975; Clark *et al.*, 1983; Donham *et al.*, 1986; Cormier *et al.*, 1990; Crook *et al.*, 1991a). The most frequently isolated bacteria included *Enterobacter agglomerans, Moraxella, Acinetobacter calcoaceticus* and *Pseudomonas* spp., and fungi included species of *Scopulariopsis, Aspergillus, Penicillium* and *Candida* (Cormier *et al.*, 1990).

Poultry houses may yield more bacteria than pig houses with up to 6.8×10^5 total bacteria, 5.6×10^5 Gram-negative bacteria and 1.9×10^3 fungi/m^3 air (Clark *et al.*, 1983). The bacteria may include *Pseudomonas, Micrococcus, Bacillus, Proteus, Staphylococcus* and *Escherichia coli* and the fungi *Aspergillus* and *Penicillium* spp. (Sauter *et al.*, 1981) sometimes including *Aspergillus flavus* and *A. fumigatus* (Chute and Barden, 1964). Numbers of bacteria may increase with the age of the birds, reaching 7.8×10^6 cfu/m^3 in a broiler house after 10 weeks (Dutkiewicz *et al.*, 1974).

WASTE DISPOSAL BUILDINGS

Bacteria and the spores of actinomycetes and fungi are often important components of the dust dispersed when household waste is handled. They may already be present in waste material at its source or may develop during its storage and subsequent processing. Their numbers and types depend on the source material, the method of storage, water content and the temperature attained through spontaneous heating. Composting is a controlled form of biodegradation in which temperature and water content are often controlled.

Domestic waste is a complex mixture of organic and inorganic materials and much of it is putrescible and readily colonized by bacteria and fungi. It may also be contaminated with fecal bacteria of human and animal origin from disposable nappies and animal feces. These and other microorganisms may grow as waste progresses through the disposal process, giving a succession of different species whose propagules can be dispersed during handling. Microbial emissions during waste handling have previously been studied in different countries (Pohjola *et al.*, 1977; Constable and Ray, 1979; Lembke and Knisely, 1980, 1985; Boutin and Moline, 1987; Boutin *et al.*, 1987; Crook *et al.*, 1987a; Nersting *et al.*, 1991; Rosas *et al.*, 1991) but there have been few attempts to compare different disposal facilities or waste from different sources handled in different ways. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Citrobacter, Enterobacter, Bacillus, Corynebacterium, Micrococcus, Mycobacterium, Nocardia* and *Streptomyces* spp. have all been reported in waste plants, numbering up to 10^3 to 10^4 cfu/m^3 air, but *Shigella* spp. have never been found and *Salmonella* has only been reported from Mexico (Rosas *et al.*, 1993). In one study, *Bacillus* spp. formed more than

half the 2.1×10^5 cfu aerobic bacteria/m^3 air with *Corynebacterium* and *Micrococcus* spp. each contributing 4% to the total but samples in the breathing zone may reveal much larger concentrations of microorganisms, up to 3×10^9 cfu total microorganisms/m^3 air in a Danish waste sorting plant (Sigsgaard *et al.*, 1990). Concentrations of microorganisms changed with distance from the source of dust and with the amount of processing activity (Glysson *et al.*, 1974). Airborne fungi may also be numerous with *Penicillium* and *Aspergillus* spp. predominant and *Penicillium* spp. numbering 5.8×10^4–5.0×10^6 spores /m^3 (Crook *et al.*, 1987a). *Cladosporium* spp., *A. fumigatus* and *A. nidulans* were present in much smaller numbers (Constable and Ray, 1979; Pohjola *et al.*, 1977). Fungi numbered 4.4×10^4 cfu/m^3 in an American waste recovery plant but samples differed greatly, particularly between seasons (Lembke and Knisely, 1980, 1985).

In a range of British waste transfer stations using different methods of handling, numbers of microorganisms differed greatly from one site to another and were affected by time of year, temperature and weather but were usually greatest when waste was moved on conveyor or by grab cranes, and particularly large when waste was separated using air classifiers, rolling drum separators and extensive conveyor belt systems. Fewer microorganisms occurred where waste was compacted than with conveyor systems and pulverizers. Water sprays over storage bunkers appeared to have little effect on numbers of airborne microorganisms (Crook *et al.*, 1987a). Most fungal and many bacterial dispersal units were in the respirable size range but most bacterial particles were non-respirable. Endotoxin levels up to 1.0 µg/m3 air were found during refuse handling (Malmros and Peterson, 1988).

Composting is chiefly performed out of doors but subsequent processing may occur indoors. The high temperatures during composting should be lethal to most pathogenic bacteria and parasites but many airborne microorganisms, especially thermophilic and thermotolerant fungi and actinomycetes may be dispersed from waste compost giving $>10^5$ cfu/m^3 air of all the major groups of microorganisms and often 10^6. However, Gram-negative bacteria and streptococci usually numbered only $>10^4$/m^3 air, and *Salmonella* sp. was never isolated. Composting changed the species composition considerably, with actinomycete numbers vastly increased to exceed numbers of all other bacteria. *Saccharopolyspora rectivirgula (Micropolyspora faeni), Saccharomonospora, Thermoactinomyces* and *Streptomyces* spp. all exceeded 10^6 cfu/m^3. *Thermomonospora* spp. were less numerous, with concentrations between 10^4 and 10^5 cfu/m^3. *Aspergillus fumigatus* was the predominant fungus in one pile, giving concentrations up to 2.7×10^6 cfu/m^3 air during processing, and *Penicillium* spp. in the second, with up to 6.8×10^5 cfu/m^3 air. Personal samplers again showed that workers were often exposed to larger concentrations of microorganisms than were revealed by static samplers (Lacey *et al.*, 1990).

SAMPLING METHODS

Determinations of the occurrence of microorganisms in buildings can only be as good as the methods used. Often insufficient attention is given to the sampling characteristics of different instruments and their limitations, the siting of samplers, the timing of samples and the way in which the catch is handled. Failure to consider these factors means that

much published work gives less reliable information than is often attributed to it. Choice of a sampler requires careful consideration of the purposes of the investigation, the information required, the characteristics of the microorganisms in the environment being studied and the sampling and trapping efficiencies of the available samplers. No one method of sampling or isolation medium is ideal for all needs.

SAMPLING VIABLE MICROORGANISMS

Settle plates, Petri dishes of agar medium exposed to the atmosphere by removing the lid for a fixed period, have been used widely in studies of the indoor environment. They are simple and easy to use but the results are difficult to interpret. Spores or cells sediment at a rate determined by the square of their aerodynamic radius, i.e. the radius of a unit density sphere sedimenting at the same rate. Consequently, small spores are effectively sampled from a much smaller volume of air than large spores and if present in the air in equal concentrations, many more of the larger particles will be collected than of the smaller. Further errors are caused by any air movement which creates a shadow with no deposition downwind of the leading edge that increases in size with wind speed until there is no deposition unless airflow becomes turbulent when deposition may become greater then expected. Much deposition may also occur in the turbulence created when the lid is removed and replaced.

Some modern hand-held samplers are very convenient to use but their trapping efficiency is often low and differs for different size particles, again distorting results. The Biotest RCS sampler has often been used in indoor studies but the sampling rate and trapping efficiency, at least in early versions, are difficult to determine. Although the manufacturer states the actual sampling rate to be 280 l/min and Macher and First (1984) found it to be 210 l/min, the ‘effective’ sampling rate is said to be is only 14% for these particles. Other tests (Macher and First, 1983) 40 l/min for 4 µg particles. This suggests that the collection efficiency suggested that efficiency declined from 100% for 15 µm particles to 60% for 4 µm and to <10% for particles of 2 µm diameter or less. The decline was particularly rapid for particles in the respirable size range (Clark *et al.*, 1981; Macher and First, 1983). However, Kang and Frank (1989) found smaller relative counts for moulds than for bacteria when comparing the RCS sampler with six-stage Andersen sampler counts. They attributed this to particles carrying bacteria having a greater density than mould spores. Estimates of concentration using the RCS sampler thus appear to be unreliable unless estimating a unif orm aerosol for which the collection efficiency is known. The Ross-Microban sieve sampler also caught fewer microorganisms than a two-stage Andersen sampler (Ren and Frank, 1992a, 1992b). The trapping efficiency of the similar SAS sampler has been determined as about 50–70% for bacteria but close to 100% for yeasts and fungi when compared with a slit sampler (Lach, 1985; Legan, 1986). However, fewer colonies were collected with SAS and RCS samplers than with slit and single stage Andersen samplers (see below) and the reproducibility of results and number of species isolated was better with the last two (Verhoeff *et al.*, 1990b). In contrast, Marchisio *et al.* (1989) found the SAS and Andersen samplers to be comparable.

Impaction and impinger samplers have the potential to collect micro organisms more efficiently than the samplers so far described. The simplest form is the slit sampler (Bourdillon *et al.*, 1941) designed for indoor studies of bacteria. Air is drawn through a

rectangular jet positioned above the agar surface of a slowly rotating Petri dish containing agar medium. Particles impact onto the agar surface and after sampling for one rotation, the dish is removed and incubated. The Andersen sampler is a form of cascade impactor in which there is a series of 'sieve' plates, each with 400 round jets which decrease in size from one plate to the next, causing successively smaller particles to be deposited in Petri dishes of agar medium placed behind each (Andersen, 1958). Air is drawn through the sampler at 28 l/min and airborne particles are deposited on the plates according to their aerodynamic size. The final stage efficiently collects particles <1 μm diameter. Different models consist of one, two or six stages. Particles deposited on the first two stages of the six-stage sampler are considered non-respirable, with the remainder being respirable; the two-stage model provides a cut-off between respirable and non-respirable particles; the single-stage model collects all particles larger than 1 μm. May (1964) suggested modification of the hole pattern of the six-stage sampler to improve collection on the upper stages and also recommended sampling without the intake cone. A large stagnation point shield should be used if there is air movement. Andersen samplers have proved useful for the isolation of actinomycetes (Lacey and Dutkiewicz, 1976).

Solomon and Gilliam (1970) proposed a simplified use of the six-stage sampler with entry cone in which only one Petri dish of agar medium was placed behind Stage 6. However, Jones *et al.* (1985) found that this gave inferior results to the standard six-stage Andersen sampler although counts from a single-stage, equivalent to stage 6 without the entry cone, correlated closely with those from the standard sampler. Two-stage samplers are also reported to give smaller counts than sixstage samplers (Gillespie *et al.,* 1981). Decreasing the number of stages loses size discrimination and the opportunity to match results to occupational hygiene criteria of inhalable, thoracic and respirable fractions of airborne dust. It also increases the risk of overloading although the number of plates used is decreased. Andersen (1958) claimed that plastic Petri dishes collected 20% fewer particles than glass dishes. However, if many samples are to be collected, glass dishes become impractical. One factor that could account for poorer recovery is the depth of agar in the plates and its separation from the impaction jets above. For optimal trapping efficiency and to obtain the size distribution between stages described by Andersen (1958), the standard glass dishes supplied by the manufacturer are filled with 27 ml agar. The dimensions of plastic dishes differ from those of glass and Tiberg *et al.* (1987) used 35 ml agar in a sampler modified for plastic dishes.

A disadvantage of impactors sampling directly onto agar is that plates are easily overloaded in heavily contaminated environments, and especially if there are only one or two stages to the sampler. This problem may be overcome by comminuting the agar from slit or Andersen sampler plates or by using a gelatine medium which can be melted and replated after dilution as necessary (Blomquist *et al.,* 1984a, 1984b). Alternatively, Sierra Marple cascade impactors, liquid impingers or filtration may be used for sampling. Solid collection media, which may be moistened with glycerol, are placed behind the impactor jets and used to collect particles in the Sierra Marple personal cascade impactor; trays to hold a gelatine collection medium have also been described (Macher and Hanson, 1987). Catches can then be washed off, diluted as necessary and then plated. Liquid impingers consist of a wide tube, which collects the sample, ending in early models in a capillary tube which dips into the liquid but which in more recent models is raised above the liquid to decrease damage to microorganisms (May and Harper, 1957). Deposition of large

particles in the right angle bend of the inlet tube may be decreased by the use of a pre-impinger (May and Druett, 1953). However sonic velocity is achieved through the capillary tube which may still damage some particles. Gentler sampling is achieved using multistage liquid impingers which collect particles in three size fractions, corresponding approximately to deposition in the human respiratory tract (May, 1966). Personal liquid impingers have also been described, with jets 1–0.42 mm diameter and sampling rates of 1–1.42 l/min (Macher and First, 1984). With all impingers, samples can be diluted as necessary before plating. Filtration provides a portable, flexible and easily managed method of sampling many airborne microorganisms. Depending on the sampler, flow rates of 2–100 l/min can be used. However for many applications, disposable aerosol monitors loaded with polycarbonate membrane filters, with suction of 2 l/min provided by a personal sampler pump, are very satisfactory (Palmgren *et al.*, 1986; Eduard *et al.*, 1990). Sampling is probably most satisfactory when monitors are exposed open-faced but after sampling the monitors can be sealed and, if necessary, mailed to a laboratory for assay. Microorganisms can be suspended in fluid within the monitor, diluted as necessary and plated. Andersen samplers, multistage liquid impingers and personal aerosol monitors give comparable counts, with most bacteria and fungi recovered with aerosol monitors (Crook *et al.*, 1987a). However, Gram-negative bacteria may be damaged by desiccation on filters. Counts of other organisms are often less from Andersen samplers than by other methods because aggregates are collected intact on Andersen sampler plates and each gives only one colony, while aggregates in samples from cyclones, filters and liquid impingers are dispersed so that each viable cell can give rise to a colony (Henningson *et al.*, 1981; Karlsson and Malmberg, 1988).

Almost all studies of the indoor air spora have involved short-period samples during periods of inactivity within the building or without reference to the amount of activity. However, numbers of airborne microorganisms may change considerably with activity that stirs up dust and longer-term sampling would seem desirable. The automatic volumetric spore trap (Hirst, 1952) is probably unsatisfactory since it was designed for large-spored plant pathogenic fungi and the inlet corresponds to the second stage of a four-stage cascade impactor (May, 1945) so that trapping efficiency declines with small particles. Longperiod samples could be collected by impingers and by filtration since heavy loading can be dealt with by greater dilution of the catch. However, care would be needed to ensure that the liquid did not evaporate excessively or that particles collected by filtration were not damaged by desiccation. The most satisfactory instrument for long-period sampling is probably the multi-stage liquid impinger since, especially in the first two stages, this treats particles more gently than other liquid impingers.

A wide range of media have been used to determine numbers of different microorganisms in the air spora (Kingston, 1971; Crook *et al.*, 1987a; Lacey and McCartney, 1992). Selective media have been used for the isolation of different groups of bacteria but these are less well developed for fungi. However, it is evident from the range of fungi isolated that a medium that allows optimum growth of xerophilic fungi should often be included. DG18 agar is a good example of such a medium which is satisfactory for the growth of *Aspergillus*, *Penidllium* and *Wallemia* spp. as well as other xerophiles (Hocking and Pitt, 1980). Malt extract and DG18 agars gave consistently high yields of fungi, both in terms of cfu/m^3 and of total number of species isolated with slit samplers and six-stage Andersen samplers and also gave best reproducibility between duplicate

parallel samples with Andersen samplers (Verhoeff *et al.*, 1990a). The use of at least two media is essential because some important hydrophilic fungi, like *Stachybotrys atra*, will not grow on DG18 while extreme xerophiles such as *Wallemia sebi*, prefer the low water activity medium. Malt extract agars containing glucose and peptone have been recommended (Burge *et al.*, 1979; Morey *et al.*, 1986b) but should not be used because the glucose encourages excessive growth of Mucorales.

Other media and incubation temperatures found satisfactory for isolating microorganisms from the air include: tryptone soya agar at 37°C for total bacteria, half-strength nutrient agar at 25 and 40°C for total bacteria and actinomycetes, half-strength tryptone soya agar +0.2% casein hydrolysate at 55°C for thermophilic actinomycetes, violet red bile glucose agar at 37°C for Gram-negative bacteria, Columbia agar base + half-strength staphylococcus selective supplement at 37°C for Grampositive bacteria, P agar at 37°C for Gram-positive cocci and 2% malt extract agar + dichloran rose bengal, chloramphenicol agar at 25 and 40°C for fungi (Crook *et al.*, 1987a).

Calibration of sampling instruments is essential to ensure that the correct sampling rates are being maintained.

SAMPLING TOTAL MICROORGANISMS

Culturing reveals only those viable microorganisms that will grow on the media used under the selected incubation conditions and can often give a misleading impression of the total numbers of cells in the air. Thus from only 0.1 to 68% of actinomycetes and from 3 to 98% of fungi on air samples f rom f arm buildings counted by light microscopy of filters grew in culture (Eduard *et al.*, 1990). However, even non-viable organisms may be important in allergic reactions and in carrying endotoxins and mycotoxins. Direct counting methods have to be used to estimate the total microorganisms concentration in the air. Methods used have included cascade impactors and filtration with polycarbonate or cellulose ester membrane filters in aerosol monitors. A four-stage cascade impactor (May, 1945) has been used widely (Lacey and Lacey, 1964, 1987; Lacey, 1971b, 1973; Lacey and Dutkiewicz, 1976) in studies of occupational environments. Filtration, using aerosol monitors loaded with polycarbonate filters and operated with personal sampler pumps as above, allows scanning electron microscopic assessment (Heikkila *et al.*, 1988; Karlsson and Malmberg, 1988; Eduard *et al.*, 1990) or acridine orange staining and epifluorescence microscopy (Palmgren *et al.*, 1986) of the catch while cellulose ester filters cleared with glycerol triacetate allow light microscopy (Darke *et al.*, 1976).

SAMPLING AIRBORNE ALLERGENS

Not all allergens are microbial in origin and while microbial allergens can be assessed using the methods described above, non-microbial allergens require different methods. Filtration has been used most commonly, with high volume samplers drawing up to 1 m^3/min through 20×30 cm glass fibre filter paper (Agarwal *et al.*, 1981, 1983) or, more recently, with 3 cm diameter glass fibre filters sampling at 17 l/min (Platts-Mills *et al.*, 1986) or 25 mm glass fibre filters sampling at 2 l/min (Price and Longbottom, 1988). Cascade impactors and multistage liquid impingers have been used to collect and size allergenic particles (PlattsMills *et al.*, 1986, 1989; Price and Longbottom, 1988). Better

recovery of scampi allergen was obtained with a Litton-type large volume electrostatic air sampler collecting into liquid than with high-volume filtration (Griffin *et al.*, 1988) and this has been confirmed in a model system using egg albumin (Lacey *et al.*, 1994). Radioallergosorbent test (RAST) inhibition assays have generally been used to detect specific IgE antibodies to the collected material in exposed subjects, and enzyme-linked immunosorbent assay (ELISA) to detect IgG antibodies. ELISA is probably more sensitive than RAST and has been used to detect house-dust mite allergen in samples collected on personal sampler filters (Price *et al.*, 1990). ELISA also allows the assay of specific components using monoclonal or polyclonal antibodies.

SAMPLING MYCOTOXINS

Glass fibre filters and a high-volume sampler have been used to sample airborne aflatoxins (Burg *et al.*, 1981) and were subsequently found more satisfactory for sampling such aerosols than cotton filters, a RCS sampler, an Andersen sampler loaded with Teflon (Dupont) discs instead of agar and a liquid impinger (Silas *et al.*, 1986). No toxin was recovered from the liquid impinger sampler and recoveries from the Andersen sampler, cotton filters and RCS sampler (assuming a 40 l/min sampling rate) averaged 23, 41 and 43% of glass fibre filter recoveries. Samples were extracted with acetonitrile: water (9:1), filtered and then re-extracted with chloroform before the filtrate was evaporated to dryness. The sample was then dissolved in chloroform and hexane added to a Sep-Pak column (Waters) and after washing with hexane and diethyl ether was eluted with chloroform/ethanol (95:5) for thin layer chromatography, with chloroform/acetone (9:1) development and confirmation using trifluoroacetic acid. Aflatoxin was quantified using a scanning fluorodensitometer with an excitation wavelength of 365 nm and an assay wavelength of 440 nm. Similar sampling methods could be used for other mycotoxins with detection and quantification by immunoassay, using monoclonal antibodies and the method described by Ramakrishna *et al.* (1990).

SAMPLING VOLATILE METABOLITES

Fungal volatiles were sampled by Miller *et al.* (1988) by passing 200 l of air filtered to 0.22 µm through clean stainless steel cartridges packed with Tenax GC support (60/80 mesh). The cartridges had been conditioned at 300°C, with a helium gas flow of 20 ml/min for 6 h and a baseline record for each cartridge determined by gas chromatography-mass spectroscopy (GC-MS) after transferring the cartridges to a desorption unit (Chemical Data System 320) interfaced to a GC-MS system (Finnigan MAT 312). The cartridges were kept sealed into glass tubes before and after sampling. As soon as possible after sampling, the cartridges were thermally desorbed and analysed.

SAMPLING ENDOTOXINS

Endotoxins have generally been collected by filtration through cellulose acetate or polyvinyl chloride, either directly with sampling rates of 1–2 l/min or using a vertical elutriator (Rylander and Morey, 1982; Rylander and Vesterlund, 1982; Rylander and Haglind, 1984; Dutkiewicz *et al.*, 1989) but an Andersen sampler, depositing onto glass

fibre filters, and a cascade impactor (May, 1945) have also been used. After exposure and weighing the filters are shaken with pyrogen-free water and endotoxin is quantified with a *Limulus* amoebocyte lysate (LAL) assay.

DISPERSAL THROUGH BUILDINGS

There have been few studies of the spread of microorganisms through buildings. Air-conditioning systems provide a potent means of dispersal of microorganisms from one part of the building to another but even without air-conditioning, microorganisms can spread rapidly. For instance, conidia of *Amorphotheca resinae* released from Petri dish cultures in the first floor hallway of a four storey building could be isolated on settle plates of creosote agar on the second, third and fourth floors within 5 min (Christensen, 1950). On the third and fourth floors, maximum numbers of colonies were grown from 5 to 10 min after release. Numbers declined with distance from the point of release but could still be detected on all floors after 20 min. When released within a room on the first floor, with the door open, they again reached the fourth floor within 5 min and even appeared in a second floor room, with the door closed, within 20 min. Spores could still be isolated at most sites 2–4 h after release. It was concluded that dispersal was aided by convection currents resulting from central heating during the winter period.

Transfer was much slower in a fully air-conditioned hospital (Lidwell, 1979). Tracer particles (potassium iodide, settling velocity about 1.5 mm/s) took 1 h or more to move from one room to another on the same floor and losses by sedimentation were up to 95%. Transfers between adjacent rooms did not exceed 10^{-2} s/m^3 and between remote rooms, 100 m apart on the same corridor, they did not exceed 10^{-6} s/m^3. Fungi were retained effectively by the filters but the large number of *Clostridium welchii* indoors was taken to indicate human sources within the building even though they were numerous outside.

HEALTH EFFECTS

BUILDING-RELATED DISEASES

Illnesses associated with buildings have been classified as sick building syndrome or building-related diseases. Sick building syndrome is generally limited to those conditions thought to have a psychological or chemical basis and generally excludes those with a microbiological basis which are referred to as building-related diseases. Inhalation of airborne microorganisms and their metabolites may cause a range of respiratory symptoms depending on the species, the circumstances of exposure and the immunological reactivity of the subject. Some are specific diseases with causes that can be identified through immunological or microbiological tests and some are recognized syndromes but with no identified cause. Others are non-specific reactions to components of the airborne dust and still others are poorly defined, e.g. chronic fatigue syndrome and increased incidence of cough, phlegm, wheeze and wheeze with dyspnoea in the presence of dampness and mould (Dales *et al.,* 1991a, 1991b), so that it is still not known whether there is a specific microbial cause. Exposure is rarely to a single organism and may be to

a range of organisms known to have different effects on the respiratory system, from plant materials such as tannins that can contribute to inflammation to microorganisms that may induce immunological responses and to metabolites such as mycotoxins that may be immunotoxic. It is thus hardly surprising that elucidation of the causes of building-related diseases and occupational lung diseases can be a complex process of elimination and supposition. Some of the components of building-related diseases are described below.

Mucous membrane irritation

The symptoms of mucous membrane irritation that are most commonly observed in workers exposed to airborne organic dusts are a dry cough, eye irritation, and irritation in the throat and nose (Rosenhall, 1990). It may be an irritant effect not involving immune responses or mediators (Richerson, 1990) and is considered to represent a response to continuous chronic exposure to organic dust, perhaps containing endotoxin, in contrast to organic dust toxic syndrome where exposure is intense and often brief. Symptoms suggestive of mucous membrane irritation have been described among about 25% of 44 garbage handlers (Sigsgaard, in press).

Bronchitis and chronic pulmonary disease

Bronchitis is typically associated with smoking but there is evidence that atopy is a predisposing factor and that it has an additive effect with smoking (Terho *et al.*, 1987; Rosenhall, 1990). The prevalence of bronchitis in atopic subjects exposed to agricultural dusts was increased two to three times over that in nonatopic while smoking caused a further similar increase. This suggests a possible allergic component in bronchitis but the role of airborne microorganisms in the disease has not been determined and the reaction is perhaps a non-specific reaction or is associated with endotoxins.

Allergic rhinitis and asthma

Allergic rhinitis and asthma occur in subjects who are constitutionally predisposed to allergy (atopic), i.e. are readily sensitized by normal, everyday exposure to airborne allergens. About 20–30% of the population are atopic and react to one or more environmental allergens, including not only fungal spores but also pollens, house-dust mites, animal furs and other materials. Occupational exposure to allergens often far exceeds what is usually considered normal, everyday exposure (i.e. that usual out of doors), but its incidence is variable, accounting for 5–15% of all asthma (Butcher and Salvaggio, 1986). Symptoms occur rapidly on exposure to the allergen with rhinitis, wheeze and asthma caused by ventilatory obstruction but there are normally no systemic symptoms. Specific IgE antibodies are formed and skin tests with relevant allergens produce rapid wheal and flare reactions. A late asthmatic response after several hours may also sometimes occur.

Extrinsic allergic alveolitis (hypersensitivity pneumonitis)

Extrinsic allergic alveolitis is a T-lymphocyte-dependent granulomatous inflammatory reaction, predominantly of the peripheral gas exchange tissue of the lung that does not depend to any great extent on constitutional predisposition (Newman Taylor, 1987). It is caused by a range of dusts containing both fungal and actinomycete spores as well as other materials, including avian and animal serum proteins. It is characteristically an occupational disease and the names given to the different forms often reflect the environment in which it occurs or the source of the antigen. Allergic alveolitis is usually associated with repeated exposure to large concentrations of spores, mostly 1–5 μm in diameter, and typically exceeding 10^6 spores/m^3 air although a minimum concentration of 10^8 may be necessary for sensitization (Rylander, 1986). Symptoms may also develop insidiously to give chronic allergic alveolitis, without previous acute symptoms, perhaps as a consequence of prolonged exposure to relatively small spore concentrations.

Acute symptoms of allergic alveolitis develop several hours after exposure to the offending dust and include chills, fever, a dry cough, malaise and, with repeated exposure, increasing breathlessness, weight loss and, eventually, permanent lung damage. Diagnosis is based on a history of exposure to the relevant antigen, clinical, radiographic and functional changes typical of the disease and the presence of precipitating IgG antibodies (precipitins) to the causal antigen. Other evidence may be provided by the presence of basal crepitant rales, impairment of pulmonary diffusing capacity, decreased arterial oxygen tension, a restrictive ventilation defect, increased lymphocytes and granulomatous infiltrations and by reproducing the disease by inhalation challenge with a relevant antigen (doPico, 1986). Precipitins are indicative of exposure to antigen but their relevance to the disease has to be proved.

A wide range of microorganisms has been implicated in different forms of allergic alveolitis (Lacey and Crook, 1988). The classic form of the disease is farmer's lung in which the thermophilic actinomycete *Saccharopolyspora rectivirgula (Faenia rectivirgula, Micropolyspora faeni)* is the principal source of antigen in the UK (Pepys *et al.*, 1963) and USA (Kurup *et al.*, 1987) although *Thermoactinomyces* spp. and *Saccharomonospora viridis* have also been implicated. With different drying practices in Finland, heating of the hay is decreased and antibody titres, which reflect exposure, are greatest to the two *Thermoactinomyces* species, *T. vulgaris* and *T. thalpophilus* (Kurup *et al.*, 1987) with little difference in the frequency of positive reactions to the two species, contrasting with earlier studies in which antibodies to *T. vulgaris* and *Eurotium umbrosum* predominated (Terho and Lacey, 1979). Kotimaa *et al.* (1984) have now found that *T. vulgaris, S. rectivirgula* and *Eurotium umbrosum* are more abundant on farms with farmer's lung patients than on those without. The roles of the different white *Thermoactinomyces* species have frequently been confused because of difficulties in their identification and taxonomy. The most frequent species in mouldy hay are *T. vulgaris* and *T. thalpophilus* but these have, at different times, been grouped in one species, also known as *T. vulgaris,* and at other times have been given different names with *T. vulgaris* being used for cultures of both species and *T. candidus* and *T. thalpophilus,* respectively being used for the other. However, priority of *T. vulgaris* over *T. candidus* has now been established and the status of *T. thalpophilus* confirmed (Lacey and Cross, 1989).

Mushroom worker's lung is another form of allergic alveolitis and although the causal agent has not been identified, thermophilic actinomycetes, especially *Thermomonospora* sp., are again abundant in the air at spawning (Lacey *et al.*, 1972). *Thermomonospora* sp. and *S. rectivirgula* are both present in domestic waste compost. In Sweden, about 50% of woodtrimmers have precipitins indicating exposure mostly to *Rhizopus*, and 10–20% were suffering from allergic alveolitis (Belin, 1987). However, the implication of fungi in allergic alveolitis of woodworkers is controversial since late asthmatic responses have been found in patients to wood dust alone (Pickering *et al.*, 1972) although Belin (1980, 1987) and Maier *et al.* (1981) consider that such symptoms can be caused by moulds. It is possible that many cases described as allergic alveolitis in the past are now best regarded as organic dust toxic syndrome or inhalation fever. Humidifier fever has been described as a form of extrinsic allergic alveolitis but part, at least, may be attributable to endotoxin exposure or regarded as an inhalation fever. The cause is ill-defined and it has been associated with a wide range of microorganisms, including bacteria, actinomycetes, fungi, amoebae and their products. Part of the sick building syndrome may also fall within this complex.

Inhalation fever

Inhalation fever has been described as a general term to include organic dust toxic syndrome (ODTS), silo unloader's disease or pulmonary mycotoxicosis and reactions to inhalation of many other substances (Rask-Andersen and Pratt, 1992). ODTS shares some features of allergic alveolitis in that intense exposure to airborne dust is necessary and results in influenza-like symptoms with leukocytosis and fever, but without prior sensitization or the formation of antibodies. Respiratory symptoms may or may not occur but there are usually no radiographic changes. Also, many workers exposed at one time may be affected. Fungi, bacteria, mycotoxins and endotoxins have all been suggested as possible causes (doPico, 1986). There is no evidence of the involvement of mycotoxins but endotoxins from Gram-negative bacteria are known to produce febrile reactions on inhalation. However, neither the aerobiology of environments in which this syndrome has occurred nor the roles of the different microorganisms have been defined.

Nine cases of occupational lung disease, one diagnosed as bronchitis and eight as atypical bronchial asthma, with organic dust toxic syndrome (ODTS) suspected in three and allergic alveolitis also in one, have been reported in a group of only 15 workers at a Danish waste sorting plant (Sigsgaard *et al.*, 1990). Symptoms suggestive of mucous membrane irritation were described among 25% of 44 garbage handlers and ODTS in six, more frequently in atopic subjects. In a British study, 28% of a group of 134 workers in waste transfer stations complained of cough and 29% of phlegm on most days or more than 3 months of the year, 13% of frequent diarrhoea, 6% of frequent chills and 6% of frequent fever and sweating which could suggest ODTS (Crook *et al.*, 1991b).

Endotoxins

Endotoxins are lipopolysaccharide components of the cell of Gramnegative bacteria which have been implicated in byssinosis, the classic occupational respiratory disease of cotton, flax and hemp workers. The toxic effect is due to the lipid A portion of the

molecule (Helander *et al.*, 1980). Inhalation of endotoxins results in a fever and there may also be local tissue, cellular and functional damage in the lung (Glysson *et al.*, 1974). Maximum acceptable levels of 10^3 Gram-negative bacteria and/or 0.1 µg endotoxin/m^3 air have been suggested (Rylander *et al.*, 1978). In printing works, there were up to 3.1×10^3 Gram-negative bacteria and 0.39 µg endotoxin/m^3 associated with an outbreak of humidifier fever (Rylander and Haglind, 1984) and up to 0.99 µg/m^3 in a garbage sorting plant where organic dust toxic syndrome had occurred in several workers (Sigsgaard *et al.*, 1990). Up to 2.5 µg endotoxin/m^3 air have been found in textile mills (Rylander and Morey, 1982) and a maximum of 5.4 µg/m3 and an average of 1.1 µg/m^3 across five farms while unloading silage (Olenchock *et al.*, 1987). Over 90% of the endotoxin on farms was carried by particles >5.8 µm in aerodynamic diameter. Similarly, Gram-negative bacteria in waste transfer sites in the UK exceeded $10^3/m^3$ air in 41% of air samples (Crook *et al.*, 1987b). Until recently, endotoxin assays, using the *Limulus* amoebocyte assay, could overestimate endotoxin concentrations if glucans from fungal cell walls were present in the sample (Morita *et al.*, 1981). However, a new assay that does not interact with glucan is now available, giving the possibility of assaying glucan levels by difference between the traditional and new tests (Endo *et al.*, 1990).

Glucans

(1→ 3)-β-D Glucans are constituents of fungal cell walls which, it is now recognized, can interfere with endotoxin assays. They are described as potent stimulators of immune complement cells and may induce inflammation at low environmental concentrations. A dose-response relationship has been found between glucan concentrations in 'sick buildings' and mucous membrane irritation, headache and chest tightness (Rylander *et al.*, 1992).

Mycotoxins and volatile metabolites

Mycotoxins are poisonous secondary metabolites from fungi, chiefly species of *Fusarium, Penicillium* and *Aspergillus.* There is evidence that aflatoxin can become airborne in spores and circumstantial evidence that other toxins may also occur in the air. Their toxicity by inhalation can be up to 40 times greater than by ingestion (Creasia *et al.*, 1987). Ingested aflatoxins may cause liver and other cancers while other mycotoxins can affect other organs or interfere with the body's defences against infections through immune suppression or cytotoxic effects on alveolar macrophages (Gerberick *et al.*, 1984; Pier and McLoughlin, 1985; Sharma, 1991).

Groundnuts and maize colonized by *Aspergillus flavus* and *A. parasiticus*, have been shown to disperse spores containing aflatoxins at harvest and when they are handled in store in the USA. Airborne dust from the maize contained up to 206 ng aflatoxins/g giving concentrations up to 107 ng aflatoxins/m^3 air, with 5–17% of particles smaller than 7µm (Burg *et al.*, 1981, 1982; Burg and Shotwell, 1984). Similarly, dust from groundnuts contained 250–400 ng aflatoxin/g, with more than 8% of particles smaller than 5 µm. With these concentrations, baggers could inhale 0.04–2.5 µg aflatoxins in a 45 h week, giving an increased risk of lung and other cancers (Van Nieuwenhuize *et al.*, 1973; Deger, 1976; Alavanja *et al.*, 1987; Dvořáčková, 1990). Secalonic acid D has been

detected in dust deposits in maize elevators and presumably could have been airborne (Ehrlich *et al.*, 1982). Fungal colonization of wood chips may also result in respiratory symptoms among workers, in which *A. fumigatus* has been implicated, with tremorgenic mycotoxins perhaps playing a role (Land *et al.*, 1987).

Mycotoxins may also have a role in illnesses, including leukemia, associated with mouldy buildings (Samson, 1985). Macrocyclic trichothecenes from *Stachybotrys atra* were considered responsible for chronic problems in one household (Croft *et al.*, 1986). The toxins were isolated from material within the house and removal of contaminated duct work, insulation and building materials cured the illness. Satratoxin H and other toxins were identified in aerosolized spores of an isolate of *S. atra* which had a mass median diameter of about 5 μm, allowing deep penetration into the lung (Sorenson *et al.*, 1987).

Volatile metabolites produced by fungi are responsible for the musty odours found in mouldy buildings and a range of symptoms have been ascribed to their inhalation (Samson, 1985; Health and Welfare Canada, 1987). These include headache, eye, nose and throat irritation, nausea and fatigue. Predominant among the volatiles produced by a range of fungi was 1-octen-3-ol, but ethyl acetate, ethanol, butanol and other short-chain alcohols and aldehydes can also be produced. The earthy odour often reported is due to 2-octen-1-ol and geosmin, the latter a product also of some actinomycetes. Chronic fatigue syndrome has been ascribed to mycotoxins but its pattern of occurrence also suggests a possible role for volatile metabolites (Auger *et al.*, 1994).

Infection

Respiratory infection by fungi is most commonly associated with *Aspergillus fumigatus* but the nature of the disease that it produces depends, like allergy, on the immunological status of the individual. In atopic subjects, mucus plugs in the airways are commonly colonized saprophytically, causing allergic bronchopulmonary aspergillosis. In others, the fungus may grow in cavities, e.g. those resulting from tuberculosis, producing a fungus ball or aspergilloma. Invasive disease occurs when the immunological defences break down as a result of underlying disease, immunosuppressant drugs or radiation therapy. The fungus is widespread in nature so that inoculum is always readily available. Bacteria may also cause infection where they originate from fecal contamination of waste.

AIR QUALITY STANDARDS

There are many problems to setting standards for air quality in buildings: not all organisms are equally allergenic or harmful to health; constitutional predisposition to allergy in the exposed person may be more important than the concentration of spores to which they are exposed; numbers out of doors frequently exceed those indoors although the species differ; occurrence of specific microorganisms indoors may indicate a constructional problem but not necessarily a health hazard; the hazards of some microbial products are, at present, little understood.

Solomon (1990) has discussed the factors to be considered in making risk assessments of airborne microbial allergens but the principles can be applied to all airborne microbial hazards. There is a need to define more clearly the components and extents of the

microbial burdens to identify outlier conditions, to assess dose responses for sensitization and health effects, to define early markers of illness and to define better the symptoms and causes of different clinical entities. Nevertheless, there are situations that are clearly undesirable. Mycotoxigenic fungi may present a high risk not only because of their toxicity but also because the toxins may be carcinogenic or they may affect the immune response of the lung to other inhaled organisms, leading to diseases apparently unrelated to the fungi. *Aspergillus fumigatus* and *Nocardia asteroides* are particular hazards in the hospital environment where there are concentrations of immunocompromised patients. *Legionella pneumophila* is also undesirable in even small numbers because of its ability to cause pneumonic disease, although the attack rate is low and underlying disease may also have a role in its occurrence. Very large numbers of airborne microorganisms ($>10^6$–10^8 cfu/m^3) also present a clear hazard of allergic alveolitis and organic dust toxic syndrome while 1000 cfu Gramnegative bacteria or >0.1 μg endotoxin/m^3 may present a risk of health effects to exposed persons. However, in other situations, the risk is less clear in the absence of dose-response data and information on the atopic status of the exposed population.

There have been several attempts to set guidelines and standards for indoor air quality with respect to microorganisms in order to prevent respiratory hazards. Some of these are listed in Table 5.2. Some suggestions clearly are on the side of caution while others are more liberal. Some may be useful to indicate dampness problems in buildings in some countries or seasons, for instance, in dry climates or when the ground is snow-covered and there are few spores in the outdoor air, while others would be unattainable in more humid or tropical environments. Until the etiology for conditions, such as humidifier fever and other buildingrelated illness, have been fully elucidated it is difficult to set standards for particular groups of organisms that truly relate to the health problems. Consequently, ACGIH has recently recommended the use of rankorder comparisons to show differences and similarities between indoors and outdoors, to indicate the source of contamination, rather than a specified threshold concentration (Burge *et al.*, 1989). A multidisciplinary, multifactorial approach is necessary to establish the species of bacteria, actinomycetes, fungi, arthropods, protozoa and other organisms both viable and non-viable that are present in particular environments, their metabolites and other constituents that might affect health, and their levels in the presence of disease outbreaks and in their absence. If the mysteries of building-related illness are to be unravelled, these determinations need to be part of an integrated approach to the cause of the disease, involving bacteriologists, mycologists, entomologists, acarologists, aerobiologists, mycotoxicologists, industrial hygienists, immunologists, clinical practitioners and statisticians.

Table 5.2 Guidelines for indoor air quality standards for microorganisms

Environment	Concentration (/m^3)	Description	Reference
General	3000 cfu *Cladosporium* 100 cfu *Alternaria*	Threshold for evoking allergic symptoms	Gravesen, 1979
Home	>1700 cfu microorganisms	Seldom exceeded in homes without problems	Wright *et al.*, 1969
Homes	5000 cfu fungi	Level exceeded most often	Hunter *et al.*,

		when surface mould	1988
Homes	4500 cfu bacteria (all year) 500 cfu fungi (winter only)	Highest normal levels in Finnish homes (not farms)	Reponen *et al.*, 1990
Homes	>50 cfu fungi	Of concern if only one species	Miller *et al.*, 1988
	<150 cfu fungi	Acceptable if mixture of species	
	<300 cfu fungi	Acceptable if mainly *Cladosporium* and other outdoor types	
Homes, offices	500 cfu fungi	Indoor source indicated if species frequency differs from outdoors	Reynolds *et al.*, 1990
Offices	1775 cfu bacteria	Need for investigation or improvement	Bourdillon *et al.*, 1948
Office	3000 cfu *Flavobacterium*	From contaminated humidifier	Rylander *et al.*, 1978
Office	5000–10000 cfu fungi	Associated with humidifier fever	Bernstein *et al.*, 1983
Offices	1000 cfu total fungi	Need for investigation or improvement	Morey, 1984
Offices	10000 cfu total fungi 500 cfu of one species from indoor source	Need for investigation or improvement	Morey *et al.*, 1986
Occupational environments	One third of outdoor level, with same species spectrum	Recommended indoor limit	Burge *et al.*, 1987
Occupational environments	1000 cfu Gram-negative bacteria 0.1 µg endotoxin	Levels at which symptoms are expected	Rylander *et al.*, 1983
Farms, factories	>1000000 spores of fungi or actinomycetes	Environments where allergic alveolitis occurs	Lacey *et al.*, 1972
Farms, factories	>100000000 cfu of fungi or actinomycetes	Required for sensitization in allergic alveolitis	Rylander, 1986

cfu, colony forming units.

CONTROL

The most reliable method of decreasing numbers of airborne spores is to control them at source by preventing mould growth or by using mould-free materials. Mould growth must be prevented by curing leaks or other underlying damp or condensation problems in the buildings by repairing the structure and improving ventilation, insulation, humidity control and heating. If these fail, fungicidal paints may need to be used. Ventilation should control the relative humidity in the building to less than 70% if conditions are to be made unsatisfactory for mould growth and it should also provide 600 or 150 l outdoor air/min, respectively, per smoking or non-smoking occupant (Morey *et al.*, 1986b). All mouldy carpeting or other furnishings must be removed. Where stored products are processed, they should be mould-free and have dried to less than 0.65 a_w before storage and then kept dry. With biotechnological processes, aerosol formation from leaks in the system or when handling the product must be prevented. If moulding and aerosols cannot

be avoided, exhaust ventilation with filtration must be applied as close as possible to the point of release to prevent dispersal throughout the building.

Air-conditioning systems need to be designed so that fresh air intakes are sited well away from exhausts and from cooling towers. Once installed, they must be maintained in scrupulously clean condition. Humidifier reservoirs, any areas with stagnant water and baffle plates need to be cleaned and disinfected frequently and filters changed regularly, especially if there is evidence of fungal colonization. Water spray humidifiers should be replaced by steam injection systems where possible (Morey *et al.,* 1986b). Filtration of intake air before circulation through roll, bag or electrostatic filters, successfully decreased counts of *Aspergillus fumigatus* within a hospital and decreased nosocomial infections (Rose and Hirsch, 1979; Streifel *et al.,* 1989). Filtration is also necessary downstream of any aerosol source before discharge of air into the office environment (Morey *et al.,* 1986b). If preventive measures fail, personal exposure may be limited by suitable respiratory protection efficient against spores down to 1 μm diameter, but this should be considered as a last resort (Lacey *et al.,* 1982).

SUMMARY

Airborne microorganisms are almost ubiquitous. The composition of this microflora depends on its source, whether from the structure or superficial colonization of the building, from humidifiers or from materials being handled, the conditions for microbial growth and the amounts of activity and ventilation. Many of the problems are not new but are achieving new prominence because of demands for better standards of living and as consequences of the building of bigger, more fully air-conditioned buildings with insufficient thought given to the design of ventilation systems. Building-related illness may result from specific, identifiable syndromes related to the immune status of the individual, e.g. asthma, or to the nature of the exposure, e.g. alveolitis, but may otherwise appear to be a non-specific reaction to the environment. Many studies of building environments have identified components of the airborne microflora but have still not unequivocally identified causes of building-related illness. The numbers and types of microorganisms in the air suggest that there are problems that need investigation but not necessarily that there is a disease problem unless allergic alveolitis and organic dust toxic syndrome is provoked. The identification of disease is likely to require multidisciplinary studies of all the factors that may be involved while their control and the prevention of new hazards requires close cooperation between biologists, architects and engineers.

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Fungal disfigurement of interior finishes

6

Olaf C.G.Adan and Robert A.Samson

INTRODUCTION

In past decades, standards for the quality of the indoor environment have been raised, more recently with a growing interest in respect of outdoor environmental effects. One may think of energy conservation measures and thermal comfort, but also of development of more ecological decorative finishes and building materials with improved durability.

Fungi play a signif icant role in the indoor environment and their implications range from health complaints, structural damage or disfigurement of building constructions, to legal consequences. Several studies are in progress to obtain quantitative data and to understand health complaints and legal aspects; the biodeterioration of interior surfaces in domestic dwellings, which usually is considered in direct relation to dampness problems caused by condensation, has been investigated extensively.

To determine causes of fungal growth in dwellings, several aspects have to be taken into consideration. One approach is to study the indoor 'climate', including temperature and ventilation, water vapour emission (and airborne transport) as a consequence of domestic activities, and to a lesser extent effects of design. Occupancy behaviour is part of most of these factors and is probably highlighted whenever the causes in a particular case are not quite clear. The impact of planning and design on risks of indoor fungal growth is revealed from statistical analyses of post-occupancy surveys in Israel (Becker, 1984), the UK, Belgium, Germany and Italy (International Energy Agency, 1991), showing that location and orientation of dwellings and occupancy density (occupants-volume ratio) clearly affect fungal problems, which is in line with theoretically anticipated trends.

Another approach is to study the quality of the building envelope,

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particularly focused on thermal bridges and air tightness. The International Energy Agency (1990) recommends minimum requirements for a so-called temperature ratio, related to an overall thermal resistance. Furthermore, in the new Dutch Building Regulations, a minimum temperature ratio is explicitly demanded to minimize risks for

fungal growth. The great interest attached to thermal bridges probably originates from the common assumption that surface condensation is a vital requirement for fungal growth. The ability of fungi to grow on interior surfaces at relative humidities below saturation has long been known (e.g. Coppock and Cookson, 1951; Block, 1953). Moreover, starting from isopleths of common indoor fungi on agar media, some simple theoretical considerations taking both temperature and air humidity into account seem to indicate that risks for fungal growth in this range should not be judged on the basis of temperature ratio alone (Adan, 1991b).

The role of material characteristics of interior finishes (nutrition and water supply) in fungal disfigurement has received only minor attention in building physics, although the impact on indoor fungal growth has been pointed out by several researchers (e.g. Morgenstern, 1982; Becker, 1984; Becker *et al.*, 1986; Grant *et al.*, 1989; Adan, 1990). In this chapter the characteristics of some modern interior decorative finishes are described.

Fungal growth on decorative material is mostly considered as a superficial phenomenon, but our knowledge of fungal attachment to the material and the different parameters involved prior to and during fungal development is only limited. To understand the interaction of fungi and finishes some recent studies using scanning electron microscopy have been performed. These are reviewed in this chapter.

Testing fungal resistance is generally limited to the decorative or outer finish. The effect of sublayers is often ignored, although it is doubtful whether this is justified in building practice. In this chapter, fungal resistance experiments of multilayered finishes are compared to fungal resistance tests for constituent materials separately. Several methods for testing fungal resistance of manufactured building materials exist. These tests are described and their implementation for domestic environments is discussed.

CHARACTERISTICS OF INTERIOR FINISHES

Interior finishes can be considered multilayered systems, consisting of one or several sublayers on the building fabric and a decorative outer layer. Furthermore, sometimes additional treatment of building constructions is applied, such as application of water repellants or consolidants, which may affect all constituent finishing materials.

PLASTERS

The sublayers of decorating material mostly consist of plasters or renders, which primarily have a levelling function preceding decorative finishing of the construction. Some plasters are also suitable for decorative purposes. Plasters may be classified in three categories:

1. Mineral plasters, based on lime gypsum or lime-cement mixtures. Hardening is a result of both hydration and carbonation; due to the presence of calcium hydrate in both cases the plaster initially is alkaline. Due to carbonation, surface alkalinity, however, is quickly reduced to values near neutral. Today mineral plasters often contain small quantities of synthetic resins to improve mechanical properties.

2. Plasters based on synthetic resins, often containing lime or gypsum additives. The hardening of these plasters is based on physical drying. Lime additives may cause discolouration (yellowing), which sometimes is confused with microbiological attack.
3. Gypsum plasters. Because hardening of these materials is based on very fast hydration (≈30 min), i.e. formation of dihydrate, retarders (e.g. tartaric acid) are used to improve applicability. Besides, several other additives are found in manufactured gypsum (Helff and Mosch, 1978; van Raalte, 1989): agents, based on cellulosic derivatives, which are commonly added to improve concrete and plaster adhesion, fine-grained sand, perlite to improve thermal properties and decrease specific weight, and hydrophilic additives (for water retention, i.e. to reduce drying rate). Elements such as some metals and phosphorus occur in minor quantities.

In the Netherlands, gypsum plasters are widely used in building practice. Application of the appropriate type of gypsum plaster is highly dependent on the building fabric. Additional treatment preceding plastering of the building fabric is sometimes required, e.g. application of water repellants in order to reduce water suction and improve hardening of the plaster.

Several material properties are important in fungal growth including the moisture properties of gypsum plasters that may be represented by sorption isotherms (equilibrium water content as related to relative humidity). Generally, gypsum plasters are highly porous, depending on the waterbinder ratio (Helff and Mosch, 1978); the micropores (diameter <0.1 μm) are, however, only a small fraction of the gypsum volume (Adan *et al.*, 1993c). Consequently, gypsum plasters usually have low water contents within the relative humidity range up to 98%, i.e. starting from values less than 1% (by mass) at 30% humidity and increasing to 2–3% (by mass) at

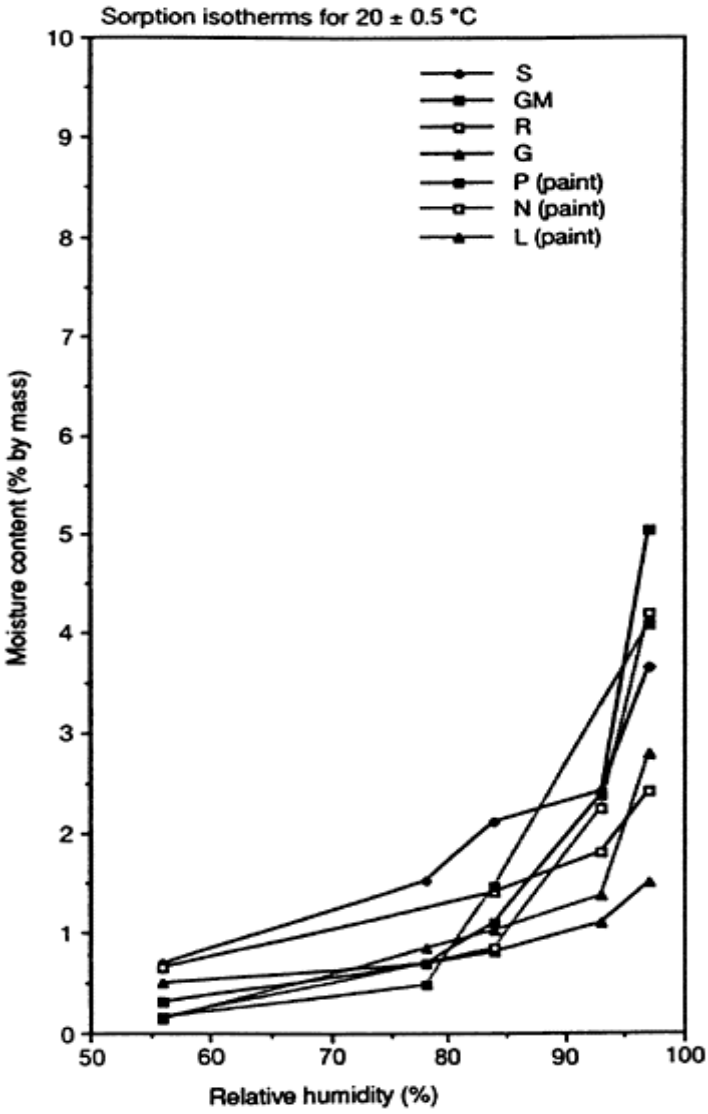

Figure 6.1 Sorption isotherms for common gypsum plasters and waterborne paints at an air temperature of 20±0.5°C (no desorption). S, common gypsum plaster, fast drying; GM, pure gypsum, with nutritive additives, used in experiments; R, common gypsum plaster, manually

handled, sublayer for decoration; G, common gypsum plaster, manually handled, may be used for decorative purposes; P, waterborne acrylic paint, porous (pigment volume concentration 60%), used in experiments; N, similar waterborne acrylic paint, virtually non-porous (pigment volume concentration 30%); L, common waterborne latex paint.

97% relative humidity (e.g. Kneule, 1964; Helff and Mosch, 1978). Figure 6.1 shows adsorption isotherms of some common Dutch gypsum plasters based on gravimetric measurement.

The surface roughness determines the interface area of fungi and substrate and may be important for adhesion of contaminants and spores. Table 6.1 summarizes values of surface roughness for common gypsum plasters, according to ISO Standards 2632/I-1977 and 2632/II-1977. Figures 6.2a and bdemonstrate morphology and texture of pure gypsum, whereas Figures 6.2c-f show gypsum plasters commonly encountered in Dutch dwellings. Obviously, additives cause a more granular texture (Figures 6.2e and f).

Table 6.1 Surface roughness for some common gypsum plasters and waterborne paints. Gypsum plasters have been prepared as prescribed

Sample	R_Z(μm)	σ(μm)	R_A(μm)	σ(μm)
MP	11	6	4	1
GM	10	3	3	1
R	16	5	4	1
G	16	6	5	1
P	4.7	–	0.9	–
N	3.9	–	0.6	–
L	6.8	–	1.3	–

This table includes two measures according to ISO Standards 2632/I-1977 and 2632/II1977:

$$R_A = \frac{1}{L}\int_0^L |\, h(x) \,|\, dx\,; \qquad R_Z = \frac{\sum_{5\ highest} h(x) + \sum_{5\ lowest} h(x)}{5}$$

where

L=traversing length along the surface, or scan length (μm)

h(x)=the deviation of the surface profile about the mean line (μm).

The surface roughness for gypsum plasters has been determined using a Hommeltester, a measuring device based on a piezo electric sensor, with resolution of 1 μm. For each specimen considered, 20 surface scans have been performed. Surface roughness for the waterborne paints has been determined using a confocal laser scanning microscope, with a lateral resolution of 0.27 (μm (Houpt and Draayer, 1989).

Nomenclature:
MP Common gypsum plaster, machine-made, used as sublayer for decoration.
GM Pure gypsum, with nutritive additives, used in experiments.
R Common gypsum plaster, manually handled, sublayer for decoration.
G Common gypsum plaster, manually handled, may be used for decorative purposes.
P Waterborne acrylic paint, porous (pigment volume concentration 60%), used in the experiments.
N Similar waterborne acrylic paint, virtually non-porous (pigment volume concentration 30%).
L Common waterborne latex paint.
σ Standard deviation (μm).

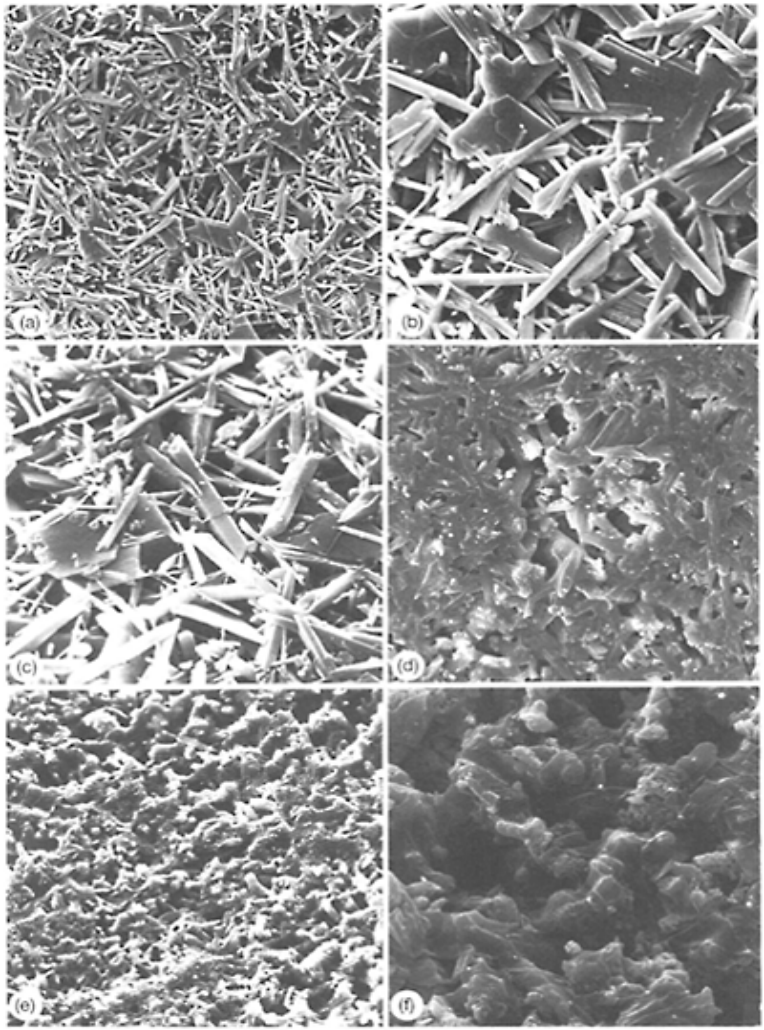

Figure 6.2 Morphology and texture of some gypsum plasters. Scanning

electron micrographs. (a) Pure gypsum (560×). (b) Pure gypsum (140×) with additional nutrients (GM in Table 6.1), used in experiments on p. 148. (c) Common gypsum plaster, machine handled, used as sublayer for decoration (1400×). (d) Common gypsum plaster, manually handled, may also be used as adhesive for gypsum building blocks (1400×). (e) Common gypsum plaster (R in Table 6.1), manually handled, sublayer for decoration (560×). (f) Common gypsum plaster (G in Table 6.1), manually handled, may be used for decorative purposes (1400×). Note the change in texture, from a crystalline towards a granular structure.

WALLPAPERS AND PAINTS

Interior surfaces can be finished with paint and/or wallpaper, ranging from woodchip to vinyl wallpapers. Wallpapers usually contain cellulosic compounds, which like the organic constituents of adhesives may supply nutrients for fungi. Paint constituents are distinguished into binders, solvents and emulsifiers, pigments, fillers and additives. Conventional solvent-borne paints often have a base of acrylic resins or the widely used alkyd resins, dissolved in organic solvents. The new waterborne paints are based on aqueous dispersions of synthetic vinyl-type binders, such as polyvinylacetate or polyvinylpropionate (copolymers, and acrylic polymers). The film formation of waterborne paints is quite different from that of solventborne paints (Bondy and Coleman, 1970). During film formation of waterborne paints, physical drying takes place; solventborne paints dry chemically, e.g. in the case of alkyd resin binder, oxidation occurs. As a consequence, porosity and pore-size distribution of both types may differ widely.

Pigments used in paints can be inorganic (e.g. white titanium dioxide) or coloured organic compounds. The impact of the pigment volume concentration (defined as the volumetric percentage of pigment present in the total solids of a paint system) on paint properties is well known. Asbeck and van Loo (1949) introduced the Critical Pigment Volume Concentration (CPVC) as a transition point in a pigment-binder system at which substantial differences in appearance and behaviour of the film will be encountered. For pigment volume concentrations below CPVC the paint is virtually non-porous, for values above CPVC, porosity increases with increasing pigment volume concentration.

Although binders used for waterborne paints, i.e. polyvinylacetate and acrylic polymers, are generally highly resistant to microbial attack, additives such as cellulosic thickeners may be counteracting (Ross, 1969; Becker *et al.*, 1986). Biocides are commonly added to prevent microbial spoilage.

In the interests of environmental care, use of solvent-thinned paints and toxic additives is being reduced (Stevens, 1991). Nevertheless, accelerated as well as natural weathering tests show that durability of waterborne paints needs to be improved (Vink, 1991). The sorption isotherms for some waterborne acrylic paints are illustrated in Figure 6.1. The curves show equilibrium moisture contents (by mass) for two pigment volume concentrations, as well as for a common latex coating. The pigment volume concentrations were chosen such that the acrylic paint film is virtually non-porous for the lowest value, and porous for the highest value. Waterborne paints generally are more hygroscopic than solventborne paints, which might be significant with respect to susceptibility to microbiological attack.

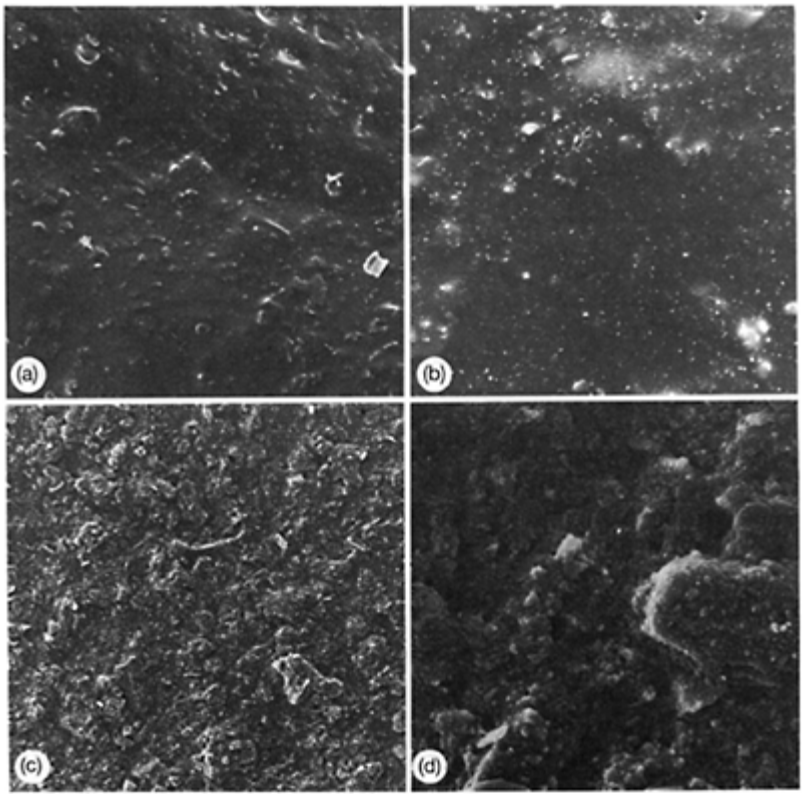

Figure 6.3 Texture of some waterborne acrylic paints. (a) 245× and (b) 1400×: acrylic paint, virtually non-

porous, pigment volume concentration 30%. (c) 245× and (d) 1400×: acrylic paint, porous, pigment volume concentration 60%.

In analogy with gypsum plasters, surface roughness has been determined for both the porous and non-porous paint (see Table 6.1). Micrographs of dried paint films in Figure 6.3 clearly show texture effects of an increased pigment volume concentration.

FUNGAL FLORA OF INDOOR ENVIRONMENTS

The fungal flora of indoor environments is generally represented by common species of the genera *Penicillium, Aspergillus* and *Cladosporium* (Beaumont, 1985; Fradkin *et al.*, 1987; Hunter *et al.*, 1988). These genera can play a significant role in allergies and other health complaints (Gravesen, 1978; Beaumont, 1985; Samson, 1985; Hunter *et al.*, 1988; Miller *et al.*, 1988). In both damp and relatively dry living and working environments, xerophilic species of the genera *Eurotium, Aspergillus* and *Wallemia* are regularly found and there are indications that these moulds also may be involved in health problems. Although the results of most studies were obtained from samples of airborne fungal propagules, species growing on surfaces of interior decorative material are identical or similar (Reenen-Hoekstra *et al.*, 1991). However, the fungal flora on surfaces may differ depending on relative humidity levels. In wet rooms such as bathrooms or kitchens, the fungal flora often consists of darkly pigmented hyphomycetes or yeasts. Common species occurring in such rooms on tiles, plaster or silicon caulking, are *Aureobasidium pullulans* and *Phoma exigua*, but also *Alternaria alternata, Aspergillus niger, Cladosporium herbarum, C. sphaerospermum, C. tenuissimum, Ulocladium* spp. and yeasts can be found. Tiefenbrunner (1990) reported *Penicillium, Fusarium, Trichoderma, Phoma* and *Cladosporium* as common genera in wet rooms, but found only low numbers of *Aspergillus* probably due to failure of isolating the species on appropriate media. Fungi, including *Acremonium strictum, Fusarium oxysporum, P. brevicompactum, P. chrysogenum* and *Ulocladium*, may even occur on wallpaint treated with fungicides.

Wallpaper may serve as a good substrate for various species: *Acremonium strictum, Aspergillus niger, A. versicolor, Cladosporium herbarum, C. sphaerospermum, Epicoccum nigrum, Fusarium solani, Gymnoascus* spp., *Penicillium aurantiogriseum, P.glabrum, P. citrinum, P. chrysogenum, P. coryophilum, Stachybotrys chartarum, Ulocladium* spp. and yeast species. True cellulolytic species, e.g. *Chaetomium* sp. may occur, but from our own experience these species are now rarely encountered except for *Stachybotrys atra.* The occurrence of *Scopulariopsis brevicaulis* on wallpaper, its tolerance of high pH, and production of the poisonous trimethylarsine has been reported in the literature and reviewed by Morton and Smith (1963). *S. brevicaulis* can be detected from airborne samples and dust samples, but has not been observed growing on wallpaper used in modern buildings.

Fungal contamination on plaster mostly occurs in rooms with high relative humidities. Sometimes the Discomycete *Pyronema domesticum* can develop on new plastered ceilings or walls and appears as a whitish to pink stain. This species is known as a pyrophilous fungus invad-ing natural fireplaces or steam-sterilized soil (Moore and Korf, 1963). During hydration of the plaster, its temperature rises and this can induce germination of the ascospores. On one occasion, *P. domesticum* was found in the Netherlands on the walls of a house destroyed by fire.

FUNGAL GROWTH ON MULTILAYERED INTERIOR FINISHES

CONSTRUCTIONAL MATERIALS

Several fungal growth experiments on building materials have been reported. The early work of Coppock and Cookson (1951) concerned decorated wood and various types of plain, white-washed and cementrendered brick. Except for red clay brick, all specimens tested showed slight to moderate growth within a 4-week period at relative humidities of 80–95% and 27°C temperature. They suggested that mould growth should be related to geometric properties of the substrate, i.e. porosity and possible pore-size distribution, although no proof could be deduced from their experiments.

PLASTERS

Manufactured plasters often contain organic additives and it is clear that these materials are susceptible to mould growth. Fungal growth on interior plasters has been examined by several researchers. Becker and Puterman (1987) found fungal growth on plain gypsum boards (within 5 days after inoculation) and on manufactured lime-cement plasters (within 9 days after inoculation), both at 97% relative humidity. Francis (1987) tested gypsum plasters in a 10-week period at relative humidities of 86, 92 and 97%. Only at 86% relative humidity did plasters remain virtually unaffected. In experiments of Herback (1990), traces of fungal growth occurred on manufactured gypsum after 5 weeks' incubation at 97% relative humidity.

Morgenstern (1982) studied the effects of polyvinylacetate (PVAC) additives of gypsum-lime plasters on the susceptibility to fungal growth. These additives are often applied to improve strength and elasticity. Experiments under conditions near saturation and at 23°C clearly demonstrated an accelerated and intensified fungal development as a consequence of PVAC addition. Initially, the alkalinity of the plaster inhibits fungal development. Owing to carbonation, pH alters towards more tolerable values. The experiments showed that PVAC additives nearly halved the time needed for entire carbonation. Besides, PVAC additives resulted in increased water content of the plaster. Both hydrolysis of PVAC and carbonation are related to this effect. Although polyvinyl acetate itself cannot be used as a carbon source by the species of fungi used in the tests, hydrolysis results in the formation of polyvinylalcohol and acetate, the latter being a possible nutrient.

DECORATIVE FINISHES

Fungal growth on finishing or decorating materials has received more attention, although it has been focused on exterior rather than on interior applications. Becker *et al.,* (1986) and Becker and Puterman (1987) studied the effect of paint porosity, as related to the pigment volume concentration (PVC), on fungal resistance of polyvinyl acetate and acrylic emulsion paints at 97% relative humidity. They concluded that the critical PVC seems to be a transition point, as fungal growth increased significantly for PVCs above this value. Grant *et al.,* (1989) studied the minimal water requirement for fungal growth on woodchip paper (either plain or emulsion painted), patterned wallpaper, vinyl wall covering and emulsion painted gypsum plaster. On emulsion painted woodchip paper, *Aspergillus versicolor* and *Penicillium chrysogenum* were able to grow at relative humidities down to 79% at 25°C. Furthermore, their study showed that the nature of the base substrate affected fungal growth on the coating, but there was no consistent pattern for all fungal species examined.

SOILING

To study effects of surface soiling with nutrient materials in the domestic situation, an artificial organic soiling is often applied (Grant *et al.,* 1989; Herback, 1990). Becker (1984) on the other hand, used samples which had been preconditioned for 6 months in domestic dwellings. Generally, the addition of an extra source of carbon, either natural or artificial, causes a reduction in minimum air humidities required for growth, or an increase in growth rate (Becker *et al.,* 1986; Grant *et al.,* 1989; Adan, 1990, 1991a; Herback, 1990).

INOCULATION

All researchers used wet inoculation by spraying an aqueous spore suspension of a mixture of fungal species, apart from Coppock and Cookson (1951), who inoculated samples by blowing a dry suspension of spores on to the surface by a gentle blast of sterile air, and Grant *et al.* (1989) who used dry camel hair brushes. Grant *et al.* (1989) reported successional colonization of papered surfaces in a case study. Consequently, they proposed a general colonization pattern, based on ranking of species according to minimum water requirements for growth.

FUNGAL GROWTH ON VARIOUS TYPES OF MATERIAL

Scanning electron microscopy (SEM) was used to obtain an overall picture of fungal growth on various types of interior finishing materials. Samples consisted of a nutritive gypsum sublayer decorated with an acrylic paint or a common wallpaper. The fungal species studied included *Penicillium chrysogenum, Aureobasidium pullulans* (which requires air humidities near saturation for growth and is typically found in bathrooms)

and *Eurotium herbariorum*, which is considered xerophilic. Aqueous spore suspensions were used f or inoculation. All samples were examined in air-dried form.

Figure 6.4 SEM micrographs showing abundant growth of *Penicillium chrysogenum* on a porous waterborne acrylic paint, base substrate gypsum, 3 weeks after inoculation at 97% relative air humidity. (a) 25×, (b) 70× and (c) 231×: cross-section of air-dried specimens, illustrating the superficial character of the phenomenon.

Figures 6.4a–c show abundant growth of *Penicillium chrysogenum* on a waterborne acrylic paint, 3 weeks after inoculation and incubation at 97±1% relative humidity and air temperature of 20±0.5°C Detailed examination of several cross-sections of different

samples showed no fungal penetration of the acrylic paint, which is consistent with observations for highly porous gypsum plasters (Adan, 1991a) and for a common latex coating in a case study of a Dutch dwelling (Adan, 1990). Furthermore, the dense biomass at the surface, measuring approximate-

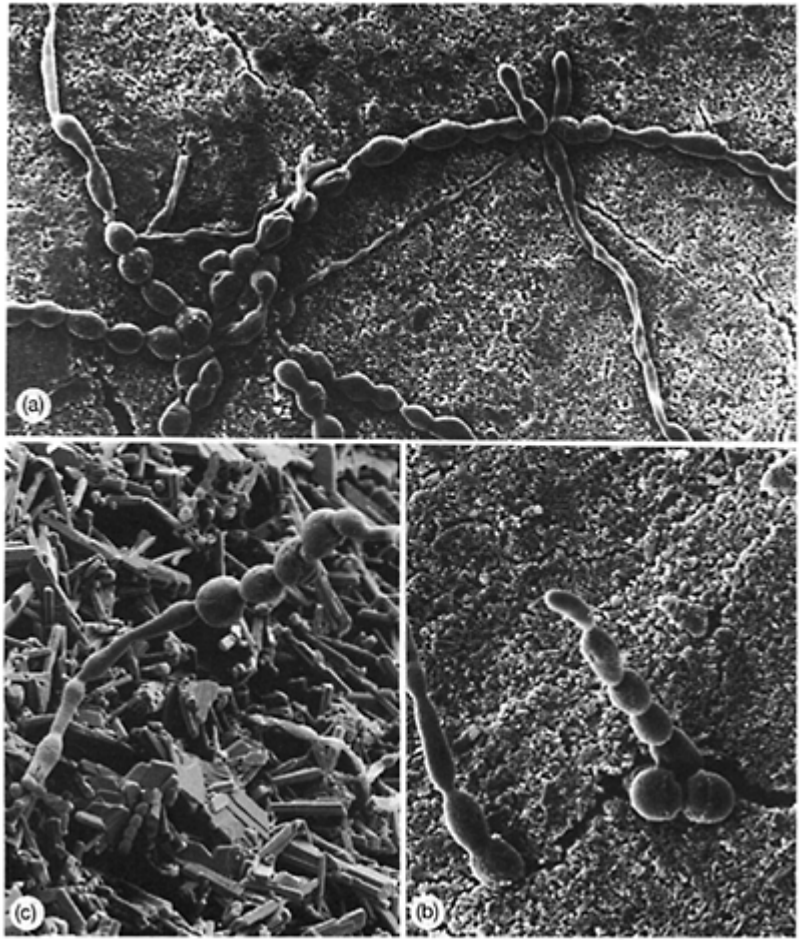

Figure 6.5 SEM micrographs showing filaments and hyphae formation of *Aureobasidium pullulans* on wallpaper ((a) 770× and (c) 1050×) and on gypsum ((b) 700×) air-dried, 3 weeks incubation at 97% relative humidity.

ly 0.2 mm in thickness, suggests effects on the surface water economy and microclimate conditions. Indeed, experiments of Waubke *et al.*, (1983) indicate that dense fungal

growth on building substrates influences both moistening and drying as a consequence of reduced vapour diffusion at the surface.

Figures 6.5a–c show *Aureobasidium pullulans*, on wallpaper (Figures 6.5a and b) and on gypsum (Figure 6.5c). Samples were incubated at 97±1% relative humidity and a temperature of 20±0.5°C and examined 3 weeks after inoculation. Formation of hyphae and filaments on wallpaper is observed in Figures 6.5a and b; Figure 6.5c shows *Aureobasidium pullulans* growing out of the gypsum substrate, and spore formation. Growth of the xerophilic species *Eurotium herbariorum* on a waterborne acrylic paint is demonstrated in Figures 6.6a–c. Conditions

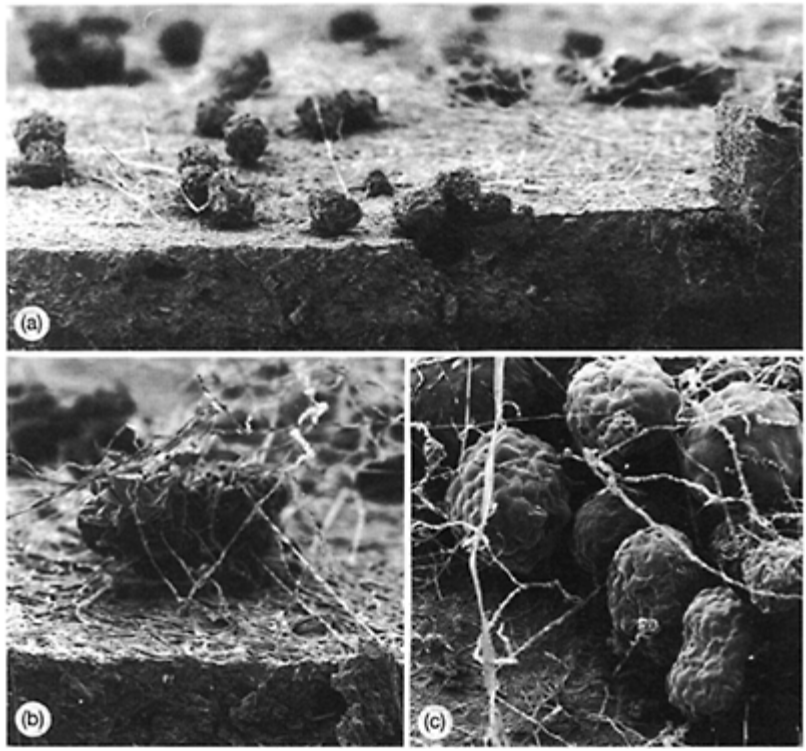

Figure 6.6 SEM micrographs of the xerophilic Ascomycete *Eurotium herbariorum* on a porous waterborne paint. (a) 105×, (b) 224× and (c) 154×: cleistothecium formation, scattered all over the surface. Specimens for SEM were air-dried, 3 weeks incubation at 86% relative humidity.

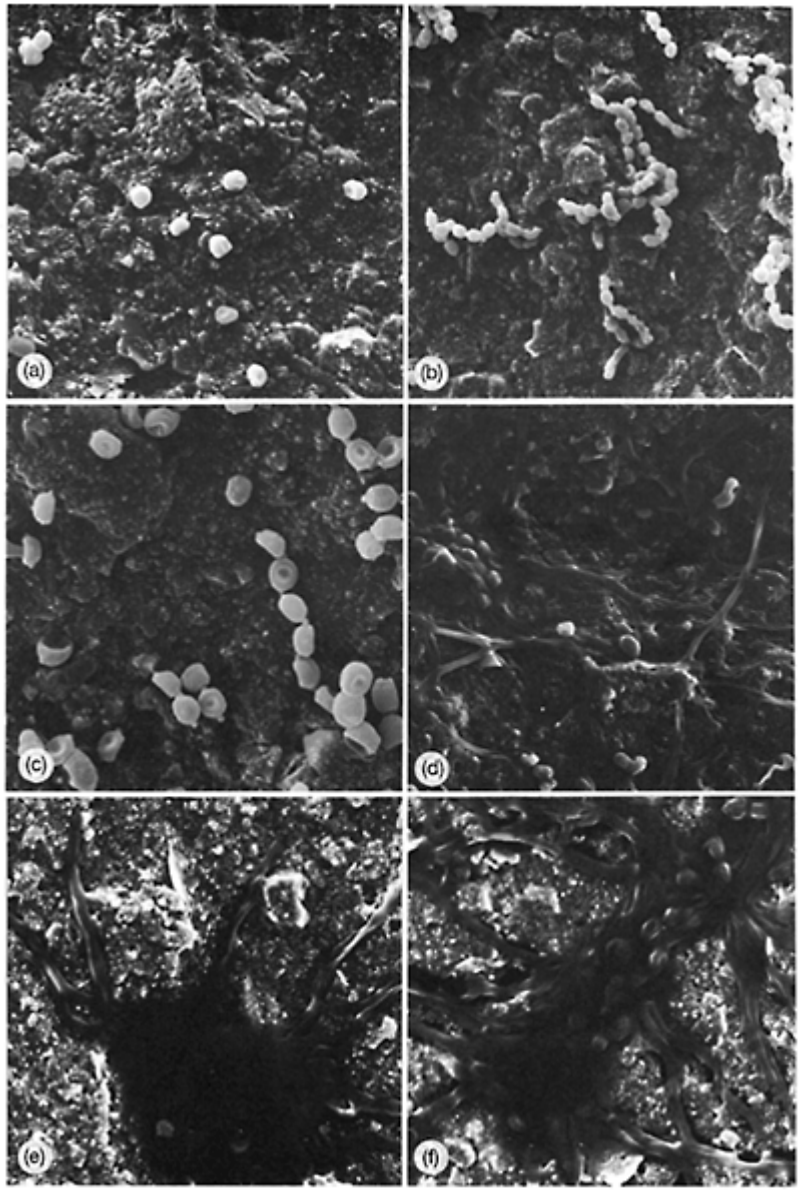

Figure 6.7 SEM micrographs of stages in growth of *Penicillium chrysogenum* on a porous waterborne acrylic paint, incubated at 97% relative humidity. Air-dried specimens. (a) 1120×: initial

situation; (b) 770× and (c) 1540×: swelling of conidia, initial germination, 18 hours incubation; (d) 770×: germination and hyphae formation, 42 hours incubation; (e) 770× and (f) 770×: continuing growth, spreading over the surface, 72 hours incubation. Note the dark spots indicating the presence of water.

during incubation were 86±1% relative humidity and 20±0.5°C temperature. Samples were examined 3 weeks after inoculation. The micrographs clearly show cleistothecium formation, not densely packed, but scattered all over the surface. The ripe fruiting bodies indicate optimal conditions for development.

DIFFERENT STAGES OF FUNGAL GROWTH ON MULTILAYERED SYSTEMS

Little is known about fungal growth and development on surfaces of interior decorative materials. Figures 6.7a–f show several stages in fungal growth in a period of 72 hours following inoculation. The substrate consists of a porous acrylic paint (pigment volume concentration 60%) on a nutritive gypsum sublayer; the sample was dried before exposition to a relative humidity of 97±1% at 20±0.5°C Figure 6.7a shows the initial situation in which the surface of the porous acrylic paint was inoculated with dry conidia of *Penicillium chrysogenum*, using a dry sterile cotton swab. Figures 6.7b and 6.7c show swelling of conidia and some initial germination after an 18 hour period. After 42 hours (Figure 6.7d) germination and hyphae formation are clearly noticeable. Continuing growth is demonstrated at 72 hours, showing fungi spreading over the surface, which elucidates the superficial character of the phenomenon. Scanning electron microscopy on air-dried specimens shows shrinkage of conidia and collapsing hyphae. Note the dark spots in Figures 6.7e and f, which indicate water attached to hyphae. See also comments on Figures 6.8 and 6.9.

Mycological specimens prepared and examined by low temperature or cryoscanning electron microscopy (LTSEM) exhibit superior preservation over those prepared by other conventional procedures (Samson *et al.,* 1990). Rapid cryofixation not only results in excellent specimen preservation: mycelium and propagules in the fully hydrated frozen state resemble the morphology of the natural state in terms of dimensions and texture, but also the presence of water can be observed. Therefore low temperature scanning electron microscopy was used in several subsequent studies of Adan *et al.,* (1993a) to examine fungal growth on the acrylic paints as well as on a plain gypsum substrate. Samples were directly prepared on SEM specimen holders and dried. They were subsequently inoculated with dry conidia of *Penicillium chrysogenum* and incubated at 97% relative humidity. The operational and experimental procedures used are described by van der Host *et al.,* (1994). Figures 6.8a–c and 6.9a–d depict fungal growth on acrylic paint and plain

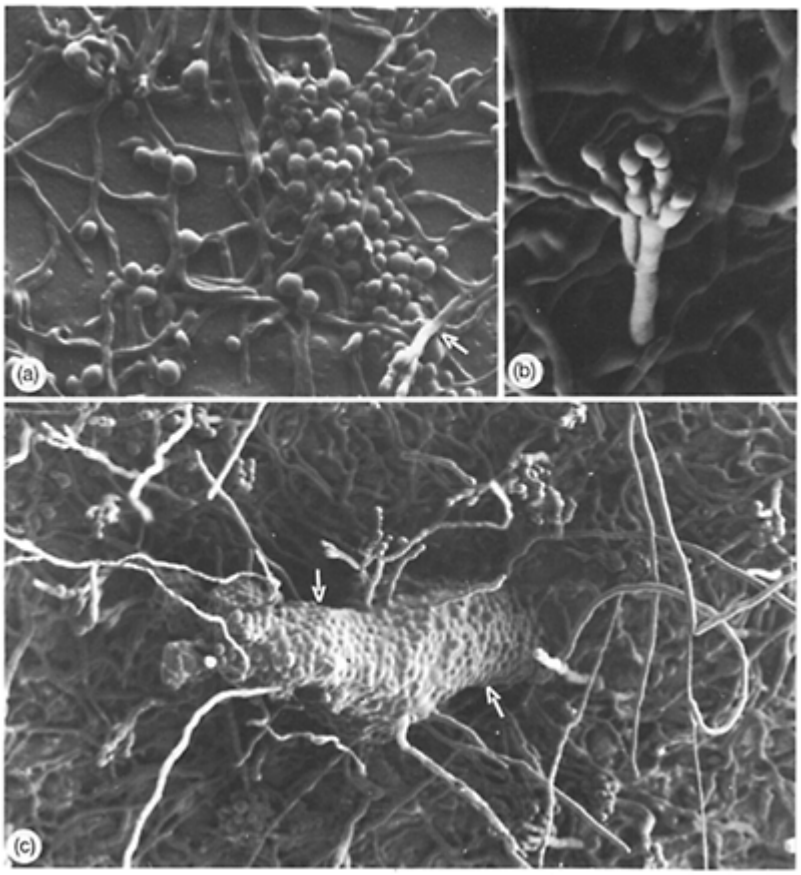

Figure 6.8 Low temperature scanning electron microscopy of *Penicillium chrysogenum* on a waterborne acrylic paint, incubated at 97% relative humidity and after 72 hours incubation. Dry conidia were inoculated by means of a sterile cotton swab. (a) 700× germination of conidia in clumps, development of hyphae and early stages of sporulation (see arrow); (b) 330× sporulating conidiophores; (c) 420× conglomerates of fungal structures and water (see arrows).

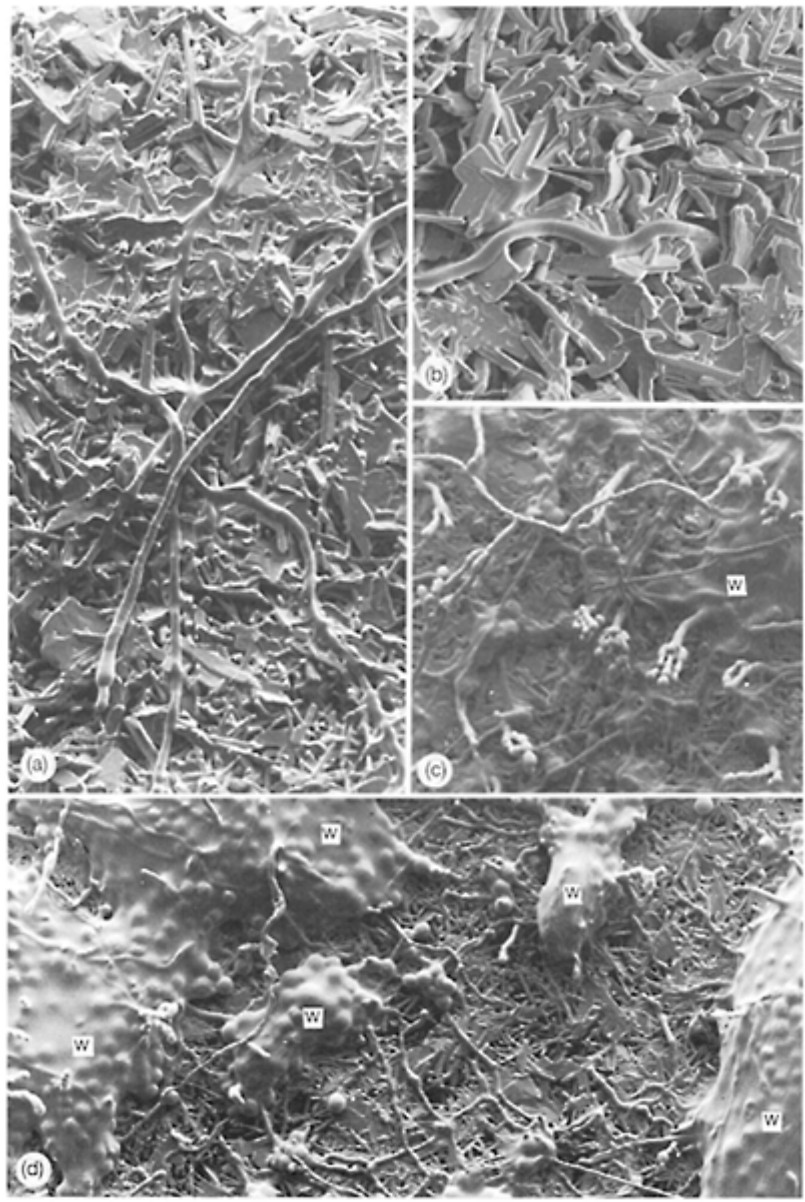

Figure 6.9 Low temperature scanning electron microscopy of *Penicillium chrysogenum* on gypsum, incubated at 97% relative humidity and after 72 hours incubation. Dry conidia were

inoculated by means of a sterile cotton swab. (a) 700× hyphae growing over the surface and (b) 1050×, penetrating or growing out of the gypsum; (c) and (d)—both 490×—swelling and germinating conidia, hyphae and sporulating conidiophores. Note the conglomerates of fungal structures in water (w).

gypsum, respectively, after a period of 72 hours incubation at room temperature. Both figures show several stages in fungal development. Figure 6.8a shows germination of conidia in clumps, development of hyphae and early stages of sporulation (arrow). Sporulating conidiophores are clearly shown in Figure 6.8b. Figure 6.8c reveals clustering of fungal structures and water in the centre of the picture. The presence of water is somewhat unexpected because dry conidia were inoculated using dry sterile cotton swabs. No water was added to the dried specimens before incubation in the 97% relative humidity environment or preceding preparation for observation in the LTSEM. In examination of air-dried specimens (Figure 6.7), collapsing hyphae introduce additional uncertainties.

Fungal growth is less abundant on the nutritive gypsum; nevertheless, in accordance with growth on acrylic paints, several stages of development are shown in the micrographs. Figure 6.9a shows hyphae growing over the surface. Note that the interface area of hyphae and the gypsum substrate is considerably smaller than the interface area of hyphae and paints. Although it was stated previously that fungal growth is a superficial phenomenon, highly porous media such as gypsum may still allow hyphae to penetrate the bulk structure. Figure 6.9b gives an example, although it is not clear whether the hyphae are penetrating into or growing out of the gypsum. Swelling and germinating conidia, hyphae and sporulating conidiophores are shown in Figures 6.9c and d. Furthermore, similar to Figure 6.8c, some coherent structures of conidia, hyphae and water are observed, from which conidiophores rise and hyphae seem to originate. The caption to Figure 6.8c is also applicable to these observations. A comprehensive picture of stages in growth of *Penicillium chrysogenum* on gypsum-based finishes is given in a subsequent LTSEM study of Adan *et al.,* (1994a). In their study, a further discussion of observations of coherent structures of fungi and water is included.

INFLUENCE OF THE BASE SUBSTRATE ON FUNGAL GROWTH

Effects of the base substrate on fungal growth on multilayered finishing systems are mostly not considered in fungal resistance tests of interior finishes. Some preliminary tests were carried out for interior finishes of common gypsum plasters, plain as well as coated with a range of decorating materials (Adan, 1991a). The material was inoculated with *Penicillium chrysogenum,* which appeared to be dominant in previous experiments (Adan, 1991a), using dry sterile cotton swabs. The extent of growth was assessed using a numerical scale, according to methods proposed by Adan *et al.,* (1994b), together with a

description of the density of growth. During the entire 12-week test period, specimens stayed in the incubators, which consisted essentially of a tank with transparent walls. The results summarized in Table 6.2 confirm earlier observations (Grant *et al.*, 1989; Adan, 1991a) that fungal growth rates on coatings may be clearly affected by the base substrate. Obviously these effects are noticed at both air humidities considered. Although previous SEM analyses suggest that fungal disfigurement should be considered a surface phenomenon, resistance to fungal attack in practice is dependent both on characteristics of the decorative layer and the base substrate. This seems to be a function of water in the hygroscopic range mediating the uptake of nutrients from sublayers.

Table 6.2 Growth of *Penicillium chrysogenum* on interior finishes

Sample	Relative humidity (%)	First signs visible	Time (weeks)			
			1	2	5	8
GZ	97	No growth	0	0	0	0
GM	97	<1	4–5S	5SM	5M–5MD	5MD–5D
GS	97	<2	0	1S	5S	5S
GZ/P	97	<1	0–2S	0–3S	3S–5SM	5S–5M
GM/P	97	<1	5M–5MD	5D	5V	5V
GZ	86	No growth	0	0	0	0
GM	86	<3	0	0	4–5S	5SM–5M
GS	86	<5	0	0	0–2S	4–5S
GZ/P	86	<4	0	0	1–3S	2–4S
GM/P	86	<4	0	0	1–3S	5SM–5M

The range of growth rates summarized in this table refers to six duplicates for each sample.
Growth rates have been assessed according to a numerical scale, including:
Coverage area: 0, no growth of 1, traces of growth, less than 1% of area; 2, 1–10% of area; 3, 10–30% of area; 4, 30–70% of area; 5, more than 70% of area. Density: S, sparse; M, medium; D, dense; V, very dense.
Nomenclature:
GZ Pure gypsum, based on calcium sulphate hemihydrate and sterile demineralized water.
GM As with GZ, with additional nutrients.
GS Common, manufactured gypsum
GZ/P Waterborne acrylic paint, porous (pvc = 60%), on pure gypsum sublayer (GZ).
GM/P as GZ/P, on nutritive gypsum (GM).

Complementary effects may occur at 97% relative humidity. Within the entire test period, coverage area on the paint is lower than coverage area on the plain nutritive gypsum, and both are significantly lower than on the nutritive gypsum coated with the acrylic paint, which exhibits explosive growth. This effect seems to be related to relative humidity, determining the water content of the paint, since at 86% relative humidity fungal growth on the coated nutritive gypsum seems inhibited compared to growth on the plain gypsum with nutritive additives. As the

Table 6.3 Outline of some fungal resistance tests

	BS 1982 (1968)		BS 3900 Part G6 (1989)	IEC 68–2 Part 2J (1988)	ASTM G21–70 (1975)	IIPEC 2.6.1 (1988)
Climate conditions	Test (b): 25±2°C, moistened soil	Test (c): 25±2°C, RH 95–100%	23±2°C. Intermittent surface condensation, switched on for 2 h, off for 10 h (RH near saturation)	20–30°C. Periodic cycling due to thermostatic control Control <1°C/h, RH>90% (no condensation)	28–30°C. RH>85%	Cyclic temperature and humidity conditions 20 h or RH 95±5% and 30±1°C, and 4 of RH 100% and 25±1°C
Fungal species	Test (b): no prescription	Test (c): *Chaetomium globosum* *Cladosporium cladosporioides* *Paecilomyces variotii* *Penicillium funicolosum* *Stachybotrys atra*	*Aspergillus versicolor* *Aureoasidium pullulans* *Cladosp. cladosporioides* *Penicillium purpurogenum* *Phoma violacea* *Rhodotorula rubra* *Sporobolomyces* *Stachybotrys chartarum*	*Aspergillus niger* *Aspergillus terreus* *Aureobasidium pullulans* *Pacilomyces variotii* *Penicillium funiculosum* *Penicillium ochrochloron* *Scopulariopsis brevicaulis* *Trichoderma viride*	*Aspergillus niger* *Chaetomium globosum* *Penicillium funiculosum* *Aureobasidium pullulans* *Trichoderma* sp.	*Aspergillus niger* *Aereobasidium pullulans* *Chaetomium globosum* *Gliocladium virens* *Penicillium funiculosum*
Inoculation	Not applicable	Spraying spore suspension (mixture aqueous) prepared on day of use, no quantitative specification; age of fungi <3 months	Spraying spore suspension 1 ml (mixture aqueous) on horizontally placed samples, concentration >104 ml; age of fungi 14 days	Spraying, dipping or painting spore suspension (mixture, aqueous) prepared on day of use, no quantitative specification; age of fungi 14–28 days	Spraying spore suspension (mixture, aqueous), no quantity specified; concentration > 10^6 ml; age of fungi 7−20 days; to be used <4 days	Spraying spore suspension (mixture, aqueous), no quantity specified; concentration > 10^6 ml; age of subcultures 9–12 days; to be used <7 days
Preconditioning	No sterilization or other pretreatment of samples	No requirements—pieces should be as clean as possible	23±2°C, RH 50±5% Paints for *interior* use: solventborne: 21 days, waterborne: 2 days Paints for *exterior* use: 7 days + artificial	In case no soiling is applied, it is permissible to clean half of samples by washing in ethanol or water	No requirements	No cleaning of the test item is permitted for 72 h prior to the test

			weathering	containing detergent		
Soiling	Not applicable	No requirements	Natural or artificial soils 0.03 g/sample applicable to paints for exterior use and for interior use with high soiling hazard	Aqueous saccharose solution used on day of preparation; no quantity specified	The specimens are placed on solidified nutrient-salt agars, both are inoculated	No requirements
Duration	12 Weeks	Min 4 weeks Max 12 weeks	12 Weeks	Without soiling: 84 days With soiling: 28 days	21 Days	28 Days
Assessment	Visual Dry weight loss Mechanical strength	Visual, ×10 magnification No rating scale	Visual, naked eye or ×25 magnification, using a numerical scale, ratings 0–5 (≈ coverage area)	Visual, naked eye and ×50 magnification, using a numerical scale, ratings 0–3 (≈ coverage area); electrical, mechanical checks	Visual, naked eye, using a numerical scale, ratings 0–4 (≈ coverage area); effects on physical optical and electrical properties (other ASTM methods)	Not specified
Control	Same tests in sterile soil or other inert material	No requirements	Control paint to check optimal growth conditions and viability	Specimens inoculated with water only Nutritive control strips to check viability	Viability test on hardened nutrient-salts agar	Viability test on hardened nutrient-salt agar; nutritive cotton ducts in test chamber to check proper conditions

relative humidity is the only variable parameter, these observations seem to indicate that the function of the paint in mediating nutrient uptake from the base substrate, may be significant to fungal resistance. This function might be affected by surface roughness, which differs widely for gypsum and paints and determines interface area of fungi and substrate. Effects of the base substrate in multilayered finishing systems, as related to geometric and hygric properties of the constituent materials have been extensively studied by Adan *et al.* (1994c) in a series of tests on gypsum-based finishes.

TEST METHODS FOR FUNGAL RESISTANCE OF MATERIALS

In spite of the increasing occurrence of fungal problems in buildings the testing of materials for resistance to fungal growth is not common practice in building physics. Several testing procedures are available nationally as Standards or Recommendations, and some are internationally accepted, but most tests concern fungal resistance of electrotechnical specimens, synthetic polymeric materials, including paints, and textiles. In this section present Standards for fungal resistance of materials applied in building practice are reviewed and compared to some selected tests for other applications.

Table 6.3 outlines Standards for fungal resistance tests:

1.The British Standard BS 1982 (BSI, 1968). In this standard three types of tests are prescribed. The following classification may be used as a guide to the choice of test that is most appropriate to the material and its proposed use:

Test a: resistance to wood-rotting *Basidiomycetes*, fungi which cause decay and breakdown of wood and other cellulosic material (dry rot fungi, e.g. *Serpula lacrymans*, and wet rot fungi, e.g. *Coniophora puteana*). Suitable for building boards including hardboards, insulating boards, plywood and chipboards. This test is not considered within the context of this chapter.

Test b: resistance to cellulose-attacking microfungi (commonly known as soft rot fungi, e.g. *Chaetomium globosum*). This test can be used on all sheet materials and is suitable for testing rot resistance of building fabrics, felts and papers. It can be carried out without the resources of a mycological laboratory and is intended to give a quick general indication of the susceptibility to rotting of building materials under damp conditions.

Test c: resistance to mould or mildew. Suitable for materials that are required to represent a decorative finish or appearance, e.g. plaster boards and sheets made of plastics. It is, however, emphasized that ‘these tests are not normally applicable to paints, distempers and other decorative finishes’.

2. The British Standard BS 3900, part G6 (BSI, 1989). This test is one of a series of standards in group G of BS 3900, which is concerned with environmental testing of paint films. It describes a method to assess the fungal resistance of paints, varnishes and lacquers, applied in the laboratory to specified panels, either as part of a multi-coat system or separately.
3. IEC Publication 68-2-10 (International Electrotechnical Commission, 1988a), to investigate fungal deterioration of assembled electrotechnical specimens. Generally fungal growth may have two effects: on the one hand the wet mycelium may cause formation of an electrically conducting path across the surface or cause a serious variation in the frequency impedance characteristics of the circuit, on the other hand fungi can yield acid products which may cause ageing effects.
4. Test 2.6.1 of the Institute for Interconnecting and Packaging Electronic Circuits (IPEC, 1988b), dealing with fungal resistance test of printed wiring materials.
5. ASTM Designation G 21–70 (ASTM, 1975). The objective of this test is ‘to determine the effect of fungi on the properties of synthetic polymeric materials in the form of moulded and fabricated articles, tubes, rods, sheets and film materials’. Therefore, it is

also applicable to paint films. The test is limited to growth rates assessment, and evaluation of effects on optical characteristics and mechanical properties.

Principles of testing are quite similar for electrotechnical specimens, paint films and building materials. Most tests are based on incubation of specimens in test cabinets under specified conditions after being inoculated with an aqueous spore suspension of a mixture of fungal species. Generally, the relative humidity is maintained at a constant level near saturation, but in some cases intermittent surface condensation is applied. Growth rates are assessed visually with the naked eye, or microscopically at magnifications up to 50×, mostly using different numerical scales with ratings referring to coverage areas.

In most tests, natural or artificial soiling is used to aid establishment of fungal growth. In this way an increased biological risk of exterior and certain interior conditions, where a high soiling hazard may be found, is simulated by providing nutrients and support for fungal spores.

Duration of tests ranges from 3–4 weeks for electrotechnical specimens to 12 weeks for building materials and decorative finishes. Inoculation is usually done by spraying, alternatively by dipping or painting; only part of the tests considered includes quantitative requirements on spore concentration and volume of spore suspension to be sprayed on the samples. Differences in initial spore concentration on the samples may cause uncertainties in interpretation of growth rates, based on assess-ment of coverage area. Almost all tests include control features to check viability of spores as well as optimum environmental conditions in the test cabinets.

Major differences between test methods are found in the mixture of fungal species used. As test results should mirror susceptibility of materials in practice, the fungal species used for resistance tests should reflect species encountered on affected spots.

SUMMARY

It is estimated (Sanders and Cornish, 1982) that at least 2.5 million dwellings of the UK housing stock (approximately 12%) are seriously affected and that a further two million dwellings have slight dampness problems. Both in the Netherlands (Honstede and Warringa, 1986) and Belgium (Hens, 1989) approximately 15% of the public housing stock is suffering from fungal and dampness problems, problems seemingly somewhat greater in rented accommodations. Similar problems are found in milder climates, such as the coastal region of Israel, which contains more than 50% of the country's population. A sample survey (Becker, 1984) showed that in 54% of the investigated population no problems of fungal growth exist, and that 19% have severe problems.

The role of material characteristics of interior finishes in fungal disfigurement (e.g. with respect to nutrition, water supply) has received only minor attention in building physics, although the impact on indoor fungal growth has been pointed out by several researchers (e.g. Morgenstern, 1982; Becker, 1984; Becker *et al.*, 1986; Grant *et al.*, 1988; Adan, 1990). Biodeterioration of paint and paint films provided a worthy challenge to microbiologists and chemists in the paint industry to develop paint preservatives. Their effort originated from fungal problems during paint manufacturing and in-can spoilage (predominantly due to bacteria; Kempson, 1976) as well as from defacement of the dried

film after application to the substrate, especially in some industrial applications where control of dampness is not possible (e.g. cellars and breweries).

However, the sole application of fungicidal control measures in dwellings to eradicate fungi has often given disappointing results (Bravery, 1985). Furthermore, the reduction of toxic additives and the application of more hygroscopic waterborne paints instead of solvent thinned (carbohydrates) paints, both of which are pursued in the scope of environmental care, will increase the importance of assessment of fungal resistance of finishing materials.

Several methods for testing fungal resistance of manufactured materials have been developed for specific purposes. Test methods for electrotechnically assembled specimens employ a small selection of fungal species chosen to attack materials used in industry. Species have been chosen such that the nature of attack may vary widely, ranging from paints and plastics to textiles and rubbers. With respect to tests for interior paints and finishes, it is concluded that except for *Aspergillus versicolor* and *A. niger* (minimum water activities for growth 0.78 and 0.77, respectively) common indoor xerophilic fungi, such as some *Eurotium* species, with minimum water activities near 0.7, are not considered. As depicted in Figure 6.6, and from our own experience, *Eurotium herbariorum* grows well on interior finishes within a 12-week test period. A method for testing fungal resistance to interior finishes, meeting these objectives, is given by Adan *et al*. (1994b).

It should be noted that although laboratory tests allow comparison of the *relative* resistance of materials to fungal attack under standardized conditions, they are not necessarily reliable indications of the resistance in building practice. Preliminary tests indicate that fungal resistance of interior finishes in practice may be significantly affected by characteristics of the base substrate. The latter is not explicitly taken into account in standardized tests however. Furthermore, although a material may be resistant to fungal attack, fungi may nevertheless grow on slight traces of dust, grease and other organic contaminants. This is simulated in the tests by application of soiling media. It may nevertheless be clarifying to distinguish stimulating effects of the clean material, as a consequence of physical and nutritive properties, and inhibiting effects due to action of fungicidal compounds, as suggested by KernerGang and Mecker (1972). Present testing procedures do not allow such a distinction, which however may become important in case of assessment and interpretation of fungicidal control measures, particularly with respect to fungal resistance of multilayered systems.

There can be no doubt that the fungal disfigurement of materials depends on the water supply to fungi. It was suggested by several researchers (Block, 1953; Ayerst, 1968; Adan, 1990) that the material intermediates the water-uptake of fungi, so material properties should play a pivotal role with respect to this requirement. Actually, this is partially taken into account in present tests for constant climate conditions; in practice, however, interior finishes are submitted to transient temperatures and humidities below saturation. In this case water storage capacity of the material can play a significant role in lengthening favourable humidity conditions. These dynamics are, however, not included in present testing procedures.

The role of water is essential for initial spore germination and subsequent fungal growth. With most microscopic techniques it is difficult to show the presence of water. Water attached to fungal mycelium and spores is seen as an amorphous mass under

conventional SEM (Figure 6.7). Both the present study and subsequent experiments (Adan *et al.*, 1994a), using low temperature SEM, show well-preserved water droplets or conglomerates of fungal structures in water. This technique offers good opportunities to study fungal growth *in situ*.

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Inspection, monitoring and environmental control of timber decay

7

Huw Lloyd and Jagjit Singh

INTRODUCTION

Wood is probably the oldest constructional and decorative material known to humans and is still widely used in buildings today (Cartwright and Findlay, 1968). Timber used in construction, either for decorative or structural purposes, is in biological terms dead wood. In nature wood on the forest floor would become wet, surrounded by organic material and colonized by decay organisms. The process of microbial colonization and decay of wood is a dynamic one in which the nature of the prevailing microenvironmental conditions is continually modified by the process of decay itself. Microorganisms such as mould, fungi, bacteria and insects degrade wood by their complex enzymatic digestive systems, eventually converting the wood to forest floor litter. This ecological succession is part of a natural cycle (Hickin, 1963, 1975; Levy, 1982). Many of these microorganisms are found both in the forest and in buildings. *Serpula lacrymans* (dry rot) is not known to live outdoors in the United Kingdom but is found wild only in the Himalayas. The common factor in this decay process is moisture. Buildings can provide specialized ecological niches and ideal microclimates for growth and proliferation of timber decay organisms, especially if they have been suffering from chronic water penetration, poor ventilation and gross neglect (Hutton, 1990a).

Keeping timber dry is the best mode of defence against these organisms. In recent times, new and older buildings have been converted into modern flats for multiple occupancy, wherein the use of traditional

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construction techniques has been ignored. Fire regulations, prevention of heat loss and creature comforts have turned buildings into sealed voids with limited ventilation, increasing the risk of timber decay. More and more reliance is placed on toxic chemicals to preserve timbers from decay, which, although of poorer quality than timber used in the past, is still almost immune to serious timber decay whilst dry (London Hazards Centre, 1989).

Once water enters a building structure and is unable to escape then timbers will absorb moisture and will become predisposed to decay. Masonry masses will absorb many tons of water and take many years to dry out. It is therefore most important to investigate where this moisture has come from and how to allow it to dissipate without allowing timber moisture contents to become dangerously high. The reliance on toxic chemicals in such situations is unsafe as they can be leached out of timbers, many of which will not be impregnated completely through their cross-section.

It is clear that timber decay in buildings can be created by defects to the building environment and can be best controlled by manipulating these environments in and around the susceptible materials (Eggins, 1968; Singh, 1989, 1990a). By reversing the conditions which brought about decay and by keeping the construction well ventilated, the integrity and structural performance of timber can be preserved for many centuries and even millenia, as proved by timber found in Egyptian pyramids, as good as new.

INSPECTION

PRELIMINARY INSPECTION AND INVESTIGATION

The main purpose of a preliminary inspection is to establish if any defects are present and what effect, if any, these have had on the building structure. The basis for any preliminary investigation must be a thorough understanding of building structure and materials, how these interact with the environment and how they are affected by adverse changes to that environment. A thorough knowledge of the organisms that cause timber decay is also a prerequisite for an accurate survey (Chapter 3).

Before the survey it is important to establish whether it is purely advisory, or with a view to full eradication of infestation, or maybe for an expert opinion in a case of litigation. Are there plans available, previous inspection reports, access to all parts of the building and services on site? These are all questions which should be answered before starting a survey.

Once on site it is usual to inspect the exteriors of the building first to determine if any obvious faults exist with drainage, high ground levels, etc. Once within the building it is then possible to marry up internal defects with the exterior defects rather than the other way around. This makes the survey more efficient. It is also best if the survey is carried out from the roof downwards, as water entry at roof level will spread out from this point down the building.

Fungal growth can act as a biological indicator of areas where timbers are located in concealed cavities and environmental conditions around that material which must be conducive to growth and development of fungi. Moisture meters are used at this stage to pinpoint any high moisture levels in masonry and timbers. Another more recent innovation is the use of canine detection of dry rot; specially trained dogs are used to search for active dry rot in a building without the need for opening up. A follow-up inspection is carried out once the building has been searched. The main advantage of this method is its speed; whole buildings can be cleared in a short period of time, which would otherwise take many hours of work (p. 165 of this chapter and Chapter 12). Once all problem areas have been pinpointed a more thorough investigation can be carried out

to determine which type of infestation is present and if any structural damage has been caused.

FULL INSPECTION

The findings from the preliminary inspections are followed up by more detailed investigation. The purpose of the detailed study is to find out the exact cause, nature and extent of decay. This is followed by assessment of the structural stability of timbers, for example wall plates, joist ends, and timber lintels. Once the cause and effect is established, then a detailed technical report can be prepared which includes recommendations and specifications. Generally, a timber decay survey of a building should start at the top of the building, because defects leading to water penetration at this level will cause water to penetrate the structure in a 'cone'-shaped area below this point.

All voids accessible for a physical inspection should be inspected and where necessary water contents taken. Inaccessible voids should be inspected with fibreoptics (p. 166). The aim is to determine the distribution and extent of all significant decay organisms in the building, the distribution of all microenvironments pre-disposing to timber decay and the building defects that cause them. The distribution of water and its movement through the structure is particularly important. The extent of significant timber decay should also be determined. Active decay organisms may not yet have caused significant timber decay. Conversely, there may be significant decay even when the decay organisms that caused it have been dead for many years. This may seem obvious, but many expensive 'treatments' are carried out on insect or fungus damaged timber that has not been infected for tens or even hundreds of years. Key factors to be noted are species and viability of decay organisms, moisture content of materials, ambient relative humidity and ventilation. Timber species and previous chemical treatments may also be significant.

It is important that the results of the investigation take into consideration the characteristics of particular methods of building construction. They should also be carefully recorded and quantified where possible. This allows analysis of the results by other experts, reduces the 'grey' area in which differences of opinion can arise and forms a basis on which future investigations can build. This recording of data is especially important in the current legal climate and photography can be especially valuable. A detailed investigation of this sort might take 5 hours for a typical three-bedroomed house.

Useful information can be gained from the occupier or owner of the property, such as the age of the finishes. This information can give an insight into the age and extent of an outbreak or whether a problem has been covered up by a previous owner. Also, whether there has been any previous treatment or, more especially, in flats, if adjacent properties have had treatment as these blocks should be taken as a whole building, not separately.

Checking the condition of structural timbers is an important part of a survey and a number of techniques are used. Useful information is gleaned on the condition of a timber, especially its surface, by prodding it with a sharp tool. Assessing the level of decay is something that comes with practice, but softening and short splintering on levering with the tool is a good measure of decay. Larger timbers such as beams and lintels can be sound tested with a hammer which would sound hollow if decayed. A more accurate method is to drill into the timber with a small diameter bit. The resistance to penetration will change when encountering softened or decayed wood and the drill bit

will jump into the timber if hollow. These techniques are usually used where the surface timber is intact and decay less obvious.

Typical defects leading to decay which should be investigated during a full survey are as follows.

Exteriors

- Chimneys should be checked for defective flashings and poor pointing. Unused chimneys should be capped but ventilated.
- Check for missing or cracked roof tiles/slates. On flat roofs check for bubbling, cracking of finishes and damage.
- Check that flashings between roof/wall connections are adequate.
- Valley gutters should be checked f or blockage, leakage and ponding.
- Hopper heads and downpipes should be checked for blockage. Are there alternative pathways for water dispersal? Is the drainage system adequate for the roof area to be drained?
- Does the building have a damp-proof course (dpc); if so, is it bridged by, for instance, high ground levels?
- Are the air bricks serving the floor voids blocked or undersized?
- In what condition is the external masonry?

Interiors

- Inspect roof spaces paying particular attention to gutter soles, rafter ends and rafter plates. Inspect flat roof spaces and check for ventilation.
- Inspect bearing ends of timbers in external wall including wall plates and lintels over windows.
- Inspect wall plates on dwarf walls in poorly ventilated floor spaces. Check for dpc material under wall plates.
- Inspect timbers directly in contact with damp masonry such as skirting boards, dry lining battens, lintels, etc.
- If areas of the building were not accessible for inspection then this should be noted for future reference.
- It is most important to establish the moisture content of timbers wherever possible, as this has a direct bearing on the level of activity of decay organisms. Readings at 20% and above will indicate that the timber is prone to decay.

The measurement and significance of water content

Timber water content might be ascertained by the use of a standard resistance-type moisture probe which measures the water content at the surface of the wood. Wood is a hygroscopic material: it absorbs and releases water with changes in the surrounding atmosphere. Up to 27% water content the water is absorbed into the cell wall material. Above this level free water fills the cell lumens. However, the water content will be subject to considerable fluctuation depending on ambient relative humidity and temperature. A rafter in a roof, for example, in summer may have a moisture content at

the surface of 16%, which might rise to over 20% in winter. This difference would not necessarily reflect increased water content resulting from a fault in the roof, but might simply arise from condensation of water from a considerable drop in temperature.

A large timber beam built into a damp wall may be above fibre saturation point within the masonry but gradually decreases in water content with distance from the wall. The core of the timber will be wetter than the surface where water is free to evaporate. Thus, dry rot is often found to have hollowed out the centre of large beams because environmental conditions favour decay and water is generated through its metabolism. Only when an equilibrium between wetting and drying has been reached, will the decay cease. It is rare to find a beam decayed along its whole length in a dry building.

The condition in the core or subsurface of a timber will remain relatively stable. It is this 'deep' water content which must be measured if results are to be meaningful. For this reason a hammer probe with insulated electrodes is used to measure the water content within the timber. Healthy roof timbers should maintain a stable core water content of between about 12% and 15%, whilst suspended floor timbers (excluding ground floors) should be between about 11% and 14%. Central heating will usually reduce this figure to around 9%.

Similarly, surface water content readings in plaster and mortar are of limited value except for purposes of comparison. A surface capacitance meter is used on plastered walls. For further investigation absolute measurements of water content are made on site by means of a carbidetype gas pressure meter. Alternatively, samples are taken back to the laboratory in sealed vials, and the water contents are measured by the oven and balance method. Dry mortar and plaster should have water contents below about 2%. At levels much above this the water content of incorporated timbers will exceed 20% and may easily reach levels at which fungal decay is likely.

NON-DESTRUCTIVE INSPECTION TECHNIQUES

The condition of concealed timbers may be deduced from the general condition and water content of the adjacent structure. Only demolition or exposure work can enable the condition of timber to be determined with certainty and this destroys what it is intended to preserve. A nondestructive approach is therefore required and, to help reduce uncertainty, instrumentation and test equipment can be useful at this stage. However, it is important to remember that all tests and instruments are only aids to the surveyor, and must be interpreted with experience and care. A slavish reliance on any technique, and failure to take into account its limitations, is a recipe for disaster.

Resistance-based moisture meters

Electrical resistance moisture meters work on the principal that the resistance between two electrodes inserted in a hygroscopic material, such as timber, varies sharply with moisture content. Very high resistances (10000 megaohm) at low moisture contents and the presence of free water in the cells above fibre saturation point make them inaccurate outside the 8–27% moisture content range. However, this is the useful range for measurements within buildings. Timber water contents above 20% indicate high humidities and/or water penetration and further investigation is necessary. Measurement

using a hammer electrode with insulated needles to measure moisture contents at depth provide a more accurate reading. The electrodes are inserted into timber and the measurement read on a digital scale.

Measurement of wall moisture contents

The most accurate way of measuring the moisture content of masonry is the oven drying method, whereby samples of the material to be tested are weighed then oven dried overnight, weighed again, and the percentage moisture content calculated. This is not, strictly speaking, non-destructive, but small samples can be taken in most instances with minimal damage. The level of hygroscopic salts can also be determined by placing the samples in a known relative humidity—usually 75%—then weighing and subtracting the dry weight. Moisture meters do not give accurate results in this medium; high readings will be given with salts, aluminium backings and certain paint finishes. A more accurate way of determing the presence of moisture rather than the salt content in a wall is to take a timber moisture content reading from a skirting board, window frame or floorboard, in contact with the suspect area. Many chemically injected damp-proof courses have been installed unnecessarily on the basis of electrically tested masonry which may be dry but have a high salt content due to some historical defect.

Gas carbide meters give good results. A sample of mortar is mixed in a sealed vessel with a powder which reacts with moisture. Pressure is created by release of gas which is measured on a calibrated scale.

Canine detection

‘Rothounds’ sniff dry rot fungus in much the same way as dogs can sniff out drugs or explosives, uncover avalanche victims, and snuffle for truffles (Figure 7.1). *Serpula lacrymans* produces specific secondary metabolites during its active growth period which dogs can be trained to detect (Singh, 1990b). Dogs can detect the scent of dry rot at a distance of several metres depending on scenting conditions. Dogs can actively search for dry rot in buildings at high speed, covering 20–50 rooms in an hour. In certain circumstances they may indicate the extent and spread of dry rot infestation. The advantage of a dog search is that they can search small inaccessible areas, roof spaces, furnished and inhabited buildings and they are completely non-destructive. The working capacity of a dog ranges from 2 to 4 hours per day.

Figure 7.1 Canine detection in a floor void.

The disadvantages of a canine search are that the dogs indicate active dry rot infestation, not dead dry rot and decay. Therefore heavily decayed but inactive outbreaks will give a weaker indication than a recent highly active outbreak that has not yet caused significant decay. Dogs will indicate areas of active dry rot even before these are visible to the naked eye as when the rot is just developing, is inside the substance of the timber, between the timber and another surface or within porous masonry. Such indications may be confirmed by comparing them with measurements of the water content of the structure or by the use of a core sampler.

Fibreoptic inspection

The correct identification of fungal material is important, as not all fungi are equally destructive. By employing high power fibreoptic instru-

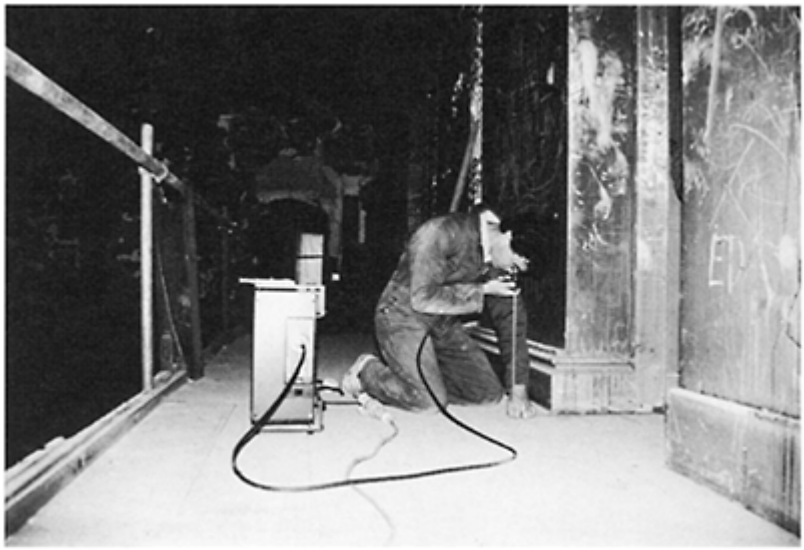

Figure 7.2 Endoscopic inspection of the floor void.

ments, the type and extent of fungal decay in concealed cavities can be assessed (Singh, 1990b). High intensity light illuminates the area under inspection through a liquid light guide and a rigid fibreoptic eye-piece (Figure 7.2). The image can then be photographed or viewed through a video system attached to the eye-piece (Figures 7.3 and 7.4). Proper use of the instruments and interpretation of results takes experience and practice, otherwise it can be easy to mistake old cobwebs for dry rot!

Fibreoptic inspection can reveal extensive decay and the consequences of water penetration. However, most wood destroying fungi will not live on the face of timber which is exposed to air movement because this produces a drying effect. It is always a possibility that a fungus, especially dry rot, is travelling behind a wall plate, for example, and is not detectable from the cavity. Fibreoptic inspection may not, therefore, find a minor attack which is developing, but it should indicate where one might be initiated so that faults can be identified and remedied. The siting of inspection holes depends on the points at risk within the room and will usually be located adjacent to balcony floors, flat roofs, cracks in rendering and other points where faults may have resulted in water penetration. Inspection may also be limited in areas of tiled and glued flooring materials and ornate or special wall coverings. Inspection holes are numbered and capped off for future use.

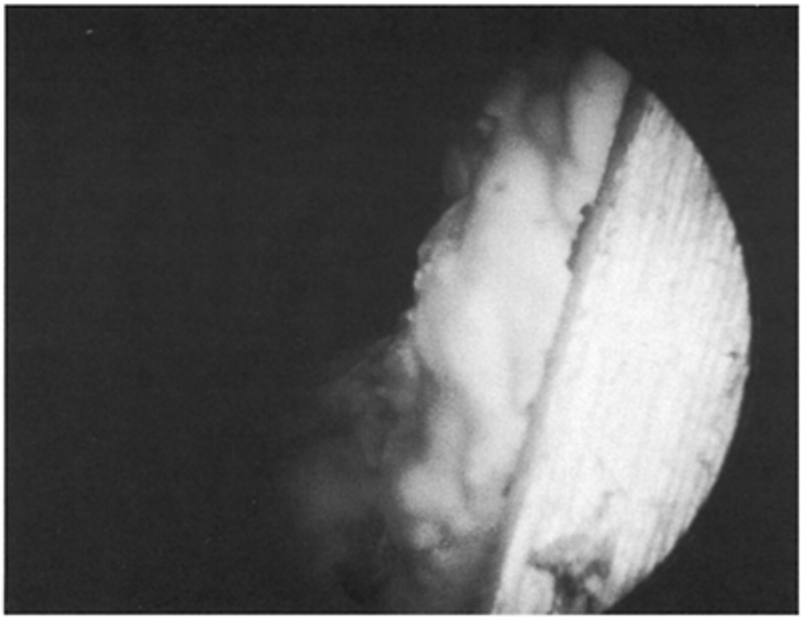

Figure 7.3 Fibreoptic inspection of rot showing ‘cottonwool’ mycelial growth.

Humidity measurement

The levels of humidity in a building can be a good pointer to potential problems. There are many portable humidity measuring meters on the market which are useful in surveying. They are often coupled to a thermometer as a direct relationship exists between the two parameters. One of the simplest types of meter is based on changes in dimension of a material with moisture. A hair or Nylon 6 is used as the sensor, which changes dimension with humidity changes. This change is transferred via a mechanical linkage to a moving pointer on a calibrated scale. More recently, sensors based on measurements of surface conducting properties have been used. The sensor element is treated with a hygroscopic salt, which can quickly become contaminated. Sensors based on bulk effects are now also used which are less prone to contamination and have a quick response time.

Air flow measurement

Air flow measurement, i.e. the amount of air movement is useful for detecting air movement under a floor space for instance. Other methods

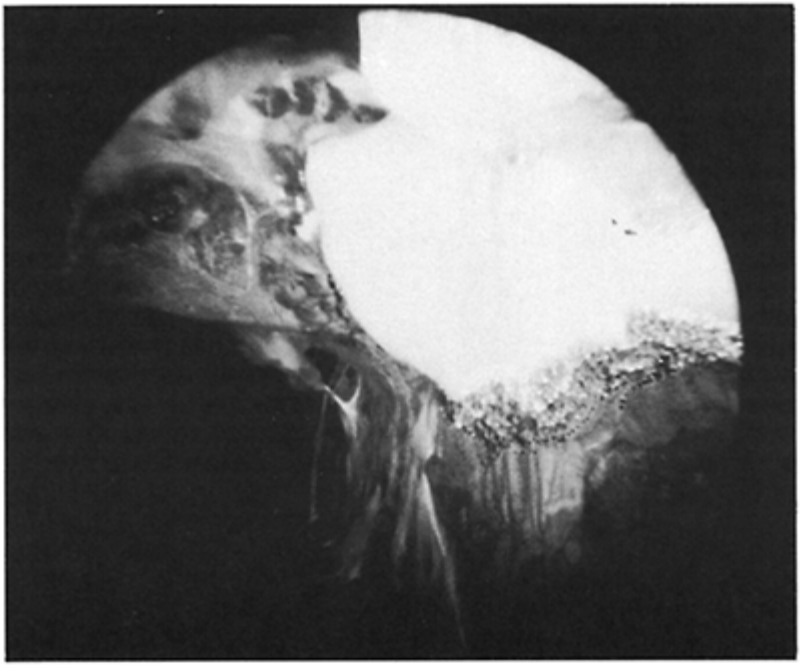

Figure 7.4 Fibreoptic inspection of rot showing fruit body and mycelial strands.

such as the use of smoke bombs to test whether a chimney flue is open or not can also be useful when determining if water penetration is a problem.

Many other non-destructive measurement techniques are available, but are either in experimental stages, expensive to use or too specialized for use in a typical timber decay survey. Some of these methods may, however, be used more widely.

Ultrasonics

This is based on the generation of high frequency sound waves in or through materials to test for differences in, for instance, density. This technique has been used to detect voids in large dimension timbers. However, a good contact with the timber is needed and a detector is needed on the opposite face. Obviously this is not possible in many instances. It is also a very specialized tool and some training would be needed to interprete results. The portable detectors are also very expensive. An alternative for detecting voids in timber is the use of a long drill bit which is bored into the timber in question. Sudden changes in resistance will indicate voids or partially decayed timber.

Television cameras

Miniature television cameras are available which can allow a remote survey of hidden structures in voids and pipework. They can be used with optical borescopes to make a recording of a survey which can be shown to a client at a later date. They are relatively inexpensive and can be hired out for use.

Other techniques are, radiofrequency detection used for detecting hidden cables; magnetometry, used for detecting metals hidden in masonry, for instance; infrared thermography for detecting hidden timbers within a masonry mass; and radiography for inspection of defects in, for instance, hidden beams.

CONTROL OF TIMBER DECAY

TRADITIONAL METHODS

Timber is a major component in most buildings. It was probably the first building material and is still widely used in new buildings. This is not surprising as it has many excellent structural and aesthetic properties as well as being an energy-efficient and renewable resource. However, timber is composed of organic materials which make it a good source of energy and many organisms have evolved to utilize it as food. Fortunately, such organisms mostly live in the forest floor where dead timber would naturally be found. This means that if they are to flourish, those indigenous to the UK require a moist, dark environment with little ventilation and a temperature between 0 and 20°C. Keeping timber out of such environments saves it from the organisms that can digest it, and effectively preserves it indefinitely. Species of trees that are heavily exposed to such conditions may evolve so that their timber is unpalatable. Thus forest hardwoods such as oak or yew are relatively decay-resistant in environments otherwise favourable to saprophytic organisms.

During the thousands of years that people have been building with timber, construction techniques have been developed that avoid conditions permitting decay. This is not difficult because environmental conditions that are comfortable and healthy for humans are unsuitable for decay organisms. Techniques were evolved to suit local materials and conditions by a process of trial and error. This knowledge was then passed on from master to apprentice and formed part of a craftsman’s understanding of his trade (McKay, 1944). In recent times, especially in the last 50 years, the old system of craft training has been destroyed, and new materials and techniques have been introduced at an ever-increasing rate. This process has been especially marked in the UK. The result is that no understanding has developed of the way in which new construction techniques affect timber decay while understanding of the older methods has been lost.

Traditional construction techniques appear diverse and complex but they can be stated as principles with universal application: minimize water penetration; ensure adequate air movement around all structures; and allow all permeable materials to ‘breathe’. Traditional buildings were also often built so that each part could be inspected, repaired or replaced without discarding and wasting the whole. Thus, historically, timber decay

was prevented by controlling the environment around the material. Indeed most buildings in the UK today were built on these principles and the timber in them continues to be preserved by these techniques.

THE USE OF PESTICIDES FOR TIMBER PRESERVATION

Remedial treatment of dry rot

The following account is taken from BRE digest 12 no 299, Richardson (1980) and Editorial (1984).

- Establish the size and significance of the attack. In particular if structural timbers are affected, carry out or arrange for a full structural survey to determine whether structural repairs are necessary and, if they are, take appropriate steps to secure structural integrity.
- Locate and eliminate sources of moisture.
- Promote rapid drying of the structure.
- Remove all rotted wood, cutting away timber approximately 300–450 mm beyond the last indications of fungus.
- Prevent further spread of the fungus within brick work and plaster by using preservatives.
- Use preservative-treated replacement timbers.
- Treat remaining sound timbers at risk with preservative (minimum two full brush coats).
- Introduce support measures (such as ventilation pathways between sound timber and wet brickwork, or, where ventilation is not possible, providing a barrier such as a damp-proof membrane or joist hangers between timber and wet brickwork).
- Do not retain dry rot-infected timber without seeking expert advice. There is always some risk in retaining infected wood which can be minimized by preservative treatment and subsequent reinspection.

Remedial treatment of wet rot

- Establish the size and significance of the attack. In particular if structural timbers are affected, carry out or arrange for a full structural survey to determine whether structural repairs are necessary and, if they are, take appropriate steps to secure structural integrity.
- Locate and eliminate sources of moisture.
- Promote rapid drying of the structure.
- Remove all rotted wood.
- Use preservative-treated replacement timbers.
- Treat remaining sound timbers which are at risk with preservative (minimum two full brush coats).

The environmental standards

Where there are actual or suspected problems of woodrot or woodboring insects in buildings, any remedial works should meet the following standards.

1. Investigation should be by an independent specialist consultant, architect, or surveyor to establish the cause and extent of damp and timber decay including the potential risk to the health of occupants before specification of remedial work. This investigation should include:

   (a) the inspection of all accessible timbers to determine whether they are subject to, or at risk from, fungal decay or insect attack;
   (b) identification of active growth of wood rotting fungi or wood decaying insects and determining whether their activity is important in each particular case.

2. Specification of remedial work should be by an independent consultant as in (1). Such specification should provide for:

   (a) the maximum conservation of materials;
   (b) the future health of the building and its occupants;
   (c) the minimal use of new materials;
   (d) the avoidance of chemical pesticides where possible;
   (e) the use of materials and techniques with minimum adverse environmental impact;
   (f) the minimum cost of the whole project including costs of the proposed works, the disturbance of occupancy, future mainten ance costs, and the costs of the safe disposal of all waste materials.

3. Remedial building works should be carried out as specified above to control the timber decay, to prevent further decay and to correct any significant building defects resulting in conditions of high moisture content or poor ventilation of timber. These should provide for:

   (a) decreasing the subsurface moisture content of all timber to below 16–18%;
   (b) isolation of timber from contact with damp masonry by an air space or damp-proof membrane;
   (c) provision of free air movement around timber in walls, roofs and suspended floors;
   (d) humidities in voids not exceeding an average relative humidity of 65%;
   (e) removal of active fungal material and any timber affected to the extent that its function is compromised or adjacent structures put at risk;
   (f) prevention of, or protection of timber from, sources of water likely to cause wetting such as overflowing gutters, leaking plumbing, condensation and rising or penetrating damp;
   (g) removal of timber damaged by insect attack to the extent that its function is compromised;
   (h) removal of all builders rubbish from voids and cavities and vacuum cleaning to remove dust.

4. The use of chemical pesticides should be avoided wherever possible. Where their use is essential to meet the requirements laid down in (2) above the following requirements should be observed:

(a) Use of fungicides should be minimal, consistent with the probability of reinfestation in the light of (3).
(b) The limitation of insecticidal treatment to the locations of significant active insect attack in the light of (3).
(c) Specific agents to be used on specific organisms only; 'combined', 'general' or 'precautionary' treatments are not to be used.
(d) Fungicides and insecticides must be currently fully approved under the Control of Pesticides Regulations, 1986. Pesticides with special dispensation or licence as of right are not to be used. As a guide products with serial numbers greater than 3000 have gone through the full HSE approval procedure.
(e) Pesticides should be applied in accordance with the manufacturer's instructions and within any regulations, codes of practice guidelines or recommendations currently recommended by the BWPA, HSE, NCC or other competent authority.
(f) The contractor applying the pesticide must certify that the treatment will not damage the health of the occupants and of the structure or wildlife in and around it.
(g) The contractor must certify that the disposal of surplus pesticide, pesticide containers and treated waste materials is safe, non-polluting, and in accordance with all current central and local government regulations and guidelines.

The use of timber pre-treated with fungicidal and insecticidal mixtures should be avoided where possible. When it is to be used the following conditions should apply:

1. Its use at each point should be specifically justified. For example ridge boards, tiling battens, sole plates, wall plates and fixing battens in contact with potentially damp surfaces or exposed to the weather should be of a rot-resistant species or pre-treated in accordance with all relevant British Wood Preserving Association recommendations and British Standards.
2. The pre-treatment pesticide used should be fully approved under all current regulations of the Control of Pesticides Regulations, 1986. Pesticides with special dispensation or licence as of right are not to be used. As a guide products with serial numbers greater than 3000 have gone through the full HSE approval procedure.
3. The pre-treatment plant carrying out the process should certify that its process and plant conforms to all central and local government regulations and recommendations and to all recommendations and codes of practice of the HSE, BWPA, NCC, NRB or other competent authority and that it submits to an annual independent Environmental Audit.
4. The contractor should certify that the pre-treated timber will not damage the health of the workers using it, the occupants of the building or the wildlife in and around it.
5. The contractor should certify that all waste material is disposed of in accordance with all current central and local government regulations and recommendations and without damaging the environment.

ENVIRONMENTAL CONTROL POLICY

Traditionally, control of timber decay organisms in infected buildings has relied heavily on extensive use of chemicals with little understanding of the biology of timber decay problems. This strategy has potential occupational and environmental disadvantages,

combined with uncertainties about its long-term effectiveness (London Hazards Centre, 1989). As with all other problems of biodeterioration the key was to discover the environmental conditions that limited growth of the organisms in question and to devise a successful treatment policy. Ecological control of timber decay is not a new strategy and has been promoted for several decades. Scientists specializing in biodeterioration provided a sound biological and technical basis for the concept and multidisciplinary practical and experimental research supports the evidence.

Because analysis of the problem was not led by the need to justify or improve any particular product or process, there was no attempt to impose a set solution on individual cases of decay. This led to the inescapable conclusion that timber decay in buildings was not caused by a deficiency of pesticides but by building failures. These resulted in the environmental conditions which favoured the various decay organisms. The main advice is to deal with the critical building defects in order to alter the environment as quickly and efficiently as possible, if necessary with the aid of artificial ventilation or dehumidification.

It has been demonstrated that appropriate environmental corrections often render pesticide treatment unnecessary and sometimes counterproductive. For instance, the pumping of large volumes of water-based fungicide into a damp wall is undesirable. This led to the formation of a policy which was called ‘the environmental control of timber decay’.

There are several components to the environmental approach: first, the non-destructive inspection of the building to locate and identify all the significant decay organisms within it; second, to devise a scheme of remedial building techniques to deal with the problem; lastly, to institute a program of building maintenance and monitoring to prevent any future problems. Using this approach, based on independent consultancy and competitive tender for works, it has been found possible to decrease the cost of remedial timber works significantly or in some cases eliminate it altogether. By avoiding destructive exposure and radical cutback techniques, it is possible to avoid extensive damage or destruction of materials and finishes. It is also possible to avoid the use of potentially hazardous and environmentally damaging pesticides and their consequential legal and management complications.

REMEDIAL BUILDING WORK AND ENVIRONMENTAL CONTROL

The most critical factors for the environmental control of decay organisms are available water and temperature. The former is dependent on such factors as moisture content, relative humidity, microventilation, and salt content. In simplistic terms, it is necessary to correct building defects leading to high moisture contents in timber and to increase ventilation around timber at risk.

In practice there are two problems: first, it is necessary to identify the signif icant building defects, and then the best techniques must be chosen to control the environment at each point. This may be achieved by analyzing the building in terms of moisture sources, moisture reservoirs and moisture sinks (Figures 1.1–1.3, Chapter 1).

It is not possible to prevent moisture entering a building entirely and often attempts to block the movement of moisture through a building structure using impermeable materials are ineffective. They may also be counter-productive as they can prevent

moisture being dissipated, resulting in high moisture levels and decay in adjacent materials. The more effective and robust approach is that used in traditional buildings. Here, porous materials are preferred and every moisture source is balanced by a moisture sink. Thus ground water may penetrate masonry but is evaporated off before it reaches timber structures. Similarly water vapour is introduced by occupancy, but is ventilated out via windows and chimneys. Failure to balance a moisture source with an appropriate sink may result in moisture moving into vulnerable materials and eventually causing decay and other problems.

Moisture reservoirs occur when a moisture source has not been balanced by a sink and water has accumulated in a porous material. Typical examples of this are to be found when thick masonry walls have been soaked by persistent leaks or when chimney breasts have been filled with rain water from uncapped chimneys. Such reservoirs may take years to dry out, even when the source has been dealt with. As a result they can act as a source of moisture for recurrent timber decay over a long period. A special case of this phenomenon occurs when large quantities of water have been used in fighting a fire.

In practice, therefore, each area of decay is associated with a building defect, resulting in an increased moisture source, a blocked or inappropriate moisture sink or a moisture reservoir. The appropriate building measures should then be specified to correct that defect. A common example might be the bridging of a damp-proof course by raised ground levels. This will act as a moisture source and may result in decay of timbers in an adjacent floor space. Lowering the ground level will cut off this source and will also provide a sink of moisture by allowing evaporation from the exposed wall. The subfloor moisture level might also be controlled by increasing the other available moisture sinks. Cleaning pre-existing airbricks or inserting additional subfloor ventilation (Figure 7.5) would be a common measure. In general it is important to increase evaporative surfaces and avoid obstructing them during refurbishment.

Another common example would be a blocked and overflowing parapet gutter acting as a moisture source. This could wet up gutter soles, joist ends and wall plates as well as any other structure in an expanding cone extending from the leak down through the building. Preventing this moisture source may require a number of measures such as increasing the capacity of down-pipes, re-lining the gutters and fitting thermostatically-controlled trace heating tape to increase free flow of snow-melt water.

Any failure in a roof finish, gutter or coping will generally result in significant water penetration into the masonry wall beneath, which will then act as a moisture reservoir. The stone or brickwork will be at risk of decay or timber in contact or embedded in the masonry will be at risk of decay as it will tend to ‘wick’ moisture from the masonry. Steps

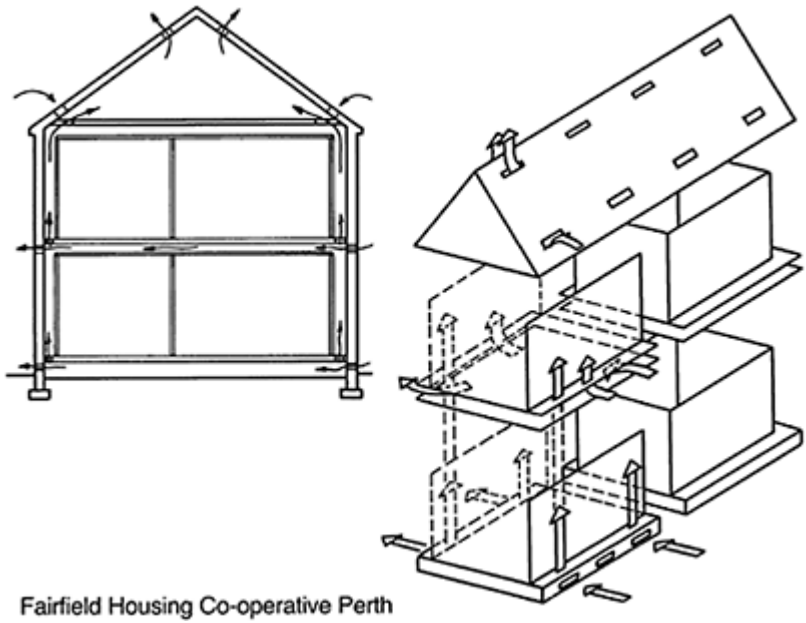

Figure 7.5 Environmental control by cross-ventilation systems of the roof void, wall void and floor void. (Courtesy of Howard Liddell, Ecological Consultancy.)

must therefore be taken to isolate in-contact timber from the masonry using such measures as damp-proof membranes or joist hangers producing an air gap. It will also be necessary to ensure that the timbers are adequately ventilated so that any moisture that is absorbed can be breathed off (Figure 7.5). Closed cavities or water-impermeable layers over timbers at risk must therefore be carefully sought out and rectified using knowledge of historic methods of construction. Bricked-in lintels and sealed-up emulsion-painted sash windows are typical examples of structures at risk in this way.

Having cut off the moisture source to a moisture reservoir and protected the ‘at-risk’ timbers, it is next necessary to provide safe ‘sinks’ for the moisture. This will ensure that the reservoir is dried out in the long term. In some cases, the reservoir can be removed entirely, for example damp pugging can be dug out and replaced. In most cases it is a matter of promoting ventilation around a wicking surface on the reservoir and ensuring that the moisture-laden air can be vented to the outside. Dry lining systems can be useful for this purpose as can the good oldfashioned chimney. Raising the temperature will promote the process of wicking and evaporation. General house heating can help, but care must be taken to ensure that water vapour does not move to other parts of the building down a temperature gradient. Heating can be especially useful if it is possible to heat the reservoir materials themselves. Special systems have been devised for heating

large section timbers and masonry, but the old-fashioned fireplace and chimney is still useful. In some cases dehumidifiers can be used in the short to medium term but care must be taken. They also require high air temperatures and high relative humidities (RHs) to extract moisture efficiently

In most cases, remedial building work is quite within the capacity of the general contractor. Most are traditional repairs, although some may take advantage of new materials or techniques such as dry lining, joist hangers and tanking. New and potentially useful products are coming onto the building market all the time, for example, time-controlled automatic fans, hollow ventilation plastic skirting boards, plastic masonry drains, roof space ventilating systems and moisture permeable paints. All such products and techniques can be used to help in making the environmental control of timber decay even more efficient and economical. All that is required is careful analysis of each situation and a little scientific understanding.

REFURBISHMENT AND MAINTENANCE PROGRAM

Refurbishment program should be aimed at drying out the building fabric to reduce the risk of future insect and fungal attacks. Masonry saturated as a result of long-term leakage, groundwater penetration, flooding or dousing after a fire, should be thoroughly dried out before the commencement of any refurbishment program. The introduction of dehumidifiers, central heating systems and adequate ventilation will encourage drying out of the fabric and can be monitored by the installation of remote sensing systems. Any new and existing healthy timber should be isolated from surrounding damp masonry. The use of hangers and future refurbishment should incorporate a maximum amount of ventilation to prevent formation of moisture pockets. If an active infestation is detected, appropriate treatments can be applied immediately to stabilize the situation until the final method of conservation and repair is decided.

Wherever possible it is preferable to institute effective inspection and preventive measures causing minimum disruption. Repairs should be carried out in the original materials for purposes of authenticity and compatibility, and because new materials and methods can sometimes create problems, such as changes in vapour diffusion, air permeability, thermal movement and chemical and electrochemical effects.

General maintenance should include frequent cleaning of gutters, hopper heads and down-pipes—a roof-heating tape can be placed along gutters and downpipes to ensure a constant water flow in snow and frost conditions. Remote sensing systems can be installed to check areas at risk so that leaks are located and remedied before they give rise to fungal attack. With careful conservation and good maintenance, a building should be under no danger from the ravages of insect infestation and wood-rotting fungi.

The investigation and building works described in the previous sections should return the building to a state of good structural and environmental health. The environmental control approach will also mean that the building is less likely to develop problems in the future. This is because the effect of minor building failures should be ‘buffered’ by the robustness of the systems established. Fortunately most traditional systems are robust in this way. This is why older buildings will tolerate a considerable amount of neglect and abuse before developing severe problems. However, the long-term health of the building

will always depend on adequate maintenance. This is no less true of buildings treated with timber preservatives.

The detailed investigation carried out as part of the environmental control policy provides an excellent basis on which to plan the most cost-effective maintenance program. Indeed the building works required for environmental control are often best integrated into such a program. Short-term ‘emergency’ measures can be taken simply to halt further decay, and measures to replace damaged structures or prevent future problems can be delayed to fit into a longer-term plan of works. This flexibility in scheduling work as a result of the environmental approach allows further savings of costs and inconvenience.

A maintenance program must also include provision for the routine inspection of all significant parts of the building at appropriate intervals. This should aim to detect and correct problems developing before they cause significant damage. Again the information gained in the investigation can be used to decide on the most cost-effective inspection intervals.

MONITORING

Often remote monitoring systems can be very useful in increasing the efficiency and decreasing the cost of maintenance programs. They can be especially useful for checking the moisture content of inaccessible timbers in roof spaces, behind decorative finishes and in walls. Systems were initially developed using paired stainless steel screws for this purpose, wired back to a junction box. Readings could be taken using a standard resistance meter.

This technique was successfully used at the Mansion House and Hampton Court Palace as well as in many more modest properties, but early systems suffered from several limitations: readings were inaccurate because of differences in the timbers; painted, treated or saltcontaminated timber gave meaningless readings; only the surface and not the more important deep moisture content could be measured; and finally the system could not be used to monitor masonry, plaster or other materials. A special miniaturized low-cost sensor has now been developed. This can be inserted into any material to the required depth, quickly and at low cost, without the problems discussed (Figure 7.6).

Sensors can be placed at all critical points after the investigation or after remedial building works. Areas can then be closed up and finishes re-applied; for example sensors may be placed in lintels, joist ends, valley gutter soles or in damp walls to monitor drying. It is important to use enough sensors and to place them with an understanding of the moisture distribution processes, because conditions can vary even in a small area. It is these local variations in conditions that produce the environmental niches which decay organisms exploit.

If more than 30 sensors are deployed, taking the readings can become onerous and this may result in human error or negligence. In these situations automatic monitoring systems become desirable and a number of specialized systems have been developed. With larger systems the wiring of sensors can also become a problem. For systems requiring 100 or more sensors, a ‘Curator’ unit is used working via a single four-core

mains cable connecting up any number of nodes, each supporting four sensors. This system can be programed with logging intervals and alarm limits for each sensor and can be read via the telephone system via its own modem. Data from the system can then be analysed using CAD and programs for statistical interpretation on a remote computer.

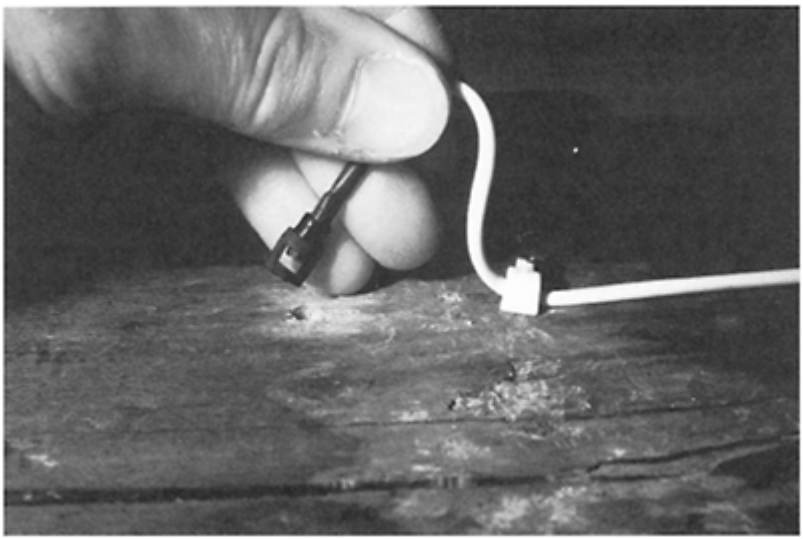

Figure 7.6 Timber moisture monitoring sensor.

TIMBER MOISTURE MONITORING SENSORS

The water content of building materials can be determined through direct and indirect methods mentioned previously. Direct methods involve removal of a sample of the material to be tested which is weighed, collected and then dried to determine its water content. This has the disadvantage of being a destructive method and it cannot be used for remote monitoring.

Indirect methods are based on measurement of characteristics that are related to the moisture level in the testing material. These involve thermal conductance, electrical capacitance and resistivity Measurement of a surrogate material in equilibrium with the first material is another method.

The use of electrical resistance moisture meters provides a quick and relatively accurate method of determining the moisture content of wood if a knowledge of their limitations is taken into account. The principal behind this is based on the changes in resistance, due to changes in moisture content, between two electrodes placed in timber. Increasing moisture content results in a reduction in electrical resistance.

Miniature sensors are fabricated from hygroscopic material which has been calibrated to match the moisture content changes in timber. They are encased in a protective plastic

sheath. The sensor is then inserted into a previously drilled hole to the required depth and the hole sealed. In most instances the sensor cable seals the hole to the outside. The sensor will fairly rapidly come into equilibrium with the atmosphere within the hole. Due to their small size the sensors can be inserted into the centre or ends of large dimension timbers allowing the best chance of detecting early defects.

MASONRY MOISTURE MONITORING SYSTEMS

These systems can be based on the direct measurement of the material or by the use of a surrogate material which changes in moisture content in a similar way to the host material. This material may be of any hygroscopic type which, providing it has been calibrated correctly, can be used as the basis of a remote sensing system.

The sensors are placed in the material to be tested at the required depth, or in an array and the hole sealed to the external atmosphere. The sensor will come to equilibrium with the relative humidity within the cavity or drilled hole and hence with the surrounding material. Single sensors can be placed at varying depths but must be sealed within the area to be measured. A series of sensors individually sealed within the drilled hole can provide a profile of readings across the material. These can either be wired up and resistance measured remotely or can be removed, weighed and oven dried to calculate their water contents. Changes in the water content of masonry can be rapid when wetted so that it could provide an early warning of building defects leading to water penetration. However drying down can take many weeks or years. The sensors deteriorate over a long period and become inaccurate.

RANGE OF MONITORING SYSTEMS

These can vary from the fully automatic systems (Figure 7.7) which can be monitored remotely via the telephone line to very simple single sensors which are read with a resistance meter.

The simple plate type of system (Figure 7.8) consists of a thin metal plate with a series of plugs installed which the resistance meter can be

plugged into and the particular sensor read. The sensors are individually wired back to the plate which can be situated any distance from the area to be monitored. The advantage of this type of system is that a number of wall areas can be monitored individually and the plate can be discretely hidden in close proximity to the area. A similar system is the switch box type which differs from the plate system in having a box with switches allowing a large number of sensors to be read at a convenient point with a moisture meter (Figure 7.9). The box however is larger than the plate and more difficult to conceal discretely.

The box system can be automated to indicate, via a flashing light, when a sensor reaches a pre-set limit, usually 20% moisture content. This rules out the need for a moisture meter, although it can still be used to check the actual moisture level. A fully automatic system based around a small microprocessor allows an unlimited number of sensors to be read in a short space of time (Figure 7.7).

The automatic type works on a ring main system which has a single cable running around the building into which are spliced ‘nodes’. Any number of nodes can be used into which are attached four sensors. The sensors can be for the measurement of moisture, temperature, and

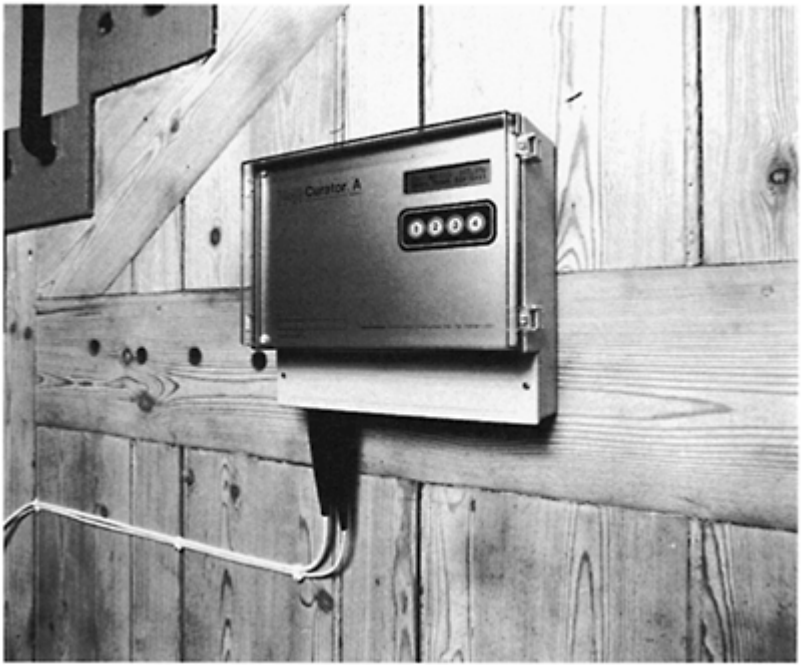

Figure 7.7 Automatic timber moisture monitoring system using a modem.

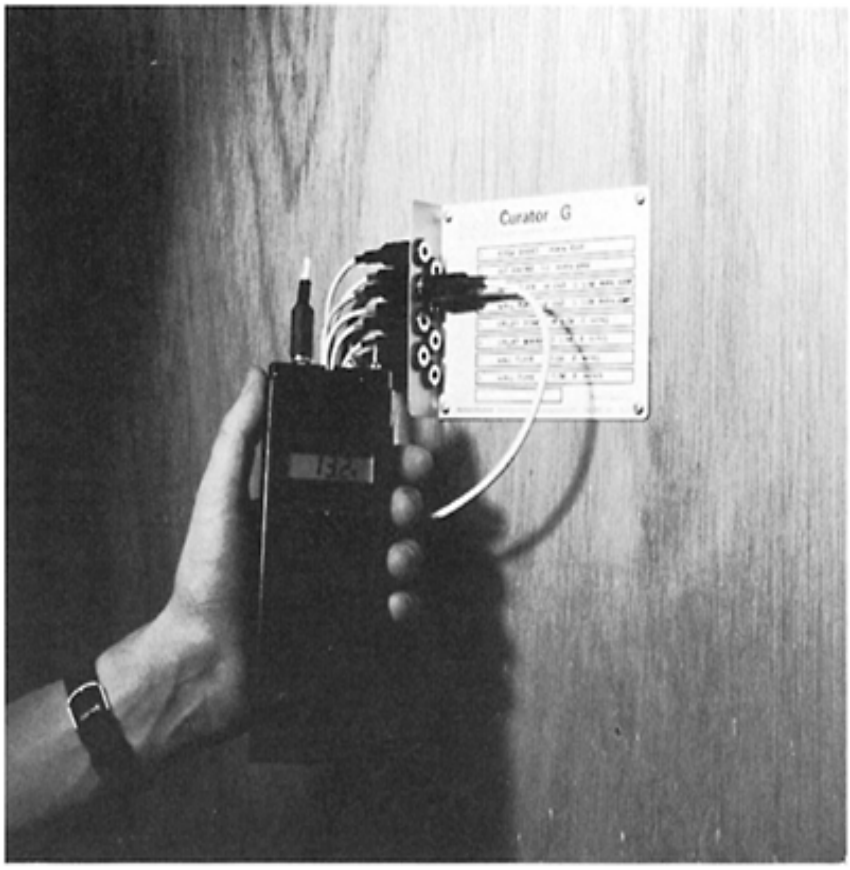

Figure 7.8 Plate type timber moisture monitoring system.

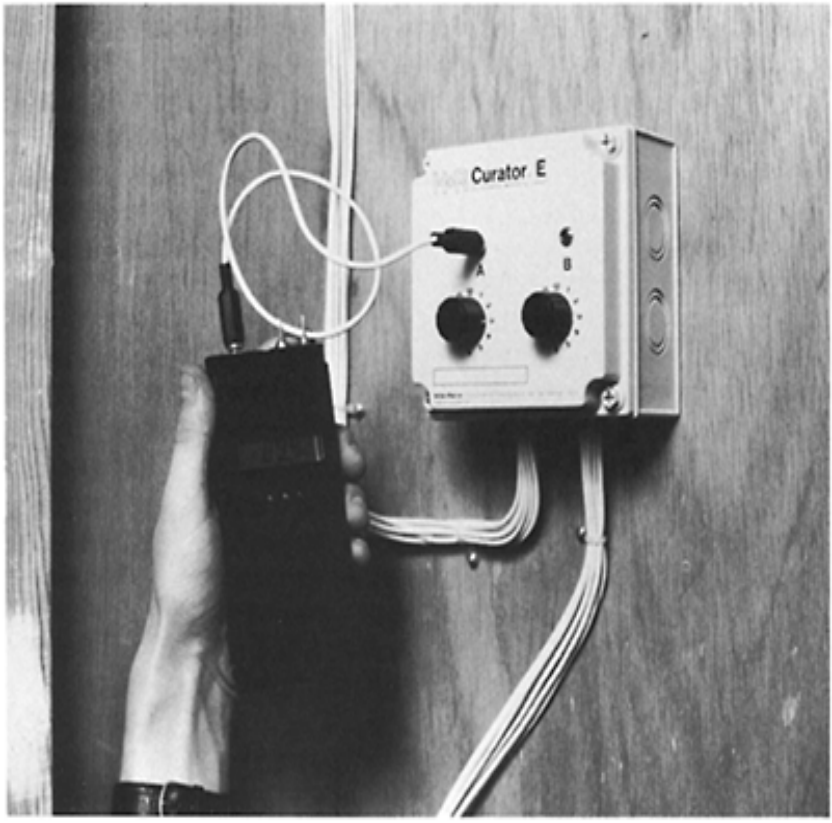

Figure 7.9 Switch box type timber moisture monitoring system.

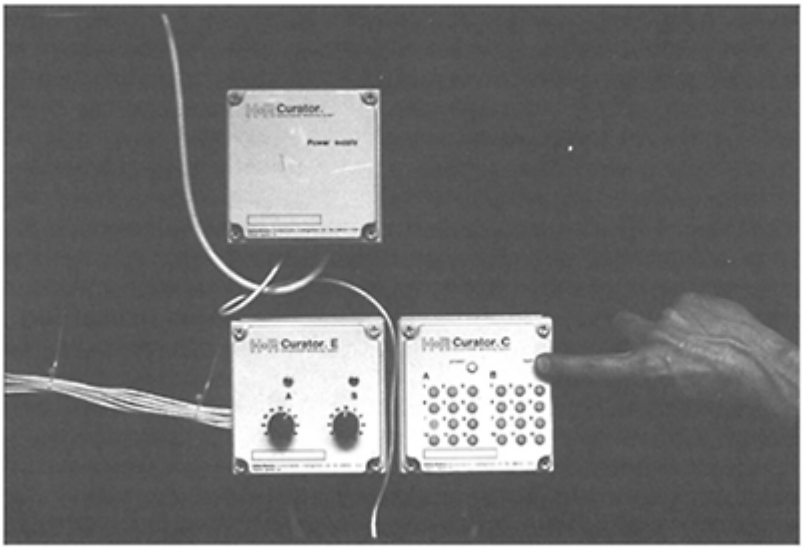

Figure 7.10 Early warning detection system.

humidity. The sensors can be individually set to alarm at different levels or switched on and off as required. The data can be read locally or via a modem onto a personal computer. The whole system can be automated and acts as an early warning to a building owner (Figure 7.10).

SUMMARY

The built environment is the product of complex interactions between the external environment, the building materials, the design, the contents, the activities in the building and the occupants, both human and commensal. To try to manipulate any one of these factors without consideration for the effects on the others can be at worst ineffective and at best inefficient and costly. Timber decay organisms cannot be eradicated even by the most Draconian pesticide treatments. However, they can only flourish in buildings if the environmental conditions are suitable. Even with the loss of traditional skill and the complexities introduced into building by new materials and uses, these conditions can easily be avoided with a little thought and scientific understanding. Indeed new materials and techniques can often be used to advantage if their properties are analysed as potential environmental controls. This more rational approach to the treatment of timber decay is really only good building practice which independent surveyors and their scientific consultants should promote in the interest of sound building and public health.

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Detection and biocontrol of wood decay organisms

8

John W.Palfreyman and Alan Bruce

INTRODUCTION

In this chapter current developments in research in two specific areas related to the biology of timber decay are discussed, namely the use of molecular methods for organism identification and the use of biological control systems as an alternative to currently used chemical preservatives.

DETECTION AND BIOCONTROL OF WOOD DECAY ORGANISMS

Increasing restrictions on the use of toxic chemicals to preserve timber resulting from both recent legislation and consumer awareness are likely to enhance the need for the development of a more targeted approach to problems associated with timber decay. Fortunately this need comes at a time when a wide range of new and developing technologies can be applied to the problem. Examples of such technologies include environmental monitoring systems, information handling systems and certain aspects of biology/biotechnology. Increased economic interest in the biological characteristics of some wood decay organisms able to biodegrade certain toxic waste products, together with legislative and consumer pressures on related (though more advanced) fields such as plant pathology, will indirectly aid developmental research into more appropriate methods for the preservation and treatment of timber.

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MOLECULAR ANALYSES AND THEIR APPLICATION TO WOOD DECAY FUNGI

INTRODUCTION

Over the last 20 years most areas of biological research have been affected by the introduction of new techniques in molecular biology. These techniques have allowed many new types of analysis to be undertaken and have had a major impact on our understanding of all types of biological process. The impact of such techniques in mycology, and in particular in the identification and detection of wood decay fungi of economic importance, forms the basis of this review. Whilst it may be considered that current technologies for identification and detection are adequate, various types of evidence indicate that this is not the case. For example, problems have occurred concerning the actual identification of specific organisms (Vigrow *et al.*, 1991a); there are difficulties in detecting incipient decay (see Schultz and Nicholas (1987) for review, though most techniques described by these authors relate to the detection of decay rather than to the detection of the organism responsible which should be possible before decay problems become apparent); there is a probable need in the future f or the use of more efficient and selective protective chemicals/biological agents and procedures resulting in a requirement to monitor the effects of such agents/procedures on decay organisms and to ascertain organism viability after selective killing techniques. The application of the techniques of molecular biology to the study of wood decay fungi will undoubtedly allow some of these issues to be confronted. Initial attempts to employ molecular biology to detect and identify wood decay organisms are discussed in this chapter. The range of organisms which can cause degradation of timber is very extensive, but for the purposes of this review only the basidiomycete organisms which cause brown or white rot of wood are discussed.

TECHNIQUES

Molecular analysis implies the analysis of biological systems at a molecular level and, whilst there are a large number of techniques available to the molecular biologist, three technologies are particularly relevant to the identification and detection of fungal microorganisms: protein analysis, immunotechnology and nucleic acid analysis. Much of this review will concentrate on the first two since applications of nucleic acid technology to wood decay fungi are currently limited.

Protein analysis

A wide range of techniques are available which could facilitate the use of protein analysis in fungal identification and detection. Foremost amongst these are various refinements of electrophoresis which allow the separation of the individual protein components of samples. After separation these proteins can be detected by various systems which include silver staining and antibody staining with either monoclonal antibodies or

polyclonal antisera (Western blotting). Specific protein subsets, such as glycoproteins, can be detected using general stains (e.g. digoxigen-based probes) or specific stains (e.g. enzyme-bound lectins).

The electrophoretic separation of proteins was greatly enhanced by the refinements of the sodium dodecyl sulphate polyacrylamide gel electrophoresis system (SDS-PAGE) introduced by Laemmli (1970) (Figure 8.1). This technique was originally used to identify the molecular weight of protein subunits and to check on the purity of proteins. However, in the last decade the high sensitivity and discriminatory ability of SDS-PAGE has led to it being applied to the analysis of complete protein extracts of various microorganisms including fungi, for example *Phytophthora* spp. (e.g. Hansen *et al.*, 1986/88). Further development of the original SDS-PAGE technique combined with isoelectric focusing of proteins has allowed the development of two-dimensional separation techniques (O'Farrell *et al.*, 1975). Using this method it is possible to identify over 2000 proteins from whole cell extracts (Strahler *et al.*, 1989). Recently introduced methods for detecting the proteins in gels after separation, in particular the silver staining methods of Switzer *et al.* (1979) and Blum *et al.* (1987), have greatly enhanced the usefulness of SDS-PAGE and now permit the detection of proteins at sub-microgram concentrations.

SDS-PAGE analyzes detergent-treated, denatured and therefore inactivated proteins. Isoenzyme analysis, an alternative type of analysis of native active proteins, has been widely used to investigate relationships between fungi pathogenic to plants and trees (e.g. *Heterobasidion annosum*, Karlsson and Stenlid, 1991) though application of this technology to detect variability in, for example, *Serpula lacrymans* strains has not yet been reported. Much earlier studies generally used non-denaturing systems, for example the isoenzyme/starch gel electrophoresis of Lawson *et al.* (1975) on the relationship between members of the genus *Polyporus;* however modern analytical methods have considerably higher discriminatory capabilities than starch gel electrophoresis.

Techniques of electrophoresis applied to extracts of organisms can give a snapshot, or fingerprint, of all the major proteins, or specific proteins, which comprise an organism at any one time. More sophisticated analyses of fractions of cells or of actual protein synthesis by cells are possible. These can give information on how an organism is changing its growth and metabolic patterns, specific information on particular protein structures and many other aspects of organism function.

The ability to fingerprint organisms allows a molecular basis for organism discrimination to be developed to supplement specific

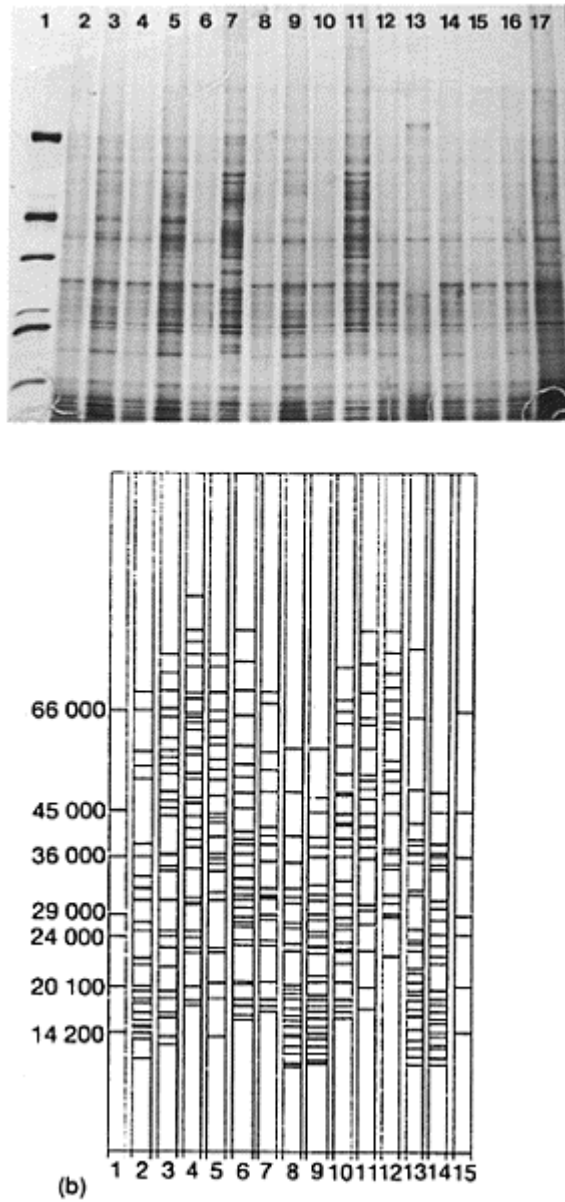

Figure 8.1 (a) SDS-PAGE analysis of various isolates of *S.lacrymans*. Even numbered tracks represent extracts of *S. lacrymans* FPRL 12C (used as a standard isolate of the organism), odd

numbered tracks represent various other isolates of *S. lacrymans.* Of particular note are isolate BF-015B (track 13), originally identified as *S. lacrymans* but now correctly identified as *S. himantioides,* and BF-050 (track 7), which in all analyses undertaken to date, except SDS-PAGE, and including a range of other molecular analyses exactly resembles *S. lacrymans.* The exact nature of isolate BF-050 remains unknown. (b) Diagrammatic representation of SDS-PAGE analysis of extracts of *S. lacrymans* and a variety of other wood decay fungi. Of particular note are track 2 *(S. lacrymans),* track 3 *(C. puteana),* track 4 *(Fibroporia vaillantii)* and track 11 *(Poria placenta).* There are obvious differences in the protein profiles (banding patterns) for these important wood decay organisms. (Reproduced with permission of *Wood Protection,* a publication of the Institute of Wood Science, UK.)

taxonomic keys, such as those developed by Nobles (1965) and Stalpers (1978) for the identification of wood decay fungi using morphological and growth characteristics. Whilst close attention to such keys can assist identification there is little doubt that misidentification and confusions do occur (see Vigrow *et al.* (1991a) and Schmidt and Kebernik (1989) for a misidentification of *Serpula himantioides* which was recently confirmed by SDS-PAGE). Analysis of proteins by SDS-PAGE has been widely used in mycological identification, originally in the field of medical mycology and more recently in plant pathology. Examples from plant pathology include the differentiation between aggressive and non-aggressive strains of the Dutch Elm disease causative organism *Ophiostoma ulmi* (Jeng and Hubbes, 1983), analysis of the taxonomic relationships between *Phialophora* spp. as determined by Maas *et al.* (1990) and confirmation of the continued speciation amongst isolates of the plant pathogen *Phytophthora megasperma* (Hansen *et al.,* 1986).

Application of protein analysis to wood decay fungi has until recently been limited to studies on individual enzymes produced by such fungi. However in recent years a range

of organisms including *Neolentinus lepideus* (Glancy, 1990), *Serpula lacrymans* (Schmidt and Kebernik, 1989; Vigrow *et al.*, 1989; Palfreyman *et al.*, 1991a) and members of the *Coniophora* genus (McDowell *et al.*, 1993) have been subjected to this technique. That it is relatively simple to visually distinguish between different wood decay fungi is shown in Figure 8.1a which represents an analysis of a range of organisms found in decayed timber in buildings. Such data can also be represented as maps (Figure 8.1b) (Palfreyman *et al.*, 1991b) and analysis of the similarity indices (by computer analysis as described by Owen *et al.* (1989) or manually as detailed by Palfreyman *et al.* (1991b) can demonstrate, numerically, the large differences in profiles between ranges of organisms even from closely related habitats. Information can also be obtained for taxonomic purposes, indeed the classification of organisms in complex genera such as *Phytophthora* currently depends upon this type of analysis. Information on taxonomic relationships between economically important wood decay fungi can be gained from SDS-PAGE, for example *S. lacrymans* and *S. himantioides*, easily distinguishable by SDS-PAGE, are more similar to each other than to any other fungi studied (Palfreyman *et al.*, 1991b) whilst members of the genus *Coniophora* (e.g. *C. marmorata* and *C. arida*) are more similar to each other than other organisms studied in different genera (McDowell *et al.*, 1993).

So can this type of analysis be of use to those involved in the preservation of wooden structures or the wooden components of buildings or their remedial treatment? Fingerprinting of organisms using SDS-PAGE is a relatively sophisticated technique which, as currently used, requires skilled technical input. However the introduction of automated protein analytical systems based on SDS-PAGE (e.g. the PHAST system of Pharmacia/LKB) greatly reduces the difficulties associated with the technique and studies in our laboratory have demonstrated the potential of this technique in the identification of wood decay fungi.

To date most studies utilizing SDS-PAGE have involved reproducible laboratory culture of target organisms followed by standardized extraction of protein components prior to analysis. During this process organisms are normally cultivated on artificial growth medium prior to analysis. In order to analyse the protein make-up of organisms in conditions more nearly approaching real life situations an analysis of *S. lacrymans* under a variety of conditions has been undertaken together with molecular comparisons of fungal decay organisms isolated at different times of infection and in different morphological forms (e.g. surface mycelia, strands, fruiting bodies, etc.). SDS-PAGE analysis on different morphological forms of *S. lacrymans* reveals differences in the molecular make-up of the organism as evidenced by similarity index values for these forms (Palfreyman *et al.*, 1991a). Whilst all isolates of *S. lacrymans* grown in liquid culture had similarity indices of approximately 100% when compared to a standard preparation of *S. lacrymans* (strain FPRL 12C) it was found that basidiocarp material had a value of 40% or less when compared to this preparation. Furthermore, comparison of material extracted from infected wood with the standard preparation also gave relatively low values. Higher values could be obtained when like was compared to like (i.e. basidiocarp material compared to basidiocarp material). Though this result might have been predicted it has now also been found that there is molecular variation within one specific morphological form of a fungus. Thus mycelial harvests of laboratory grown *S. lacrymans* and other important wood decay organisms display differences in molecular

profiles according to the position within the hyphal mat from which the material is extracted. This presumably represents differences in the metabolic activity of the organism at different growing stages within a colony (Vigrow *et al.*, 1991b; Vigrow, 1992).

Direct analysis of infected wood material from the field would represent the simplest application of SDS-PAGE. However when such samples are analyzed no recognizable banding patterns were found (Palfreyman, unpublished observations; Jellison, personal communication) and identification by this method is therefore problematic. Stained gels of decayed wood material show, instead of banding patterns of the type shown in Figure 8.1a, smears which may represent degraded pro-teins, highly modified proteins or the presence of complex interfering molecules from the degraded wood. Simple methods to remove these smears by use of specific detergents or altering the pH of gel buffers have little effect and suggest that the direct identification of fungi by SDS-PAGE from infected wood samples is unlikely to be possible without first isolating the organism.

Overall it can be considered that the highly complex profiles produced by SDS-PAGE analysis give too much information for simple rapid analysis and that the technique is too sensitive to the status of the sample for it to act as a primary testing system. However as a final arbiter in situations where there is disagreement between experts and where an isolate of the organism in dispute can be prepared the system is ideal for establishing identity.

Immunotechnology

Immunotechnology represents a large set of techniques which are based on the exquisite ability of the mammalian immunological system to produce antibodies which can react specifically with particular molecular shapes (antigens). After appropriate processing such antibodies can be used as molecular probes in a range of techniques. Antibodies produced *in vitro* (monoclonal) or *in vivo* (polyclonal) can be used in techniques which include sophisticated staining procedures such as Western blotting or immunocytochemistry, sensitive assay systems for almost any type of biological molecule or set of molecules (e.g. whole organisms), mechanistic studies on antigen function and simple enduser detection and assay kit systems.

SDS-PAGE can give valuable data on organism identification, but the technique will always be complex and is unlikely to work on infected wood. To overcome these problems a number of groups have been investigating the use of immunotechnology to detect decay fungi in harvested wood (Goodell and Jellison, 1986; Palfreyman *et al.*, 1987; Breuil *et al.*, 1988) and pathogenic fungi in trees (e.g. *O. ulmi;* Dewey and Brasier, 1988). Results of the former studies have indicated that the detection of *Poria placenta* by an enzyme-linked immunoassay is possible (Goodell and Jellison, 1986) though unidentified components of undecayed wood appeared to have an inhibitory effect on the assay system used (Jellison and Goodell, 1989). Palfreyman *et al.* (1987) showed that a simple dot-blot immunoassay could detect extracts of liquid culture grown *N. lepideus* and that whilst cross-reactivity of the antiserum with other organisms was extensive this could be reduced by selective cross-absorption. Further studies (Palfreyman *et al.*, 1988) indicated that the dot-blot method, whilst essentially a qualitative procedure, could easily be modified to produce quantitative assessments of antigen. Breuil *et al.* (1988) reported

that enzyme-linked immunoassay technology (ELISA) could be used to detect sap staining in unseasoned lumber, again showing that cross-absorption could reduce unwanted reactions, and suggested that the use of ELISA could be of practical value. In addition to unwanted cross-reactivities a second problem with the application of immunotechnology to some wood decay organisms was identified both in these early studies (Palfreyman *et al.*, 1987) and more recently (A.P.Koch, personal communication), namely the apparent ability of many wood decay fungi to non-specifically bind immunoglobulins. The basis of this binding is unclear and unless it can be overcome the usefulness of immunoassays will be limited.

Even when using a very non-specific polyclonal antiserum raised against a whole cell extract of the dry rot organism *S. lacrymans* specific organism identification is possible provided the type of analysis used can show sufficient discrimination. Thus Western blotting using this antiserum can not only differentiate between widely different organisms such as *S. lacrymans* and *C. puteana* but also between *S. lacrymans* and *S. himantioides* (Vigrow *et al.*, 1991b). However it is interesting to note that a specific isolate of *S. lacrymans* (BF-050), which according to Palfreyman *et al.* (1991b) showed marked molecular differences on SDS-PAGE to the standard *S. lacrymans* profile, gave Western blotting profiles similar to the standard. This indicates that the discriminating power of the latter technique may not be as great as SDS-PAGE. However since the Western blotting profiles are much easier to analyse than the very complex SDSPAGE profiles this lack of discriminating power may not be a disadvantage. Why *S. lacrymans* BF-050 shows a different SDS-PAGE profile to all other isolates of the organism studied in this laboratory to date has not as yet been identified though the geographical origin of this isolate, Australia, may be significant.

Whilst the production of specific immunological reagents may not be simple the application of reagents, once they have been produced, to the detection and identification of decay organisms should be relatively simple. Techniques such as ELISA as used for *O. ulmi* (Dewey and Brasier, 1988), *Poria placenta* (Goodell and Jellison, 1986) and *Ophiostoma* spp. (Breuil *et al.*, 1988) and dot-blot as used by Dewey *et al.* (1989) for *Humicola lanuginosa* and Glancy *et al.* (1989) for *N. lepideus* represent methods that can be used easily by untrained personnel. However whilst these methods are of great use in many types of system the whole range of forms in which fungal microorganisms can be represented may present problems for the potential immunoassayer, e.g. an organism such as *S. lacrymans* can be present as surface mycelia, mycelia within infected wood, strands, spores or basidiocarps (fruit bodies). If a non-expert is taking a sample for potential immunoassay will the sample chosen react with a specific antibody reagent? The scope of appropriate material for particular immunoassays will need to be defined and broad range antibodies produced.

A model system where useful data was generated by a simple immunological test was reported by Glancy *et al.* (1989). Analysis of a simple immunoassay system for *N. lepideus* was carried out by investigation of the antigenicity of core extracts taken from inoculated pole stubs. Poles were harvested at different times after infection and the traditional method of mycological evaluation (plating out cores on malt extract agar containing benomyl to decrease the growth of non-basidiomycetes) was compared with extraction and subsequent immunoassay using a simple polyclonal antisera. As previous experience would have indicated, many more immunologically-positive samples were

found than samples producing basidiomycete isolates, though the majority of mycologically active samples were associated with immunologically-active samples. Indeed, though a number of different organisms were extracted from the poles the only statistically positive correlation was between *N. lepideus* and the immunologically positive samples. Refining of this assay to reduce the number of positives, assuming that a proportion of these were false positives, would probably require the production of a monoclonal antibody based assay system.

A major advance in immunotechnology came in 1974 with the production of monoclonal antibodies which offered the possibility of much higher reagent specificity. The production of monoclonal antibodies against fungi was hampered in the first instance by the extreme antigenicity of such organisms and even though useful monoclonal antibodies were reported using isolated proteins as immunogens the production of reagent antibodies of high specificity using unpurified immunogens proved difficult. The introduction of the exoantigen technology of Kaufmann and Standard (1987) to monoclonal antibody production by Dewey *et al.* (1989) as applied to *H. lanuginosa* has resulted in a reliable method for reagent production. Both Kaufmann and Dewey used the extracellular proteins of fungal decay organisms as immunogens since antibodies produced in this manner are found to be generally of higher specificity. The reason for this is not known but may simply reflect the simpler antigenic nature of so-called exoantigens as compared to whole cell extracts. Other methods for producing specific monoclonal antibodies which are likely to be employed in the future include the use of immunomodulating drugs such as cyclophosphamide to restrict the production of cross-reacting antibodies (Matthew and Sandrock, 1987) and *in vitro* immunizing protocols. Such techniques should allow the production of high specificity reagents which would overcome problems of cross-reactivity. However, to what extent problems associated with the non-specific binding of antibodies can be overcome remains to be seen. Preliminary studies in this laboratory indicate that whilst this problem may be minimal with some organisms, e.g. *Heterobasidion annosum* and *C. puteana*, there are others, in particular *S. lacrymans*, which may be able to non-specifically bind both polyclonal and monoclonal antibodies with sufficient avidity to make analysis difficult.

Nucleic acid analysis

The ability to analyse large nucleic acid molecules (i.e. DNA and RNA) in detail dates from the application of restriction enzymes to transform large molecules to small reproducible fragments susceptible to electrophoresis and cloning. Identification and detection of fungi from nucleic acid analysis has recently been undertaken using the techniques of restriction length fragment polymorphism (RFLP) analysis and the polymerase chain reaction (PCR). The former technique depends upon the detection, using restriction enzymes, of the slight differences in nucleic acid sequence found between closely-related organisms (see Michelmore and Hulbert, 1987, for review), the latter on the amplification of specific DNA sequences and their analysis by electrophoretic or blotting techniques. Also recently introduced is the pulsed field electrophoresis method which allows analysis of whole chromosomes from organisms. Whilst this technique has so far made no impact on wood decay fungi its application to the identification of yeast isolates is well advanced (Johnson, 1990).

The differential expression of proteins and antigens during the fungal life cycle indicates that problems are likely to occur when using these types of molecule as the basis for an identification procedure. A more rigorous analysis could be based on the genetic make-up of an organism which is inherently more stable than the products of gene expression. Most work to date on the use of nucleic acids to identify organisms has centred on RFLP analysis of the relatively small mitochondrial genome. An advantage of this technique for the mycologist is that it does not require the use of specific DNA probes and therefore no sequence information is required. For example the identification of aggressive strains of *O. ulmi* has been proposed by Jeng *et al.* (1991) using diagnostic RFLPs from purified mitochondrial DNA. Whilst SDS-PAGE can also achieve this identification it is sensitive to environmental conditions which may cause modification of the fungal metabolism *in vitro*. An alternative use of RFLPs uses radioactively-labelled conserved DNA sequences associated with ribosomal DNA (rDNA) as probes to detect RFLPs associated with the less conserved spacer regions between the repeating rDNA sequences. To date there are no reports of the applications of either technique to economically important wood decay organisms.

An alternative strategy is to amplify specific, or random, DNA sequences using the PCR reaction, and random sequence amplification has been applied to distinguish between human tissue samples (via specific polymorphisms) and plant cultivars (Caetano-Annolles *et al.*, 1991). Application of this new technique to wood decay fungi is now underway and isolates of different strains of *H. annosum* have been typed (Galbraith and Palfreyman, unpublished data) and differences shown between the amplified DNA of the major decay organisms of structural timbers (Palfreyman and McCrae, unpublished data).

CONCLUSIONS

The molecular studies detailed above support the hypothesis that the application of molecular biology to the identification and detection of wood decay organisms should increase the accuracy of determinations of this type. For reasons detailed in the introduction it seems certain that increased accuracy is likely to be an important criterion of future developments.

BIOLOGICAL CONTROL FOR WOOD AND WOOD PRODUCTS

INTRODUCTION

Given appropriate environmental conditions which allow the growth and development of microorganisms, finished wood products are unfortunately subject to decay by a wide array of fungal, and in some cases bacterial, biodeteriogens. The type and extent of the decay will be determined by the particular use to which the wood product is placed, as well as the natural durability of the timber species employed.

As part of the European standardization program for wood and wood products a new standard (BS 335–1 1992) has been introduced which classifies wood products into classes based on the decay hazard to which they are likely to be exposed. This

classification is then taken into consideration when the wood products are treated with preservatives. Hazard class 1 represents timber in an environment where the moisture conditions will remain permanently below 18% and therefore not favourable for fungal development or decay. Hazard class 4, by contrast, represents wood in high hazard decay conditions where it is permanently exposed to wetting and retains a moisture content in excess of 20% for almost its entire service life (e.g. fence posts). The natural decay resistance of timber types differ greatly between species with the heartwood of species generally more resistant to decay than sapwood due to the presence of fungitoxic extractives (Desch, 1981). With the increasing scarcity and cost of the more durable timbers, however, faster growing softwood species with less inherent resistance to decay are increasingly used after appropriate chemical treatment.

THE PROBLEM

Since wood is a good nutrient source for many types of organisms it has an inherent decay potential. However control of wood decay organisms can be achieved by manipulation of the wood ecosystem so that it no longer favours the growth and development of the decay-causing organisms. This can be achieved in a variety of ways.

Traditionally wood has been protected by the addition of toxic chemicals thereby making the environment unsuitable for the organisms. This approach has dominated the wood preservation industry over the last 200 years with a wide array of water-, oil- and solventborne preservatives in use for a wide range of wood products. In more recent years increasing concern over the safety of many extensively used preservatives has been accompanied by more stringent legislative control over the production, use and disposal of toxic wood preservatives (Greaves, 1987). A re-evaluation of many preservative formulations now gives greater emphasis to the permanence, leachability and environmental dangers associated with the chemicals. With these considerations in mind the chemical preservation industry is currently developing new less toxic preservatives and is re-examining the usefulness of some of the more acceptable currently used preservatives for high hazard decay applications.

An alternative chemical approach involves the use of chemical agents to modify the molecular structure of the wood (Kumar and Agarwal, 1983) thereby increasing its resistance to degradation without leaving behind chemical residues which might create environmental problems during the service life of the timber.

Another, perhaps complementary, strategy to the use of chemicals would consist of altering the physical conditions of the wood substrate so that it is no longer suitable for growth of the biodeteriogen and in some instances may even result in eradication of established decay organisms. Examples of methods employing this approach would be to ensure that wood is dried out and maintained in a dry condition or by the application of elevated temperatures to achieve the eradication of decay agents (Koch *et al.*, 1989). While these methods might be appropriate for internal wooden structures they are often impractical for use on many wooden products exposed to fluctuating conditions in exterior use situations.

Both chemical treatment and temperature and moisture control involve manipulation of the abiotic conditions of the wood ecosystem. A third approach to control decay would be to alter the biotic status of the system. This strategy, termed biological control,

involves the application of antagonistic organisms which reduce the ability of the decay agents to colonize or biodeteriorate the wood substrate. Biological control relies on the inoculum potential and natural combative mechanisms of organisms to achieve successful control of decay fungi. It is fair to say that this strategy to wood decay control has received little attention in the past from the wood preservation industry whose products, though highly successful, are in most cases chemically based. With the increasing environmentalist pressure in recent years to reduce the use of toxic chemicals wherever possible, biological control has emerged as an environmentally safer technology for wood decay control and research interest has been stimulated.

THE AGRICULTURAL MODEL

Similar environmental concerns about the use of pesticides in agriculture have in recent years resulted in extensive research into biological control for the suppression of important plant pathogens (Campbell, 1989; Whipps and Lumsden, 1990). It is fair to say that the insight gained by researchers in this field has acted as a very valuable resource for the study of the biological control of wood decay fungi. Many of the organisms applied as antagonists of wood decay fungi have been selected for study because of their successful exploitation in agriculture. Whilst research in agriculture may act as a valuable model for the development of biocontrol agents to prevent timber decay it is, however, important to note that a number of very significant differences exist between the requirements for control in the two situations.

Period of protection

Agricultural research has shown that soils may remain suppressive to disease for a number of years (Baker and Cook, 1982), although it is usually sufficient for the control system to be effective merely over one growing season. By contrast, and depending on the particular application of the wood, biological control systems may have to remain effective for anything up to 40 years to compete favourably with current chemical wood preservatives. This means that either the control agent must remain viable over this period or it must produce some form of metabolite which will remain *in situ* for the expected field life of the product. There are instances, however, where wood must be protected from fungal attack for relatively short time intervals (e.g. wood chip piles prior to pulping) and it may be that these applications will be the first to exploit biological control.

Efficacy of the system

With many agricultural crops it may be acceptable simply to have a reduction in the crop yield provided other priorities are satisfied. For example, the use of biological control could result in a 95% yield compared with chemical treatments but this reduced yield may be acceptable if it is achieved without the risk of environmental pollution and is cost-efficient. Such an ‘acceptable failure’ philosophy is unlikely to be acceptable for wooden structures where the strength of the material can be dramatically reduced even though the activities of the decay organisms have been largely controlled. If the strength

properties of structural timbers are reduced then the safety of the structure will often be compromised—clearly an unacceptable situation.

Availability of nutrients for biocontrol agents

While soil would never be classified as an ecosystem with an abundance of available nutrients, biological control agents in agricultural systems can nevertheless exist on the regular supply of nutrients released from plants in the form of root and leaf exudates and can therefore colonize the substrate to be protected. Wood by contrast does not contain a renewable supply of nutrients to sustain a biocontrol agent. Biological control agents cannot afford to use any of the structural carbohydrates of the wood which they are designed to protect and so must exist on either the soluble sugars left in the timber after drying (provided these have not been previously removed by leaching) or be applied together with extraneous nutrients.

Delivery system technology

The development of appropriate delivery systems for agricultural applications of biological control agents has not been particularly difficult since many control agents can be manufactured in a form which can be applied using delivery systems similar to those already developed for chemical pesticides, e.g. seed coatings or spray inocula. By contrast it is most unlikely that a similar transfer of technology could be used when applying biocontrol agents to many wooden products. Chemical preservatives are often applied to timber at high temperatures, with the use of pressure, and these processes are unlikely to be suitable for the application of living organisms. However, in their favour, biological control agents are living organisms and if applied under conditions favouring growth may be able to colonize substances and effectively deliver themselves to the appropriate position.

Target specificity

One of the most important differences between biological control systems in agriculture and wood is the range of target pests which must be controlled. The prevailing environmental conditions will determine the range of biodeteriogens which will attack a wooden product and similarly will play an important role in selecting which pathogens will attack a particular plant species. Living plants, however, also have a range of constitutive and inducible defences which help to reduce the range of pathogens which are able to colonize them. Once timber is felled and processed these defences are removed and subsequently the range of organisms able to attack the timber is increased. This in turn makes it more difficult to develop biological control systems for the material since one of the major attractive features of biocontrol agents are the specificity with which they act.

Due to particular selective pressures, however, there are some instances where wooden structures are attacked largely by single fungal species, e.g. *Serpula lacrymans* dry rot decay in buildings or internal decay in creosoted distribution poles which is principally

caused by *Neolentinus lepideus*. It may well be that these or other similar specific wood decay problems will be the initial targets f or commercial biological control systems.

CONTROL OF TIMBER DECAY

Much research has been undertaken in recent years to develop biological control systems for the protection of various wooden products. Freitag *et al.* (1991) give a comprehensive review of the research and identify a very important differentiation between 'bioprotectants' which act as prophylactic control agents and are applied prior to the onset of any decay and 'biocontrol agents' which are used to eradicate established decay in wooden structures. These authors have also identified the likely requirements for biological control agents in each circumstance.

Many of the initial studies on biological control of wood decay focused on protection of growing trees and stumps against pathogens which often also cause decay of the felled timber. Targets for these control systems have included *Heterobasidion annosum* (Rishbeth, 1963; Ricard, 1970), *Chondrostereum purpureum* (Corke, 1974; Mercer and Kirk, 1984a, 1984b), *Phellinus weirii* (Nelson and Thies, 1985) and *Ophiostoma ulmi* (Atkins and Fairhurst, 1987). While these examples might reasonably be considered to be agricultural applications of biological control more recent research has concentrated on control of decay in finished wood products.

Much of this work on harvested and seasoned wood has concentrated on the use of *Trichoderma* spp. as potential antagonists of a wide range of wood decay organisms (Bruce, 1983; Morris, 1983; Bettucci *et al.*, 1988; Highley and Ricard, 1988; Murmanis *et al.*, 1988; Siefert *et al.*, 1988; Morrell and Sexton, 1990). Other microorganisms have also been examined by different researchers, including bacteria (Preston *et al.*, 1982; Bernier, 1986; Benko, 1988, 1989) and other mould fungi (Morris and Dickinson, 1981; Siefert *et al.*, 1988; Highley, 1989). *Trichoderma* is chosen as a potential antagonist by many researchers because members of this genus have been extensively studied as antagonists of plant pathogens in agriculture (Papavizas, 1985) and early studies using this organism in wooden poles gave encouraging results (Ricard, 1976). Ricard reported that *Trichoderma* spp. could become established in a very high proportion of poles artificially inoculated with this biocontrol fungus.

Trichoderma spp. have also been found to exhibit a wide variety of mechanisms by which they can inhibit other fungal species, some or all of which might be active against wood decay fungi. These include: production of fungistatic and fungicidal volatiles (Dennis and Webster, 1971; Bruce *et al.*, 1984a); production of lytic enzymes including chitinase and β-1, 3-glucanase as part of a mycoparasitic process (Elad *et al.*, 1982; Murmanis *et al.*, 1988); production of siderophores to compete for available iron (Srinivasan, 1993); a high inoculum potential associated with removal of available sugars (Hulme and Shields, 1970) and the production of a wide variety of soluble metabolites with fungitoxic properties as reviewed by Taylor (1986). This extensive arsenal which *Trichoderma* spp. are known collectively to possess, allied with their history of antagonism against a wide range of plant pathogens, suggests that they are an appropriate choice for study against wood decay fungi. Unfortunately much of the research examining the modes of action of *Trichoderma* against wood decay fungi has been done using laboratory cultures which bear only limited similarity to field conditions.

A MODEL SYSTEM

While laboratory studies to screen potential control agents and elucidate their antagonistic mechanisms are an essential part of the research and development of any biocontrol system they must be reinforced with information from long-term field performance trials. Unfortunately there are many instances where control agents have exhibited good antagonistic capabilities when tested on agar media yet have failed to produce control when tested in the field.

Work at Dundee Institute of Technology over the last 10 years has examined the use of *Trichoderma* spp. to control decay in a particularly high hazard situation, i.e. internal decay in creosoted distribution poles. The research has examined both mechanistic aspects (Bruce and King, 1983; Bruce *et al.*, 1984a; Bruce and Highley, 1991) and field performance (Bruce, 1983; Bruce and King, 1986a, 1986b; Bruce *et al.*, 1990). The work can therefore be considered as an important case study of the use of *Trichoderma* for control of wood decay. This application of biocontrol is particularly appropriate since the decay fungus *Neolentinus lepideus* is predominantly responsible for internal decay in this creosoted product and so should be an ideal target for a specific biocontrol agent.

Internal decay of creosoted distribution poles is a major problem for the electrical supply industry with decay being difficult to detect, especially in the early stages. Current treatments involve the application of chemicals into the groundline regions many of which are known to leach into the surrounding soil environment. While the development of a biological control system for this application is attractive, it is a significant challenge. To introduce a control agent such as *Trichoderma* into an established ecosystem where it is not commonly found, and yet expect it to produce long-term control comparable with that achieved by chemical biocides, is asking a lot. Another constraint is the lack of available nutrients in the poles where any soluble sugars are likely to have been leached by rainwater after many years' exposure to the elements.

Field trials were set up to determine:

1. whether *Trichoderma* could become established in poles artificially inoculated with the control agent;
2. if the control of *Neolentinus lepideus* achieved in laboratory culture could be reproduced in poles under field conditions;
3. the longevity of any protection.

Field studies on 200 creosoted poles inoculated with Binab FYT pellets (a biocontrol product manufactured by Bioinnovation AB Binab, Sigtuna, Sweden, containing spores and mycelial fragments of two *Trichoderma* spp.) resulted in successful establishment of *Trichoderma* in the groundline region of greater than 90% of all poles at three different geographic locations in the UK within 4 years of pole inoculation (Bruce and King, 1986a). The small numbers of poles in which *Trichoderma* was not established were found to be either too dry or too wet to permit fungal growth and as such were not at risk from decay.

A second experiment set up to monitor the extent of *Trichoderma* colonization and decay control in the field showed that spread of the *Trichoderma* throughout the decay-susceptible groundline region was variable and appeared to be limited by the populations of non-decay fungi already resident in the poles (Bruce and King, 1986b). This experiment involved the inoculation of poles with both *Trichoderma* and *Neolentinus*

lepideus. After incubation periods of up to 18 months the poles were sacrificially sampled and numerous samples were isolated and cultured onto selective media. Identification of isolates allowed the spread of *Trichoderma, Neolentinus lepideus* and other pole inhabitants to be mapped with the aid of a computer program (Bruce *et al.,* 1984a). While inoculation of poles with *Trichoderma* did produce a significant reduction in the incidence of decay compared with control populations, complete control was not achieved.

It was hoped that with extended incubation in the poles the *Trichoderma* might continue to spread and thereby improve the levels of control. However, sacrificial sampling of a smaller number of poles 6 years after inoculation revealed that levels of colonization by the *Trichoderma* were no greater than those found 18 months after inoculation and indeed the control of decay in these poles was lower than that achieved earlier (Bruce *et al.,* 1990). Once again the *Trichoderma* had failed to spread throughout the regions of the poles which were inhabited by other resident organisms.

Despite the failure of the *Trichoderma* to completely control the decay fungus after extended field exposure this later experiment did produce a number of very important results. It established that *Trichoderma* could remain viable in the poles over extended periods under field conditions. More importantly wood removed from regions of poles colonized by *Trichoderma* were found to be resistant to decay (Bruce *et al.,* 1991). Wood blocks cut from those parts of the groundline regions of the poles which had been shown by isolation and mapping studies to be totally colonized by *Trichoderma* were exposed to a limited range of wood decay

Table 8.1 Mean percentage weight loss in blocks removed from *Trichoderma*colonized regions of a biologically-treated creosoted distribution pole after 12 weeks' exposure to Basidiomycete fungi. Controls were unsterile material from regions of pole interiors not colonized by *Trichoderma* (from Bruce *et al.,* 1991)

Block type	% Weight loss produced by			
	N. lepideus (Pole isolate)	*N. lepideus* (BAM 20)	*A. carbonica* (MAD 141)	*T. versicolor* (MAD 697)
Test samples			,	
*Trichoderma*infected region of pole	2.2±2.1	1.3±0.8	1.4±0.3	6.2+0.9
After steam sterilization	52.5±3.2	29.5±4.8	15.2±5.6	8.4+2.2
Controls			,	
Region of pole not infected by *Trichoderma*	45.7±6.2	28.7±8.2	N.T.	5.4±3.3
After steam sterilization	44.8±3.8	30.5±1.9	20.2±5.3	12.2±1.6

N.T., Not tested.

fungi using the standard American soil block test for measuring the decay resistance of preservative-treated wood. The results (Table 8.1) clearly show that the *Trichoderma*-

infected wood gives total protection against the brown rot decay fungi *Neolentinus lepideus* and *Antrodia carbonica* but is not completely effective against the white rot fungus *Trametes versicolor*. This result has shown for the first time that, providing adequate colonization by *Trichoderma* can be achieved, the fungus can protect wood from decay for extended periods under field conditions. The protective effect was lost, however, when the *Trichoderma* was killed by steam sterilization of the blocks prior to their exposure to the decay fungi. This indicates that the protection is perhaps dependent on the viability of the control agent or is due to a metabolite which is not particularly heat stable.

FUTURE RESEARCH

Before biological control systems can be fully exploited commercially further research is required. Major research objectives to overcome the problems detailed above and those identified by other studies in this field are:

1. systematic screening of other potential antagonistic organisms;
2. identification of the *in situ* modes of action of control agents;
3. development of appropriate formulations and delivery systems.

Only a limited number of potential antagonists of wood decay fungi have been studied to date and a more extensive and systematic screening of possible control agents is therefore required. It is reasonable to assume that a more systematic and comprehensive screening regimen would result in the discovery of many more promising isolates. However in order for such screening to produce useful results interaction conditions must be carefully controlled. Our experiences have shown that the outcome of interactions between *Trichoderma* isolates and target wood decay fungi can often depend on the nutrient status of the medium. Freitag and Morrell (1990) have developed a small wafer assay for screening potential antagonists. This, or an agar media that more closely resembles the nutrient environment found in wood, may provide a screening system more accurately reflecting the antagonist's control capabilities.

Only after the mode of action of the biocontrol agent in the wood has been established will it be possible to develop more appropriate screening methods to examine closely related isolates, or indeed to enhance the antagonistic abilities of control strains in an attempt to maximize their efficiency. While many modes of antagonism have been shown by *Trichoderma* spp. against wood decay fungi, most of the research has been undertaken in artificial culture. Work at this laboratory has shown that *Trichoderma* isolates are capable of inhibiting the growth of wood decay fungi by volatiles, soluble metabolites, lytic enzymes and can produce siderophores which may have a role in the biocontrol process. However, whether these modes of action are individually, or jointly, responsible for the control achieved *in situ* in wood remains to be established. Since the nutrient status of the medium has been found to have a pronounced effect on the levels of volatiles, metabolites and enzymes produced by *Trichoderma* isolates (Srinivasan *et al.*, 1992) it is most unlikely that many of these compounds will be produced under the limited nutrient conditions present in wood.

The final though nevertheless crucial stage in the commercialization of any biological control system, is the development of an appropriate formulation of the active ingredient

together with an effective delivery system. As mentioned earlier this presents a particular problem for biological control agents for use in wood since few technologies are currently available for this purpose. Our experience from field trials with *Trichoderma* was that the spread of the organism in poles was limited and that this failure adversely affected the control achieved. While a multiple injection of the Binab product would almost certainly have improved the colonization potential of the fungus, the addition of selective nutrients to the inoculum might also have enhanced the spread of the *Trichoderma*. An additional approach could be the application of the control agent with an environmentally acceptable chemical as some form of integrated control.

Any new concept such as biological control, which it is hoped will have a long-term effect, is likely to be perceived with a degree of suspicion, especially as biological systems are often considered as essentially ephemeral. While biological control of wood products may be limited, at least in the first instance, to specific applications there is little doubt that with further research it can fill an important niche left by the removal of toxic chemicals for wood preservation. It is important, however, to ensure that all biological control products are fully researched prior to commercial use in order to avoid expensive failures that would only act to diminish the confidence of the wood preservation industry in the concept of biological control.

SUMMARY

The identification of fungi causing damage to timbers in buildings and in a variety of other situations has traditionally relied upon observation of morphology, use of complex keys and experience. In this chapter the possibility of using techniques of molecular biology based upon protein, antigenic and nucleic acid methodologies is explored. Analysis of a range of organisms including the dry rot organism *Serpula lacrymans* and the organism causing destruction of distribution poles *Neolentinus lepideus* are discussed. Currently, molecular methods can only act as an adjunct to traditional techniques, but the further development of, in particular, immunotechnology and PCR technology offers great promise for the future.

Though biological control systems have been successfully used for the control of some specific plant pathogens, the potential for bioprotection of wooden structures by fungal control agents has not yet been fully investigated. This chapter highlights the particular difficulties associated with the development of biocontrol systems for wood protection and briefly reviews the previous work undertaken in this field at Dundee Institute of Technology as well as highlighting the benefits of the technology at a time of increasing environmental concern over the use of toxic wood preservatives.

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Chemical control of fungal decay in buildings

9

E.Austin Hilditch

INTRODUCTION

Decay in buildings only occurs when and where there is a high level of damp. Always the first step in eliminating decay is discovery of the reason for dampness followed by its elimination. Damp gets into buildings in many ways; elimination and prevention is mostly by building works, but for rising damp the most common remedy is the installation of a chemical damp-proof course.

The second step in the elimination of decay entails the removal of decayed and infected timber, followed by chemical treatment. The purpose of chemical treatment is to sterilize and protect sound timber that is to be left in place, and to sterilize infected walls, grounds, etc. Both parts are essential; despite some misconceptions, chemical treatment has never been advocated or practised as the only measure against decay; only exceptionally is decayed timber treated and retained. Examples are small-scale shallow wet rot where treatment may penetrate fully into the infected timber, or for historic panelling, works of art and the like.

Preservative pretreatment of timber before installation in a new building is recommended in situations where experience has shown there to be a high risk of decay. Following decay all replacement timbers should be treated.

MODERN INDUSTRY AND ITS GROWTH

Historically, eradication of decay in buildings was by repair and replacement by local builders. Use of chemicals commenced in the mid-1800s,

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but it was not until more effective products based on organic solvent carriers became available in the second quarter of the 20th century that use became widespread. Preservatives became available to the general public and to the building tradesman in the 1930s, through companies such as Cuprinol and Solignum.

Today treatment of decay, for any large outbreak, is largely a specialist industry, founded in the 1930s with the establishment of companies such as Rentokil and Richardson & Starling. It grew in the years following the Second World War with the need to make good the results of wartime neglect; further growth followed the introduction, by most building societies, of a requirement that surveys examined for both rot and woodworm, and making treatment a condition of mortgage. By the late 1960s the industry had acquired something like its present shape.

Most specialist companies are members of the British Wood Preserving and Damp-Proofing Association, but many other firms carry out remedial work of this type on a regular, or occasional basis; estimates of the total size of the present industry are difficult. A few companies operate nationally, most are regional. The basic strength of the industry, and the quality of its work, is demonstrated by the giving of long-term guarantees (usually 20 or 30 years) on work carried out, a practice started in the 1960s and now near universal. Guarantees are issued by individual companies. Independent back-up, that becomes effective if a company cannot meet its obligations, can be obtained through the Guarantee Protection Trust, providing that the company carrying out the work is a member.

BASICS OF REMEDIAL TREATMENT

Decay is caused by fungi; fungi require water to grow, and wood will only decay if sufficiently wet. For wet rot the moisture content must be above 30%, and for dry rot* above 20% (moisture as a percentage of the dry wood). Decay will continue and spread at these levels but spore germination is only likely at even higher moisture contents; moisture will exceed 30% (fibre saturation) if the wood is wetted by liquid water, directly, or through contact with wet ground, masonry and the like; rarely by absorption of moisture from the air.

In an unheated building, without defect and with some ventilation the moisture content of timber should not exceed 18% in the winter. For

* The term **dry rot** is used in this chapter exclusively for decay by *Serpula lacrymans*. Historically it was used for decay by several fungi, but from about 1960 the term has increasingly been used with this restricted meaning. Initially it was more usual to use the term **true dry rot** to be specific accepting that dry rot also had a wider meaning; this is now less common. The wider meaning is still sometimes used in non-technical works and in other countries; there may sometimes be confusion.

heated dwellings 14% is more typical, falling to 9% with full central heating. The presence of decay is therefore an indication that excessive water is getting into the building.

If infected wood is dried below the threshold level, active fungal growth and hence the progress of decay will cease. It is sometimes argued that if dampness is fully overcome there is no need for further measures, but the fungi will only die if conditions remain dry enough for sufficiently long. Different fungal species differ greatly in their ability to survive; in adverse conditions some die quickly but some, notably dry rot, can survive for 10 years in favourable conditions. Wherever fruiting bodies have been produced spores will abound (especially with dry rot). Spores are better able to survive dry conditions than

mycelium. Once any decay has occurred in a building it should be presumed that there will be residual infection from mycelium-infected timber, masonry, etc; spores will grow and reinitiate decay if a favourable moisture level is again reached. Hence the essential need for both the removal of all infected timber and other materials and full sterilization and protection of remaining timber.

With outbreaks of dry rot, which is both more invasive and able to grow at lower moisture levels than other f ungi, inclusion of full chemical treatment is essential. For extensive wet rot or when it is in essential structural timbers full treatment is advisable, but wet rot is often limited to a small area or even only part of a piece of timber; with small isolated occurrences it may be acceptable to limit treatment, removing all decayed timber and replacing it with pretreated timber. Exceptionally, with wet rot, never with dry rot, decayed pockets may be left in place and saturated with preservative; this is not the best practice, but economics may make it acceptable. In any place where current codes recommend use of treated timbers, or if there is any prospect of recurrence of damp, treatment should be in full.

Reliance on damp-proofing alone carries with it substantial risks. Buildings can take a long time to dry out, particularly if walls are very thick. Regrowth of surviving fungi is a risk during this period, if dampproofing measures are ineffective, or if there is subsequent entry of damp by another route. Preservative treatment is essential for an assured cure, the added cost of a preservative treatment is seldom more than a small proportion of total costs. The cost of a future recurrence can be large.

Chemical treatment is carried out within a building for several reasons: (i) to eradicate any fungi that has only just started to grow into timber that does not yet appear decayed, (ii) to kill any mycelium missed in cleaning up, (iii) to sterilize surfaces contaminated with spores, (iv) to contain mycelium in walls (dry rot) and (v) to provide a level of protection against future infection and attack.

ERADICATING DECAY

INSPECTION

The first step is a detailed inspection, the prime objectives of which are:

1. to find the reason for the dampness;
2. to determine the type and full extent of decay and infection;
3. to determine what structural repairs and other works are needed;
4. to determine the nature and extent of chemical treatment needed.

Table 9.1 Treatment of decay

Step	Wet or dry rot	Dry rot
1	Determine full extent of attack on timber. Locate cause of damp	Determine extent of infection of walls, sub-floors, etc.
2	Cure dampness. Include provision of ventilation where necessary	
3*	Cut out and remove all decayed	Extend cutting out to include 0.5 m of apparently sound

	timber	timber
4		Strip off infected plaster, extending to 0.6 m beyond observed growth Clean surface of masonry, brickwork, etc. removing any growth
5	Clean up and remove all infected material and dirt	
6*	Treat all timber that is to be left in place	Treat infected masonry sub-soils, etc.
7*	Complete building repairs. Use preservative-treated timber for replacement	So far as possible avoid fixing timber into walls in infected area

* The order in which these steps are carried out may be dictated by structural considerations.

It is good practice to identify the source of dampness first; often, but not always, this is fairly obvious. With decay in ground floors dampness is often due to moisture rising up walls from the ground; this may be aggravated by condensation on the cold, damp surface. A line of efflorescent salts at the periphery is a strong indication of rising damp. Ground floor timbers may also get wet enough to decay where there is no, or inadequate, ventilation to the sub-floor. Condensation often allows growth of surface moulds, but decay is only likely to occur from condensation alone if there is persistent, heavy condensation on the timber (unless coupled with another source), or if condensation on impermeable surfaces, e.g. tiles, runs down onto wood. It can be difficult to be certain of the source of condensation; it might be far removed from the effect.

Prevention of rising damp involves the installation of a damp-proof course (dpc), usually chemical. Work necessary to prevent entry of water in other ways and to restore and ensure maintenance of dry conditions may include the treatment of external walls with water-repellent chemicals to prevent rain penetration, while general repairs and the like may entail use of mastics or coatings based on bitumen, silicone or other synthetic polymer sealants. Adequate ventilation should be provided around all timbers especially below suspended ground floors as a precaution against future problems even where it is not the main cause of dampness at the time of inspection.

Dry rot *(Serpula lacrymans)* must be distinguished from wet rot. It is not essential to determine the specific species or even type of wet rot (white or brown), all wet rots are treated in the same way whereas dry rot requires specific treatment. Moulds and other non-decay fungi must be differentiated from decay; moulds spoil finishes and may damage fabrics, paper and other materials but do not cause wood decay and do not, usually, damage building structures in any other way. Their presence indicates damp conditions, it may only need a marginal worsening, or more time, for decay; damp should be cured and mould cleaned off.

In any building that is generally damp there may be several separate outbreaks; initial inspection needs to encompass the whole building. Finding the extent of decay commonly necessitates opening up woodwork, probing through paint, lifting floor boards, and so on.

Dry rot hyphae almost always grow deep into walls, spread over sub-floor soil and into other inert materials. Commonly, growth will extend 2–3 m from any wood and exceptionally it may reach 10 m. Determining the extent of this growth is essential as both timber and masonry must be treated. Opening up should be more extensive than

with wet rot, especially examining sub-floors, looking behind panelling and plaster, probing for lintels or other timber hidden behind plaster, finding the extent of growth in cavities and along any service or other ducts, and so on. Examination should be on both sides of infected walls, and should extend into joining properties.

PREPARATIONS

Timber that is decayed should be cut out. Cutting out should extend into apparently sound wood since hyphae are likely to have spread into timber that is not yet obviously decayed; with dry rot cutting out should extend 500 mm beyond any apparent infection. Where structural timbers are removed, temporary support may have to be provided; if replacement is made before chemical treatment ensure that new timbers do not mask original timber from treatment.

With dry rot all plaster should be removed from infected wall areas and for 600 mm beyond any growth.

The last step before chemical treatment is a general clean up to clear the building of decayed wood, fungal bodies and any debris that might be a source of infection, and making timber and walls ready for treatment. Remove any surface coatings that might prevent acceptance of treatment, remove insulating or similar materials and dirt that might absorb the fluid, etc.

TREATMENT OF REMAINING TIMBER

Obviously, timbers in place in a building can only be treated *in situ*. This limits choice to methods such as spray or (f or DIY application) brush. These methods provide the lowest level of protection of any method in use; preservatives must have good penetrating power to compensate, thus only organic solvent type preservatives are used; penetration is sufficient to kill any fungal infection on, or just into, superficially sound timbers.

Greater protection than is provided by a spray treatment can be obtained by injection of liquids, or application of pastes. These are more expensive but can achieve through penetration and correspondingly high protection. They can also be used where it is necessary to avoid spray mist. Application must be to all surfaces, including ends, which often presents practical problems. Achievement of levels of treatment specified by manufacturers for individual products is essential for effective treatment. Industrial methods of treatment cannot be used *in situ*.

Spray

The commonest way of treating timber in place in buildings is by spraying all accessible surfaces until the required treatment is obtained or no further fluid is absorbed. In most ordinary timbers this is effective for dealing with insect attack, but has limitations in fungal eradication and prevention.

Spraying is carried out using a low pressure pump delivering to a course-jetted spray head, usually on a lance (1 m). Spray should be held sufficiently close to the timber to avoid excessive overspray but not so close as to cause bounce back.

Several passes may be necessary to get sufficient absorption. Target treatment rates should be around 250 ml/m^2 of timber surface*. Penetration into pine sapwood can be expected to be 5–10 mm, but is much less in most other timbers. It is perhaps only 1–2 mm in oak heartwood, but even industrial treatments cannot improve on this.

* All treatment rates relate to prepared timber; rates for rough sawn are around double.

The effectiveness of spray treatment is partly dependent on the operator, but properly applied, is generally satisfactory for the purposes discussed here. Where surfaces cannot be opened up for spray treatment, and the timbers are judged to be at risk, injection or pastes should be used. Risks during application are mainly associated with spray drift, and include health, fire and staining of plaster or other materials through overspray.

Injection

Injection is carried out by drilling holes into the timber and feeding preservative fluid into these holes. The number and depth of holes must be judged for each particular situation being careful not to weaken the timber.

For top surfaces fluid can be fed in through a funnel allowing gravity to drive penetration but it is more usual in professional treatments to put a plastic nipple with an integral valve into the hole, to connect to a pressure pump and to inject under pressure. This technique is widely used for treatment of wet rot in window frames. Holes, 10 mm diameter (to match nipple), are drilled to within 10 mm of the far side of the wood; holes are positioned about 65 mm from joints and at intervals of 300 mm along the length to be treated. On pieces wider than about 75 mm the line of holes should be staggered.

The amount of fluid used varies greatly, perhaps exceeding 100 l/m^3 in sapwood or infected timber.

Paste*

Preservatives can be formulated into a thick paste which will adhere to timber surfaces. They can be pasted on thickly; they break down slowly releasing preservative fluid onto the timber surface; penetration continues as long as there is fluid available; very deep penetration results.

Application can be by brush, trowel, sealant gun or similar device and can be in bands or as an overall coating. The amount put on should be:

For interior timber, not in contact with ground	
with little or no infection	16 kg/m^3
with slight fungal attack	32 kg/m^3
For timber in contact with ground or with moderate decay	80 kg/m^3

While either pastes or injection may be used to halt decay, they cannot restore lost strength in already degraded timber.

* These pastes are technically described as **bodied mayonnaise emulsions;** until recently this term was used commercially; because of the possibility of confusion with salad mayonnaise this description is now deprecated.

TREATMENT OF MASONRY (DRY ROT)

Dry rot *(Serpula lacrymans)* differs from all other fungi in that it will grow for great distances across or into materials from which it draws no nourishment. In particular it spreads from timber to walls and through walls to other timbers. Unless infection in walls and other materials is killed or contained it may grow and initiate a further outbreak. In my experience, failure of a dry rot eradication is more often due to inadequate wall treatment than any other cause.

There is no practical way of ensuring that all dry rot strands within a wall are killed, chemical treatment is aimed at killing fungus in the outer parts of the wall and placing a barrier around the infection in the centre of the wall so that, even if reactivated, the fungus will not be able to grow out to reach and infect wood.

Walls vary greatly in their construction; treatment must be adapted to each specific wall. To prevent further spread up or along the wall, treatment around the periphery must be to the full breadth of the wall: holes are bored at 250 mm intervals 300 mm above and around infection. For single leaf walls, less than 250 mm thick, boring need be from one side only extending to within 50 mm of the far side; for thicker walls drilling needs to be from both sides. For cavity walls both leaves must be drilled and treated separately. A dry rot killer specifically formulated for use on walls is then fed into the holes to irrigate the wall in depth; the amount of fluid needed varies considerably with construction of the wall. Commonly a pump and lance, as used for dpc installation, is employed.

Within the affected area the whole surface must be treated to prevent spread outwards. The surface is flooded with fluid at a rate of about 1 l/m^2 by spray or brush. If infection of the wall is very severe then the whole area should be drilled (600–900 mm spacing) and irrigated with fluid. Solid concrete sub-floors, grounds and subsoil should be sprayed freely.

Growth within the cavity of cavity walls or along any ducts must be killed. Ensure that the treated zone extends beyond the limit of growth in the cavity, this may be further than that on the outer surface. Remove bricks at intervals and spray in the cavity.

TREATMENT OF RISING DAMP (DAMP-PROOFING)

Dampness low on a ground floor extending from ground level to a height of 1.2 m or less is most likely to be due to ground moisture rising in the wall. Progressively this rising damp will carry hygroscopic salts up the wall, so making worse a situation which may be further exacerbated by condensation on the cold surface.

For most of this century a physical damp-proof course (dpc) of lead, copper, slate, bituminous felt or plastic has been built in during construction. Rising damp may be found in older properties or where the original dpc has deteriorated or been bridged. (If bridged, the best cure is removal of the bridge.)

Rising damp can lead to either dry or wet rot in ground floor timbers; decay is confined to near beam ends in the case of wet rot, but may extend over the whole floor in dry rot infestations.

Installation of a dpc

Chemical dpcs are put in place by injecting suitable chemicals in solution into the wall, so as to form a continuous, impermeable barrier *in situ.* Installation is in the same position as for a physical course, that is, about 150 mm above ground level and below any timbers.

Holes are drilled into the wall in a horizontal plane over the length of the affected wall (BSI, 1985). The exact pattern of drilling depends on the construction and on the type of product and method of injection being used; typically, for a brick wall to be injected at low pressure with a water-based product, holes (10–16 mm diameter) are drilled into a horizontal mortar course 150–170 mm apart, avoiding any hole coinciding with a vertical joint. Single block walls, less than 460 mm thick, can be drilled from one side only. Thicker walls are drilled from both sides, with both drillings extending beyond the centre. Injection is by pressure pump through a short lance (with pressure tight seal) inserted into the hole; nominal pressure should be around 350 kPa (50 psi).

The amount of fluid injected (which should be metered) depends on the thickness of the wall and on its construction:

Wall thickness (mm)	115	230	345	600
Litres/m	0.75	1.5	2.25	3.9

Where a solvent-based product is to be used, drilling is into the bricks and high pressure injection may be preferred; holes are drilled at intervals of 120 mm; walls less than 120 mm thick are injected from one side, thicker ones from both sides. Injection is at a pressure of around 700 kPa (100 psi), and should continue until fluid is seen coming out all along.

Some bricks are too dense for satisfactory injection into the bricks, injection should then be into two adjacent horizontal mortar courses and the connecting vertical joint at intervals of 75 mm. Rubble and some other walls may present problems. With any method both leaves of cavity walls must be treated.

TREATMENT OF NEW AND REPLACEMENT TIMBER

BASICS

The resistance of treated timber to decay depends primarily on:

1. the preservative;
2. the amount of preservative;
3. the distribution of preservative in the wood, especially the depth of penetration.

In practice both the amount and distribution of preservative are determined by the method of treatment.

By its nature timber only absorbs liquids slowly; deep penetration, such as is necessary for protection in high hazard environments, can only be obtained by prolonged soaking or by forcing in under pressure. The basic method and detailed treatment schedule should be chosen in accordance with intended use, the degree of protection required and the type of preservative chosen. Other factors related to the end use may also be relevant to the choice.

DOUBLE VACUUM

Double vacuum methods are currently the preferred methods for industrial pretreatment with organic solvent preservatives and are only used to apply this type of preservative. Steps in the double vacuum treatment of timber are:

1. put timber into a closed vessel;
2. draw vacuum (initial vacuum);
3. fill vessel with preservative;
4. release vacuum;
5. hold at atmospheric pressure, or under applied pressure for double vacuum pressure (impregnation stage);
6. release pressure (if applied), remove fluid from vessel;
7. draw vacuum (final vacuum);
8. release vacuum, withdraw timber.

The initial vacuum creates a vacuum in the timber: when released, this pulls fluid into the timber. Hydraulic pressure may be applied if deeper penetration is required. The second vacuum pulls some preservative out of the timber, reducing loading but not penetration.

The penetrations and loadings of preservative to give protection for any specific hazard situations, or to achieve required treatment in timbers of different treatability are obtained by varying the time and level of vacuum or pressure of the various stages; for easily treatable timbers, like European redwood for ordinary use, the treatment schedule is an initial vacuum of −0.33 bar held for 3 minutes, an impregnation time of 3 minutes at atmospheric pressure and a final vacuum of −0.88 bar held for 20 minutes. For less readily treated timbers or for use in higher hazard situations the initial vacuum period is prolonged and impregnation is at 1 or 2 bar pressure held for up to 1 hour.

Schedules for the treatment of timber for use in new construction are detailed in British Standards 6576 and 5589 (BSI, 1989, 1992). There are no standard recommendations for repair timbers; with wet rot, treatment as for moderate hazard conditions (hazard class 2), or as appropriate for the same timbers in a new building, seems appropriate; with treatment as for high hazard (class 3) for dry rot, and as for very high hazard (class 4) if damp is expected to persist or recur.

Fluid uptake typically ranges from 25 to 50 l/m^3, depending on the schedule and the profile of the timber.

PRESSURE

Pressure treatment techniques are similar to those of the double vacuum/pressure method, but hydraulic pressures during the impregnation stage are much higher (up to 14 bar) and held for longer; typically an initial vacuum of −0.8 bar held for 30 minutes, impregnation at 12.4 bar pressure held for 60, 90 or 120 minutes, according to the intended use, and a final vacuum of −0.8 bar held for 15 minutes (BSI, 1989). The only preservatives applied by pressure for building timbers are waterborne.

Uptake is in the range 150 to 300 l solution/m^3, giving standard retentions of 4.0 or 5.3 kg/m^3 of the preservative salts.

IMMERSION

Immersion treatment is simply the total immersion of timber in the preservative; it is used with organic solvent preservers, but not with waterborne ones. The penetration and absorption of preservative is related to the immersion time. The minimum time for any purpose should be 3 minutes giving uptake of around 125 ml/m^2; for dry rot replacement the minimum time should be 10 minutes with uptake around 250 ml/m^2. For more severe situations, such as timber in contact with wet masonry, time should be 1 hour or more; large amounts of preservative are absorbed (40 l/m^3); drying may take some weeks; use before dry may result in bleeding into plaster or other absorbent materials; painting and varnishing may be impeded.

WOOD PRESERVATIVES

Conventionally, wood preservatives are considered in three groups Figure 9.1: tar oil (creosote), waterborne and organic solvent. Creosote is not used in buildings, mainly on account of its strong, persistent odour and its oily, dirty nature. For both organic solvent- and waterborne preservers the active preservative chemicals are dissolved in a carrier liquid. Some compositions used in remedial treatment are atypical and are best considered as separate types—pastes and emulsions.

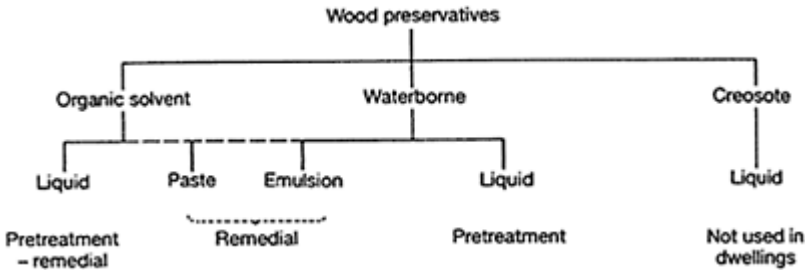

Figure 9.1 Types of wood preservative.

ORGANIC SOLVENT PRESERVERS

Organic solvent preservatives have been the backbone of the remedial treatment industry, for both decay and insects, since its inception; this results from the advantages of ready penetration, absence of swelling, or distortion, rapid drying (relative to water) and cleanliness, giving efficacy greater than any other preservative type when applied by methods available for use in treatment of timbers in place in a structure.

For *in situ* use application is by spray or injection, for pretreatment by double vacuum or immersion. Brush or spray pretreatment is acceptable for amateur use, but for professional use one of the more effective methods is preferred.

Water penetrates particularly slowly; organic solvents of the type used in wood preservatives penetrate more readily; for this reason waterborne preservatives are only applied by high pressure methods, while organic solvent preservatives may be applied by less drastic means, including immersion; techniques employing a combination of vacuum and low pressure are preferred nowadays. For professional treatment, spray and similar techniques are only recommended for timbers already in place in a building.

Solvent

For almost all products in current use, the solvent is a petroleum fraction derived from crude oil, similar to white spirit or paraffin, being primarily a mixture of saturated, aliphatic hydrocarbons with up to 17% aromatics (not including benzene), boiling range between 140 and 270°C. They are flammable.

The nature of wood is such that no liquid penetrates rapidly; non-polar organic solvents penetrate much more rapidly than water; accordingly the depth of penetration obtained with any specific treatment is greater. Three minute immersion in a typical organic solvent gives penetration around 6 mm; for water it is less than 1 mm. The greater efficacy resulting from greater penetration allows organic solventbased products to be applied by simple ‘portable’ methods such as spray or brush.

After application the solvent evaporates; drying time depends on both the specific properties of the solvent in any particular product and on the drying conditions. For most products timber will appear surface dry in a few hours, and in good, well-ventilated situations with temperatures of 15–20°C will be largely dry in 3–5 days with final drying in 7–10 days. In an unventilated, unheated building in the winter drying may extend over a month or more. These solvents allow the use of a range of effective, permanent active ingredients, and the inclusion of water repellents, colours and auxiliary components.

A further feature of solvents is the absence of any swelling or warping, such as might follow impregnation with water; this is of major value in pretreatment of accurately machined timber such as window frames and is sometimes of some benefit in *in situ* treatments.

Active ingredients

The range of active ingredients that have been used for treatment of decay in buildings in the last 10–15 years is quite small. The two most widely used have been pentachlorophenol (PCP) and tributyltin oxide (TBTO), use of which is now restricted. Chemicals now in use include acypetacs-zinc (3% Zn) and other zinc salts of high

molecular weight organic acids (naphthenate, octoate, versatate), organoboron ester (trihexylene glycol biborate), IPBC (3-iodo-2-propenyl-*n*-butyl carbamate), TCMTB (2-(thiocyanomethylthio)benzothiazole), and azaconazole.

Pentachlorophenol

Pentachlorophenol (PCP) is a broad spectrum fungicide with some insecticidal properties, eminently suited to wood preservation. It came into use for this purpose around 1930; it is very economical and became the most common active ingredient for organic solvent preservatives throughout the world. It was widely and generally used for *in situ* treatment of wet or dry rot in Britain, but under the Control of Pesticides Regulations (CPR) application in buildings is now restricted to the treatment of dry rot or cubic rot fungi* in buildings of artistic, historic or cultural importance or where emergency treatment is necessary. Where use is proposed notification of intended treatment must be sent to the Building Research Station before treatment is commenced. Use is permitted for industrial pretreatment of timbers for certain uses.

Tributyltin oxide

Tributyltin oxide (TBTO) was used for both pretreatment and remedial treatment of timber extensively from the late 1960s to 1990. It is a highly effective fungicide particularly easy to formulate and over-paint. Under CPR it is only used in paste products in buildings, or for the pretreatment of timbers for some uses.

Zinc carboxylates

Zinc naphthenate has been in use since the early 1900s. It is an effective fungicide used at a concentration of 3% Zn. Over the last 10–15 years the zinc salts of other high molecular weight carboxylic acids have come into use; foremost amongst these is acypetacs zinc which uses a combination of different acids, conferring some advantage over salts of single acids. The main fungitoxic effect is due to the zinc but with a significant contribution from the acid.

Boron esters

Trihexylene diglycol biborate is used for remedial treatment but not for pretreatment. The activity approximates to the equivalent amount of boric acid. Use for remedial treatment is at a concentration of 1% (as boric acid).

Other ingredients

Recently developed materials coming into use for remedial treatment include: IPBC (3-iodo-propenyl-*n*-butyl carbamate); TCMTB

* The courts have yet to determine the meaning of this term, in this context, or when treatment can be considered an emergency.

(2-(thiocyanomethylthio)benzothiazole); and azaconazole. Related materials such as tebuconazole and propiconazole are being considered.

PASTES

Pastes, as used for remedial treatment, are an ingenious devise for obtaining deep penetration. They can be put onto the sides or undersides of beams where they may take over a week to break down fully. Thus, they provide a supply of liquid preserver during this period; penetration then compares to that obtained by long soak.

Essentially, these pastes are oil-in-water emulsions containing around 80% oil; the structure results from the compression of the dispersed oil droplets. The solvent used is non-volatile. Evaporation of water causes emulsion break down with the release of oil on to the surface; evaporation of water is controlled by formation of a skin.

The oil, which is the carrier for the active ingredients, is an organic solvent derived from petroleum; it is in many ways similar to that used in conventional preservers, but is less volatile, so that it does not evaporate before maximum penetration has been obtained. As a consequence, wood cannot be painted or finished. This is only occasionally important in remedial use against decay, but is a factor in limiting wider use. The active ingredient now used is tributyl tin oxide which has superseded pentachlorophenol.

EMULSIONS

Emulsions, by which is meant free-flowing emulsions with a low proportion of solvent (typically 10–20%), are widely used for *in situ* treatment against insects, but because of minimal penetration are not suitable for use against decay.

Recently, microemulsions (emulsions in which the dispersed oil droplets are extremely small) have been developed for wood treatment. Penetration is greater than for conventional emulsions but it remains to be demonstrated if it is adequate for decay treatments. Should other considerations lead to greater emphasis on immediate sterilant effect and less on long-term protection, penetration will become less important and emulsions may then be more effective.

WATERBORNE WOOD PRESERVATIVES

Waterborne preservatives are simply solutions of chemicals in water. Because of the slow penetration of water into wood they are applied by high pressure (12–14 bar). The equipment needed is such that their use is only possible for industrial pretreatment. Only one type of waterborne preservative is used significantly in the UK: CCA, a combination of compounds of copper, chromium and arsenic. Several ‘types’ are in use, differences being in the exact compounds used and in their ratios; a typical composition is copper sulfate 35%, sodium dichromate 45% and arsenic pentoxide 20% (BSI, 1987). For application they are used as a 2 or 3% solution. After application a chemical reaction takes place whereby the active ingredients become insoluble and so permanent in the wood.

MASONRY STERILANTS—DRY ROT

Most treatment of walls to eradicate or contain dry rot is with simple aqueous solutions. Over the years the most commonly used active ingredient has been sodium pentachlorophenate, usually at a concentration of 5%. This is now only used in certain situations. For general use alternatives are sodium 2-phenylphenoxide and several quaternary alkyl ammonium compounds.

Organic solvent preservatives as used for timber treatment are sometimes used for wall treatment. Large volumes are used and the drying period is prolonged; this has never been good practise in occupied buildings and even in unoccupied ones there seems no valid reason for general use since water-based materials are fully effective.

DAMP-PROOFING COMPOSITIONS

Three types of product are in common use, silicone or polyoxoaluminium stearate dissolved in an organic solvent such as white spirit, and methyl siliconate dispersed in water.

Silicone resins (polymeric methyl silanes) are applied as solutions, typically at 5% concentration in white spirit or a similar solvent. These were the first materials to be used for injection dpcs (in the 1960s); the industry was established on their proven performance. Most of those now in use are ‘universal’ in that they can be used on any common masonry material, but some qualities, especially those first used, give poor performance on limestone.

Siliconates, e.g. sodium methyl siliconate, are soluble in water and are applied as aqueous solutions. On drying these first react with carbon dioxide in the air to form methyl silicic acid which then polymerizes to insoluble polyalkyl silicic acid. These are universal and are usable on brick and natural or reconstituted stone, including limestone. Poly oxoaluminium stearate may be represented as $(O{=}Al{-}St)_n$ where St is a stearate radical. It is applied as a 7.5% solution in white spirit. All these chemicals are inert materials now having proven performance beyond 25 years.

Relatively large amounts of liquid are injected into walls, especially if thick; accordingly, drying is prolonged. Water-based products may be preferred over solvent-based ones if a building is occupied and if solvent vapour needs to be minimized. On the other hand, injection of large amounts of water into an already wet wall will prolong restoration of dry conditions and may delay other repairs. Solvent products are found to penetrate better into some types of wall and the choice between the two types is partly based on the experience of the applicator and partly on requirements of individual jobs.

HEALTH AND ENVIRONMENT

For over a century the safety of wood preservatives has been a consideration in their use; none of the products used over the last 20–30 years have been proven harmful to people when properly used, or, except in the isolated case of bats, to have caused damage to the environment through proper use as a wood preservative. Conversely there have been

accidents, careless or improper use and some of the chemicals used have been found to have detrimental effects through use for other purposes, some have been the target for allegations that have not been fully refuted (for example, pentachlorophenol); prudently some have been replaced. As with other chemicals the emphasis is now on proving safety by extensive testing by the present standards drawn up in light of greatly increased present knowledge.

The potential of any chemical to affect humans, animals, plants or other organisms is highly specific to individual substances; the intrinsic potential of an individual material to cause harmful effects must be considered alongside the nature of these effects, and the risk that they will occur. Risks are related to use: where used, how used, how much is used, how much is needed to cause harm and so on; materials should only be considered unsafe if, used for a proper purpose, in a proper way and with due care, harm still results.

Wood preservatives are pesticides but, from an environmental standpoint, there is a major difference between their use and that of most other pesticides, especially in agriculture. Wood preservatives are, by intent and design, put into wood, they are designed to stay there for perhaps 50 or more years, they employ chemicals with long-term persistence, they persist in the wood. There is no proper use that entails application to people, food, animals or the general environment. They must, of course, be handled properly; any release to the environment is the result of accident, misuse, improper or inadequate disposal, often through ignorance combined with failure to effect proper controls.

Wood preservatives are liquids that dry after application and are conveniently considered in two parts:

1. That part that is permanently retained in the wood after application. This includes the active fungicides and any other ingredient included to improve or modify performance.
2. The carrier liquid, which carries the permanent components into the wood and which, during or soon after treatment, evaporates.

There is thus a clear difference between the two parts. In normal use only the carrier evaporates to the atmosphere; other components are retained in the wood for its life, and at the end of its life will have been degraded, or will be burned and destroyed or dispersed in minute amounts. Only during application is there any hazard from the whole product.

Local damage to the environment results when product is discharged to rivers, ground and the like through accident or dumping. Prevention of such pollution is essential; much legislation is aimed at prevention or containment. Some dumping of liquid waste to ground is still legally permitted; there is a need for provision of other facilities, and for more education, training and control of individuals. Accidents and improper disposal can only be reduced by a combination of knowledge and acceptance of individual responsibility; this is of particular importance with treatments in buildings where products are used on a site with no containing or disposal facilities and with limited supervision.

A total environmental assessment of wood preservatives, as with any other chemical, must include manufacture, distribution and storage. In these respects wood preservatives are no different to other chemicals. These factors are outside the scope of this chapter.

SOLVENTS

Solvents used in wood preservatives evaporate into the atmosphere. They do not pose any threat to the upper atmosphere. Potentially they could contribute to photochemical smog and similar effects in the lower troposphere, but the amounts used are so small in relation to other uses that effects are of no consequence.

Limited, short exposure is not harmful to humans; greater or more prolonged exposure may be discomforting but causes no permanent harm. Only with gross or persistent exposure over many years is there a possibility of more serious effects.

Solvents in the environment

Solvents evaporate into the atmosphere where they break down fairly quickly under photochemical action to carbon dioxide and water; this takes place in the lower atmosphere and typically the half-life for degradation is less than one day (Milner and Gindre, 1990). With such rapid degradation there is no contribution to the greenhouse effect, as happens with small hydrocarbons, such as methane, nor is there any involvement in ozone layer depletion. (The extent, causes and consequences of these is, in any case, still a matter of debate.) Some ozone and oxides of nitrogen may be produced during degradation which might contribute to photochemical smog if other conditions for its formation exist, but again the amounts are so minute compared to, say car emissions that effects can only be negligible.

The amount of solvent put into the environment as a result of the remedial treatment of timber is only a small proportion of the total. In the UK usage is probably below 3 million litres a year (2000 tonnes as carbon); this amount of petrol would allow the whole of the adult population of the country to drive an average car for about 1/2 mile. Total UK emissions of CO_2 from liquid fuels is 48 million tonnes a year (as carbon) (UN, 1989/90) and from all fossil fuels 141 million tonnes. Against this background remedial treatment emissions comprise 0.004% and 0.0015% of liquid and total fuels, respectively; the whole of the solvent used in wood preservation for all purposes, around 30 million litres, is only 0.04% and 0.015%. This amount is so small that emissions due to wood preservation cannot credibly be considered to be of any consequence to any of the atmospheric effects.

Human exposure to solvents

Limited exposure to hydrocarbon solvent vapour appears to produce no ill effects; with greater exposure, effects are headaches, nausea and similar, which disappear when exposure ceases. Beyond these immediate effects they are not generally regarded as a significant threat to human health. (More serious effects can be experienced with concentrations encountered inside storage tanks and the like, which are above those encountered in preservative use.) Ventilation during use and until fully dry will minimize any effect.

Some evidence has appeared over the last few years that operators in some occupations, exposed to solvents over many years, may suffer damage to the central nervous system (Danish painters' syndrome). Many of the studies are complicated or confused by other factors and the evidence for the effects of white spirit type solvents

must currently be taken as indicative rather than conclusive. One study (Mikkelsen, 1988) likens the effect to that of habitual high alcohol intake and suggests that the risk increases only with exposure at a daily average of over 100 ppm for 6 years. Ensuring ventilation has always been considered essential, these indications only emphasize its importance. The occupa-tional exposure standard (HSE, 1991) currently set by the Health and Safety Executive for atmospheric concentration of white spirit is 100 ppm (575 mg/m^3) (time weighted average over 8 hours). A specific standard has not been set for the broader fraction solvents mostly used in wood preservatives; until it is, that for white spirit seems a reasonable guide.

During application inside buildings, atmospheric concentration can be very high both from evaporating solvent and from spray mist. The greatest hazard is spray mist, but it falls out very quickly, leaving vapour as the only potential hazard. Vapour concentrations vary and depend on: rate of evaporation, surface area, amount of fluid used and level of ventilation; the rate of evaporation is effected by temperature and by the volatility of the specific solvent.

Vapour concentration is highest during and immediately after application, decreasing progressively until drying is complete. There is minimal data on operator exposure levels in remedial work, but considering long periods on opening up and other work with no exposure, and providing that safety practices are observed (both during and after spraying) effects of long-term exposure should be avoided. More data would, however, give more confidence. Whatever the level of effect, emphasis is now put on reducing exposure of all workers to solvents. After completion of treatment, in a well-ventilated place in the summer, atmospheric concentrations fall rapidly and may reach a level where there need be no concern. In the colder part of winter, however, with limited ventilation, a level that is unacceptable may persist for a few weeks.

When treatment is carried out in an occupied house, or when a house is occupied before the solvent is dry, then there will be a period during which the occupiers are exposed. This period will be far too short for those effects that may follow long-term occupational exposure to be involved. To avoid the discomfort of the short-term effects, sufficient ventilation should be maintained fully until dry. This is particularly essential if there are babies, children, asthmatics or others with conditions that may make them more susceptible to small amounts. It must also be recognized that some people have a near paranoia about chemicals, and that their smell may initiate worry and distress; always emphasize ventilation, not only in the site of treatment but also in the whole of the building and any connected property. Once fully dry there is obviously no on-going risk to occupiers from the solvent. There is some variation in drying time amongst proprietary products.

Solvents have a degreasing effect on the skin and frequent contact may cause dermatitis. The solvent is also likely to increase the rate at which other ingredients in a formulation are transferred across the skin.

Fire

The solvents used are flammable; at ambient temperatures* vapours do not ignite readily, their flashpoint is around 40°C in a small enclosed space (closed cup) and 60°C in an open space (open cup). Accordingly, vapour concentration does not become high enough

for an explosive mixture to be formed. However, any combination of heating and source of ignition must be avoided until drying is complete, as local heating can raise the temperature above flashpoint.

Fine mists behave as flammable vapours at any temperature even below the flashpoint; during spraying and until all spray mist has dispersed full precautions against ignition must always be taken. Course sprays should be used and so far as is possible, atomized mists avoided. The surface of timber, especially if rough sawn, acts as a wick and while wet with fluid is easily ignited, thus naked flames, sparks and the like must be avoided in the vicinity of treated timber until it is fully dry.

FUNGICIDES

A range of fungicides, insecticides and other permanent ingredients are used in wood preservatives. The effects of over-exposure or environmental contamination are individual to the specific material used. Those now in use have passed scrutiny by the Health and Safety Commission under the Control of Pesticide Regulations and are considered safe for use, provided they are used for the approved purpose and that stated precautions are observed.

Active ingredients are used in relatively low concentrations: in the product typically 1–15%, with that remaining in the wood typically 0.1–1%. The potential hazard from product or treated wood is reduced accordingly. Normally active ingredients remain in the wood for several decades, and are thus inaccessible.

Pentachlorophenol

PCP became perhaps the most extensively used fungicide in preservatives for a multitude of purposes, with some use as a herbicide. As a consequence, it has become widely distributed in the environment. For this reason a WHO report (WHO, 1987) in 1987 recommended reduction in usage. In most uses PCP has now been replaced, but with few alternatives proven suitable for long-term wood preservation, complete re-

[*] At, say 30°C, temperatures in roof spaces on hot summer days in UK may, exceptionally, exceed 40°C, as indeed they may in hotter climates. At these temperatures vapours may ignite if there is an ignition source.

placement presents a major problem. PCP has a slight volatility which results in some loss from the immediate surface. It may be detectable in the air in treated locations.

The effects of PCP on human health remain contentious. There have been a number of poisoning incidents through accident or gross disregard of care in use. Media campaigns in the UK, Germany and other countries have made widespread allegations regarding the safety of PCP; many of these could not be subjected to proper investigation, those that could, generally, did not stand up. Green politics in Germany have become deeply involved and it is now almost impossible to sort out real effects, impartially and without prejudice. There is some reasonable suspicion but no convincing evidence that harm actually results from proper use.

The EEC has proposed a ban on PCP (Council Directive, 1991); giving effect to this in the UK the Health and Safety Executive has revoked approvals under the Control of Pesticides Regulations for marketing or use of PCP-containing products for amateur or professional use, except for professional use against dry rot (not against wet rot). Use in industrial plants is permitted.

Tributyltin oxide

Tributyltin oxide (TBTO) became subject to environmental concern through its use in prevention of fouling on ships and consequent harm to marine life, particularly certain shellfish. Proper use as a wood preservative should not result in marine pollution except through accident or improper disposal. Animal studies gave concern about a potential immunotoxic and teratogenic effect. Insufficient data were available on levels of exposure to fully assess the risk so the HSE withdrew approvals for all amateur use and for all professional use except in pastes in 1990. Use in industrial plants is permitted.

Other ingredients

Those now in use have passed scrutiny by the Health and Safety Commission under the Control of Pesticide Regulations and are considered safe for use, provided they are used for the approved purpose and that stated precautions are observed.

SAFETY

The basic principles of avoiding ill-effects from any chemical are simple: keep them out of, and off, the body, i.e. do not inhale, do not ingest, do not allow onto the skin or in the eyes. For most chemicals, small amounts will not have a harmful effect; safe use means ensuring that any chemical that does get into the body does not exceed the safety limit. Responsibility for safety rests with the user; it might be said that ‘chemicals are neither safe nor dangerous, it is the user that makes them so’.

SAFETY LEGISLATION

Producers have long recommended safety precautions to be taken in using their products; for around 20 years the industry cooperated with the government in a voluntary safety scheme (Pesticide Safety Precautions Scheme; PSPS). This has now been replaced by a body of legislation aimed at not only ensuring that full and proper instructions are given but also that they are observed.

Legislation is partly under the Health and Safety at Work Act, 1974 and associated regulations, particularly the Control of Substances Hazardous to Health Regulations, 1988, and partly under the Food and Environmental Protection Act, 1985 and the Control of Pesticides Regulations, 1986*.

Products must have government consent before they are sold or used. The whole framework starts with the assessment of products and the nature and degree of any hazard and the associated risk; products considered to present excessive danger or on which

information is inadequate will not be granted consent. Workers must be trained, supervised and monitored for their job. A range of guidance notes, training recommendations and codes of practice have been published (HSE, 1989, 1990, 1991b, 1991c).

OPERATOR SAFETY

Those who use any material as part of their daily lives for many years are at greatest risk from it; they are likely to be exposed to higher amounts, more frequently than casual users. The risk is correspondingly greater and the importance of observing safe working practices and taking appropriate precautions cannot be over-emphasized. Safety precautions are often simple, prime responsibility can only rest with the individual operator, but proper training is essential. Management at all levels must provide training, must support and supervise them in practice, and must act against any worker persistently refusing to wear protective clothing or disregarding other safety procedures; in the extreme such persons must not be employed on this type of work. On site the most important personal precautions are: ensuring adequate ventilation, wearing protective overalls, gloves and hats, with eye protection

* All available from HMSO, London.

and masks while spraying. Other precautions to be taken on site are to prevent contamination of food, water tanks and the like and to avoid environmental pollution through spills or disposal.

Fire is always a risk when using solvent-based products, from start of application to final drying, but it is greatest in the early stages. Stringent precautions must be taken, anything that could initiate a fire being rigidly avoided.

For occasional users manufacturers' recommendations, which are always given on labels, must be read, and if followed will ensure safety.

BY-STANDER SAFETY

Any one not involved in a treatment, or not wearing proper protective clothing, should be kept well away during application. After treatment is complete no one, unless wearing gloves, should touch treated wood until it is dry; for children and animals the best way is to keep them away.

It is further necessary to ensure that occupiers are not exposed to excessive solvent vapour during drying: generally it is recommended that rooms are not occupied for 48 hours after treatment, and then that windows are kept open until all smell has disappeared. The whole house, especially bedrooms, should be ventilated until drying is complete; this may take a long time in winter. Extensive work, involving large amounts of fluid, is preferably not carried out in occupied houses, unless the working and living parts can be isolated. In all circumstances occupiers must be warned of the need for ventilation and of the need to avoid starting a fire.

Where treatment is in part of a terrace, block of flats or in one of a pair of semidetached dwellings neighbours must be warned before treatment is commenced, especially if the structure is such that they may be inconvenienced by the treatment.

CONCLUSIONS

The use of chemicals for treatment or prevention of decay is, in many instances, essential if reliable remedies are required. The present drive to avoid use of chemicals stems from the 'chemical phobia' that has become common following publicity from extreme environmental activists; arguments are often unbalanced, dependent on innuendo, with little or no valid support f or claims. Government and industry put their house in order some 20 years ago with the establishment of formal procedures for assessing the safety of materials, but it must be conceded that environmental pressures have resulted in an increase in the attention given to the detailed and scientific assessment of products, in the balancing of hazard and risk, and in attention to safe practices. Under present legislation restrictions are placed on materials for which dangers are apparent, or for which hard data is lacking; great emphasis is placed on detailed communication and training on methods of use, safe practices, disposal and so on. Trends are right, understanding of hazards and risks is increasing, values change, and through all this it is necessary to continue to be assured that preservatives can be used safely, that users know how to use them both safely and effectively and to be sure that there is no detrimental effect on anything except the fungi that they are designed to control.

In biblical times treatment of dry rot (leprosy of the house) (*Leviticus*, **14:**33–55) was a matter for the priesthood and initially involved taking down the infected walls and rebuilding with new materials, then if there was a recurrence, to break down the whole house and dump it in an unclean place.

Today a specialized industry exists which regards eradication of dry rot or any other decay as an everyday matter; confidence in their work is such that the giving of warranties of free retreatment in the event of any recurrence within 30 years is normal commercial practice. In part this is no doubt due to better knowledge and attention to elimination of damp, but in large part this achievement is attributable to the use of chemical compositions for treatment and sterilization of timber and masonry. Discontinue this part and the whole must be questioned.

Dry rot is still the most serious fungal pest of buildings. Its eradication calls for walls to be treated chemically; surface sterilization by flame is not adequate to prevent dry rot spreading out of the wall at some future time. Indeed, in the absence of a reliable chemical containment of dry rot in a wall the only reliable remedy may still be the biblical one of taking down and removing walls, foundations, subsoil, etc. and, in the final analysis, accepting that some houses may be in a terminal condition.

With dry rot that has developed to a fruiting stage, spores are likely to be widespread in the house; if there is any case for changing present practices it is for wider sterilization and cleaning—the biblical routine required washing of clothes of anyone who had slept in an infected house. It would be useful if more specific investigations were carried out into the importance of sterilization versus long-term protection for those timbers that

remain in place and are treated in place. Should emphasis shift to sterilization this could change views on acceptable chemicals and formulations.

Many wet rot outbreaks are small and localized. They may be adequately dealt with by building methods only. Treatment of replacement timbers by industrial methods is always preferable. The use of durable hardwoods is not generally a practical alternative.

With this outlook there should be confidence in the continued use of chemicals for the protection and control of fungi in buildings so long as fungi continue to attack building timbers.

SUMMARY

Methods and materials used for the remedial treatment of decay in buildings using chemical wood preservatives are outlined. Health, safety and environmental aspects are reviewed.

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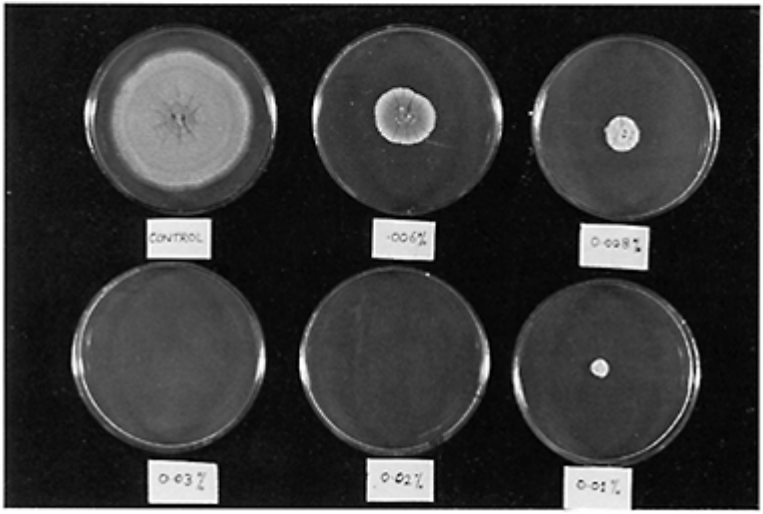
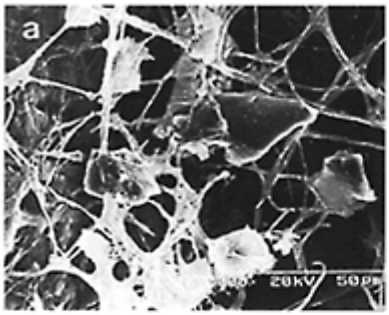
A view of Ajanta Cave no. 10 showing fungal deterioration of wall paintings.

Enlarged view of deteriorated cave paintings.

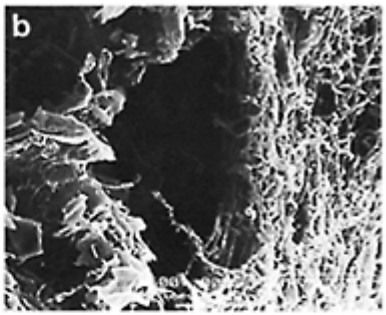


Efficacy of orthophenylphenol against *Aspergillus terreus*.

Micrographs showing some characteristic mechanics of fungal growth

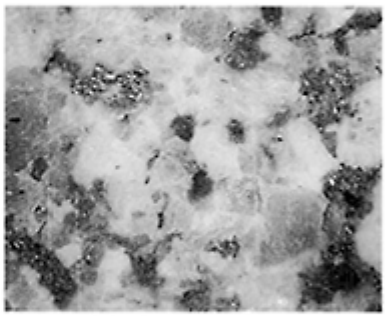


(a) *P. frequentans* growing on sandstone; the mycelium traps, and drags across, mineral particles.

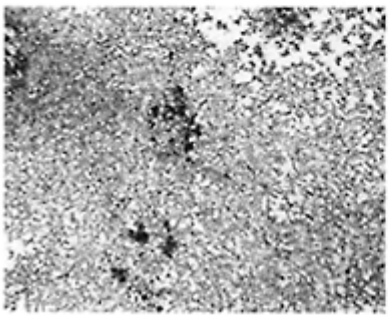


(b) *P. frequentans'* hyphae spread over the rock surface.

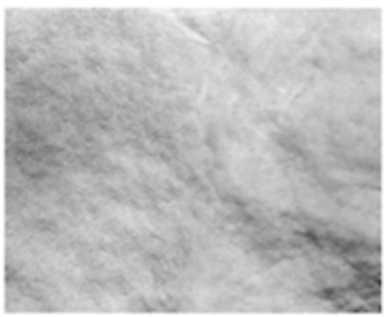
(c) General view of surface granite infested by *P. frequentans*.



(d) Control sample of granite which was not inoculated with the fungus.



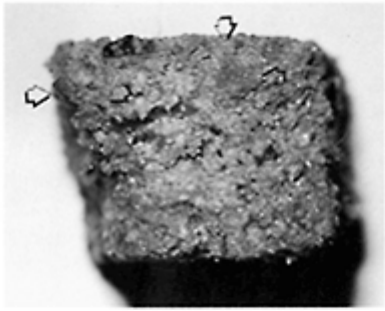
(e) *C. cladosporoides* growing on limestone.



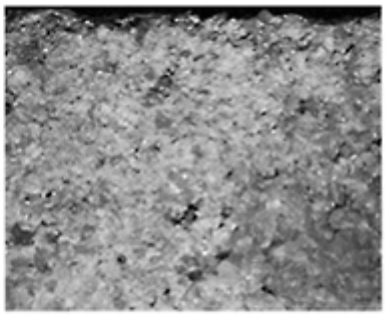
(f) Control sample of limestone.

Parts (a) and (b) were obtained by SEM, the rest by stereomicroscopy.

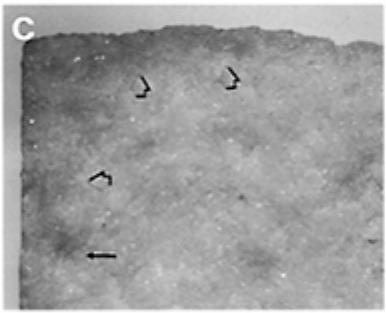
Photographs revealing fungal activity on rock surfaces by red TPF (2, 3, 5-triphenyl formazan) after incubation in TTC



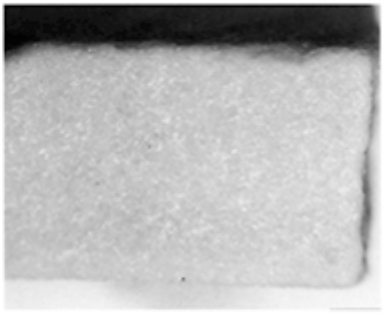
(a) *P. frequentans* growing on sandstone.



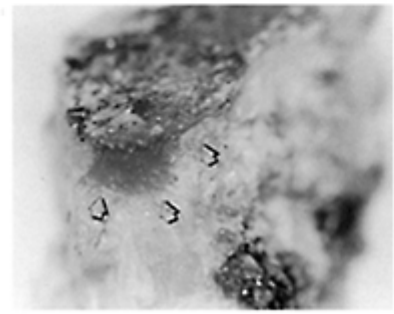
(b) Control samples not inoculated with the fungus.



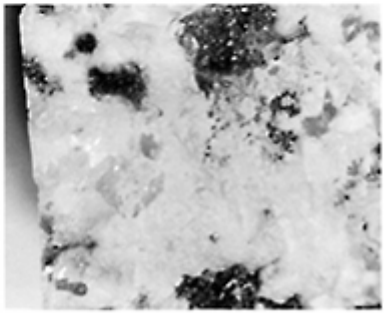
(c) Coloured areas of *P. frequentans* growth over the limestone profile.



(d) Control limestone.



(e) Demonstration of *C. cladosporoides* growth several millimetres under the granite surface.



(f) Granite sample without the fungus after the TTC treatment.

Biodeterioration of wall paintings: the Indian experience

10

Kundan Lal Garg and Shashi Dhawan

INTRODUCTION

Deterioration of wall paintings caused by biological agents is a serious problem throughout the world. They can deteriorate under the influence of biotic and abiotic factors in the environment unless special measures are taken to conserve them. For example, in the Lascaux cave in France the green infection of wall paintings has been reported to be due to bacteria carried on the shoes and sweat of visitors (Lefevre, 1974); consequently the caves have been closed to visitors. The microorganisms develop on the paintings only if the relative humidity of the surroundings exceeds 75% and temperature ranges between 5 and 40°C The early stages of attack by biological agents are relatively easy to control, but once such an attack is widespread it is extremely difficult to deal with.

Murals are made by a range of different techniques. With pure fresco, the paint is applied to wet lime plaster. Calcium carbonate, formed during drying of the plaster, consolidates the pigment particles. When conserving damaged frescoes, imperfections in the painting are repainted with distemper paints containing various binders (Doerner, 1971). The impact of physical/chemical factors in the deterioration of wall paintings, both of the paintworks and of the underlying materials (plaster, stone) has been studied extensively. However, serious damage may also be caused by biological organisms (Tonolo and Giacobini, 1963; Petushkova and Lyalikova, 1986).

Fungi grow on paintings only when the relative humidity exceeds 70%. Several hyphomycetes have been isolated from paint films and surfaces

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(Ross *et al.*, 1968; Denizel *et al.*, 1974) but damage is caused only by a few of these. The species involved are specific to the conditions under which deterioration occurs. *Aureobasidium pullulans, Cladosporium herbarum* and species of *Alternaria, Penicillium* and *Aspergillus* are common on exterior paint surfaces in temperate climates. Of these, *Aureobasidium pullulans* is the most important in causing deterioration of the paint. It is

tolerant of high temperatures and grows over a wide pH range, is resistant to strong sunlight, and has a low requirement for water.

Sometimes, a sudden change of environmental conditions can have a deleterious effect on conserved artefacts. For example, about 300 decorative tumuli located in the Kyushu, Kinki, Kanto and Tohoku regions of Japan were excavated, and everything that had been well preserved in a stable environment underground for ten centuries or more was suddenly exposed to violent changes of temperature, humidity, light, water, air and organisms (Arai, 1984). These tumuli are invaluable historical sources in understanding Japan's 'Kofun' or Tumulus age. However, various kinds of microorganisms began to grow on the murals following excavation causing surface stain, discoloration and flaking. Arai (1984) believed that the very humid environment (95–100% relative humidity) of unexcavated tumuli contributed to the control of microorganisms.

Strzelczyk (1981), in his extensive studies of the microorganisms growing on paintings, reported that although the communities contain a large variety of microorganisms, only few of them develop and damage objects. This was demonstrated by comparing the microflora identified directly under the microscope with that obtained from culture media inoculated with samples of artifact overgrown with microorganisms. To determine the level of microbial contamination of wall paintings and the characteristics of its mycoflora, use is made of a range of quick tests which give extensive and reliable information. Microbial activity can be determined by the presence of adenosine triphosphate (ATP), components of cell walls and membrane lipids and by the activity of glycolysis fermentations. Special staining techniques are being investigated, utilizing fluorescent dyes to detect viable and metabolically active cells in the material, as well as indirect immunofluorescence, scanning electron microscopes (SEM) and methods based on changes in the electrical resistance of the substrate during the development of microorganisms (McCarthy, 1989). However, apart from the work described by Curri (1979), these have been used only infrequently for the investigation of biological damage to works of art, since they require either a large amount of the material to be investigated or equipment not available to restoration agencies.

FACTORS AFFECTING BIODETERIORATION OF WALL PAINTINGS

VISITORS

Microorganisms, including fungi and bacteria, can be carried into caves by air movements and by visitors. The latter disperse bacteria during movement and by breathing, and fungi via sand particles attached to the shoes. Deterioration of rock surfaces in caves may also be caused by bacteria and algae. Condensation water can contribute to deterioration of paintings through its interaction with carbon dioxide (to form carbonic acid) and other biological agents derived from visitors and suspended in the air (Torraca, 1984).

VEGETATION

Plant roots, passing through cracks in the rock structure and reaching the tomb or cave chambers, may cause considerable damage to wall paintings, often resulting in the detachment of the surface rendering by mechanical action. Control of such vegetation is not very difficult but it must be repeated regularly (Giacobini and Barcellona, 1967; Bettini and Villa, 1976; Torraca, 1984).

MOISTURE/HUMIDITY

One of the most important agents of deterioration is the seepage of water through cave walls (Agrawal and Singh, 1975, 1976). Water seepage through crevices and cracks in rocks and walls can penetrate plaster and damage paint. In the presence of water, microorganisms grow rapidly, attacking the binding medium. A systematic study of these microorganisms on Indian wall paintings has been little studied (Tilak *et al.*, 1970; Agrawal *et al.*, 1988). High humidity is also important because it allows microbial attack of wall paintings. Wall paintings have always been vulnerable to high humidity, and thus to damage by microorganisms favoured by it. Construction faults in buildings, resulting from neglect or war damage, contribute to leakage and wall dampness, and favour the development of microorganisms. Excess water first causes swelling, then deformation and cracking of paintings.

Ground water permeating through the walls of buildings and caves can carry salts that are transported from the soil onto the surface of paintings, where they crystallize and promote or inhibit development of microorganisms. Saturated salts can render water less available. Stopping the circulation of ground water in the walls of old buildings is a very difficult task and is often not feasible, because of the excessive cost.

Microorganisms differ in their requirements for available water and temperature, while the availability of water in a substrate, at a given water content, changes with temperature (Ayerst, 1968). Tolerance for low and, with xerophilic fungi, for high humidities is greatest at optimum temperatures while the temperature range permitting growth of a species is greatest at optimum humidity or water activity (a_w). The response of microorganisms to low humidity is greatly affected by the content of nutrients in the substrate which can also affect a_w. High concentrations of nutrients increase the tolerance of microorganisms to low humidity (Ayerst, 1968), perhaps due to increased production of water in respiration.

DEVELOPMENT AND DISTRIBUTION OF MICROORGANISMS AND MECHANISM OF DETERIORATION ON WALL PAINTINGS

Biological changes on murals can be described as:

1. deterioration caused by the activity of autotrophic microorganisms (algae and lichens);
2. deterioration caused by the activity of heterotrophic microorganisms (bacteria, fungi and actinomycetes).

AUTOTROPHIC MICROORGANISMS

High humidity (above 95%) of the plaster and the presence of sunlight promote development of algae on wall paintings. Tonolo and Giacobini (1963) reported that green algae predominate on murals, although they do not list the genera represented. Sokoll (1977) however, identified blue-green algae, green algae and diatoms on plasters and wall paintings (Table 10.1).

Table 10.1 Autotrophic microorganisms isolated from wall paintings and plaster (Sokoll, 1977)

Group	Algal species
Chlorophyta	*Chlorella ellipsoidea, C. vulgaris, Chlorosarcinopsis minor, Gleotila protogenita, Microthamnion strictissimum, Protococcus viridis, Ulothrix punctata, U. zonata*
Cyanophyta	*Microcoleus delicatulus, Microcystis parietina, Oscillatoria brevis, O. irrigua, O. pseudogeminata, Phormidium autumnale, P. bohneri, P. valderianum, Symploca muralis*
Chrysophyta	*Botryochloris minima, Fragilaria spp., Heterodendron* spp., *Pascheri* spp.
Pyrrophyta	*Ceratium hirundinella, Pediastrum* spp.

Strzelczyk (1981) reported that algal growth can be heavy, covering large areas of plaster with a green coat, but that it sometimes avoids areas coated with toxic paints. Sunlight is indispensible for the development of algae but it has a harmful effect on most heterotrophs because of its ultraviolet content. Algal growth on areas exposed to sunlight causes stains of various shades of green, from very dark or almost black, to yellow-green. The shade depends on the predominant species, which in turn largely depends on climatic conditions, especially temperature. Skinner (1971) found that illuminated painted areas in temperate climates became covered with green algae (species of *Pleurococcus, Protococcus* and *Trentepholia*), while under tropical conditions, blue-green algae of the genera *Oscillatoria* and *Scytonema* predominated (Skinner, 1971).

The effects of algae on the surfaces of murals is not limited to the surface, but the paint may be destroyed by organic acids (acetic, glycolic, lactic, oxalic, pyruvic and succinic) secreted by the organisms (Levin, 1962). Algae can also excrete large quantities of sugars and amino acids, which promote the development of bacteria and fungi. Dematiaceous fungi frequently accompany algae because they are resistant to UV radiation through their pigmentation, and contribute to the destruction of murals.

HETEROTROPHIC MICROORGANISMS

Growth of heterotrophic microorganisms (fungi and bacteria) is attributable to dampness of the walls, high ambient humidities and to the presence of organic compounds derived from dust deposits, by infiltration of ground water, or from paints, binders, etc. Paintings made in pure fresco should not, in principle, be attacked by heterotrophic microorganisms, since they do not contain organic compounds in their structure. However, paintings made using the mixed technique *(al secco)* are more frequently attacked by heterotrophic microorganisms. Deterioration of murals by heterotrophic

microorganisms is largely the result of accumulation of dust on the paintings, which absorbs water (Tilak *et al.*, 1970).

Arai (1984) identified nine species of fungi from the Takamatsuzuka murals in March 1975. Using standard methods, *Doratomyces, Fusarium, Cladosporium* and *Mucor* spp. predominated in these samples, and a few *Trichoderma* and *Penicillium* spp. were present. Arai (1984) also isolated microorganisms inside a virgin tumulus. Most numerous in the air were Basidiomycetes and *Penicillium,* followed by *Alternaria, Trichoderma, Aspergillus, Cladosporium* and *Pestalotia*. Among the bacteria, *Bacillus* and *Micrococcus* were the most common together with some actinomycetes. Emoto *et al.* (1983) analyzed the numbers and types of fungi and their activity during the excavation and closure of the Torazuka murals. Fungicidal and germicidal treatments were taken to prevent the Torazuka murals from further damage by microorganisms.

The growth of microorganisms on parts of the wall surface and props in the stone chambers of Ozuka tomb (Fukuoka) and Chibusan tomb (Kumamoto) and in the air, both outside and inside Ozuka tomb were studied by Emoto and Emoto (1974). They reported seven species of fungi in the stone chamber of Ozuka tomb, six species of atmospheric microorganisms and nine species of fungi in the stone chamber of Chibusan tomb (Table 10.2). The microorganisms found on the walls included those with black colonies (*Gliocladium virens, Gliomastix* sp.) and one with green colonies *(Trichoderma viride)* which grew rapidly Another species *(Gliocladium roseum)* produced red stains in colonized areas. Since many of these microorganisms may be brought there by people or in the air, it is necessary to establish measures for protecting the tombs against possible infection whenever they are investigated or inspected (Emoto and Emoto, 1974).

Table 10.2 Microorganisms that deteriorate wall paintings

	Microorganisms	Isolated from	Reference
1	*Aspergillus* spp., *Cephalosporium* spp., *Penicillium* spp., *Sarcina lutea,* fungi from Dematiaceae group, Streptomycetes	Frescoes of ancient Ostia: the Church of St Kliment in Rome; frescoes of Sinorelli	Tonolo and Giacobini, 1963
2	*Aspergillus* spp., *Cephalosporium acremonium, Mucor mucedo, Oospora crustacea, Penicillium* spp., *Pullularia pullulans, Pyronema domesticum, Rhizopus nigricans, Sporotrichum roseum, Stemphylium* spp., *Torula murorum*	Wall paintings in Polish palaces and Roman-Catholic churches	Wazny, 1965
3	*Alternaria* spp., *Aspergillus candidus, Cephalosporium coremioides, Circinella conica, Cladosporium atroseptum, C. epiphyllum, Fusarium* spp., *Penicillium paxilli, P. puberulum, Sporotrichum bombycinum, Trichoderma roseum,* Mycelia sterilia	Paintings of temples of Tsminda-Nikolazi and Tsalindzhikha in Georgia, USSR	Voronina, 1966
4	*Cladosporium epiphyllum, Mucor* spp., *Sporotrichum bombycinum,* Mycelia sterilia	Wall paintings of Pozhaisky monastery in Lithuania	Voronina, 1966
5	*Aspergillus spp., Glio*cladium spp., Mucor spp., *Penicillium citrinum, P. camemberti, P. decumbens, P. frequentans, P. purpurogenum, P. restrictum,*	Frescoes in Florence after the flood of 1966	Gargani, 1968

Stemphylium spp.

	Microorganisms	Isolated from	Reference
6	*Aspergillus versicolor, Bispora menzellii, B.pusilla, Cephalosporium acremonium, Cladosporium cladosporioides, C. elatum, C. sphaerospermum, Fusidium viride, Penicillium meleagrinum, Plicaria muralis, Sporotrichum gorlenkoanum, Stemphylium ilicis, Verticillium lateritium*	Wall paintings of Novgorod Kostroma, Vladimir, Moscow	Kuritzyna, 1968
7	*Aspergillus spp., Camposporium* spp., *Cladosporium* spp., *Curvularia spp., Helminthosporium* spp., *Nigrospora spp., Pithomyces*	Aeroflora of Ajanta wall paintings, India	Tilak *et al.*, 1970
8	*Alternaria* spp., *Aspergillus* spp., spp., *Pseudotorula* spp. *Cladosporium* spp., *Curvularia* spp., *Helminthosporium* spp.,	Aeroflora of Ellora wall paintings, India	Tilak *et al.*, 1970
9	*Alternaria tenuis, Aspergillus Nigrospora* spp., *Pithomyces* spp. *amstelodami, A. niger, A. versicolor, Botryotrichum artogri seum, B. piluliferum, Chaetomium murorum, C. globosum, C. indicum, Circinella sydowi, Cladosporium herbarum, Cunninghamella echinulata, Geotrichum candidum, Mucor spinosus, Penicillium lilacinum, Scopulariopsis brevicaulis, Stachybotris atra, S. cilindrospora, Stemphylium periforme, Torula herbarum, Trichoderma viride*	Paintings of 14–17th century monasteries of Northern Moldavia, Voronet, Sucevita, Humor, Arbore, Patraati, Varatec, Agapia, Putna, the Cozia monastery, the princely church at Curtea de Arges	Savulescu and Ionita, 1971
10	Species of *Arthrobacter, Bacillus, Micrococcus, Pseudomonas, Mycobacterium, Sarcina lutea*	Wall paintings of the Northern Moldavia and Cozia monastery	Lazar, 1971
11	*Alternaria spp., Chaetomium* spp., Actinomycetes, spore-forming bacteria, deteriorating cellulose	Paintings of Church of Saint Petka Samardzhiyska, Saint Panteleymon in village of Boyan, Church of holy Archangel in village of Arbanasi, Bulgaria	Slavova, 1972
12	*Cladosporium* spp., *Streptomyces* spp., coccal bacteria	Frescoes in cathedrals, tombs, burial vaults, Italy	Bassi and Giacobini, 1973

	Microorganisms	Isolated from	Reference
13	*Aspergillus echinulatus, A. niger, A. repens, Chaetomium herbarum, Stemphylium macrosporoideum, S. piriforme*	Wall paintings of 14–17th century monasteries of Northern Moldavia (Hurnor, Moldavita, Sucevita, Voronet, Arbore, Petrauti, Parhauti, Varatech)	Ionita, 1973
14	Species of *Arthrobacter, Bacillus, Pseudomonas*	Paintings of monasteries of Northern Moldavia (Humor, Voronet, Arbore, Moldavita, Sucevita, Parhauty, Patrauti, Neamt Secu, Schity, Sihasrrieri)	Lazar and Dumitru, 1973
15	*Alternaria alternata, Cladosporium* spp., *C.*	Wall paintings of Ozuka tomb	Emoto and

	herbarum, Epicoccum purpurascens, Gliocladium roseum, G. virens, Gliomastix spp., *Penicillium* spp., *P. citrinum, P. citreo-viride, P. janthinellum, P. oxalicum, P. purpurogenum, Trichoderma viride, Verticillium spp., Streptomyces* spp.	(Fukuoka), Chibusan tomb (Kumamoto), Japan	Emoto, 1974
16	*Acremonium roseum, Aspergillus echinulatus, Penicillium chrysogenum, Stemphylium piriforme,* organotrophic bacteria	Wall paintings, Romania	Istudor *et al.,* 1976
17	Filamentous fungi, yeasts, organotrophic bacteria	Paintings of Etruscan tombs, belfrey in Arezzo, Italy	Curri, 1979
18	*Thiobacillus thioparsus, T. thioxidans,* thionic bacteria, organotrophic bacteria, Actinomycetes, Micromycetes	Frescoes of the Rajion Palace in Milan, Italy	Sorlini *et al.,* 1979
19	*Penicillium chermisinum, P. implicatum, Sporotrichum grisellum, Trichoderma viride, Arthrobacter spp.*	Paintings of Roman Catholic church in Dembna Podgolyanskom, Poland	Smyk, 1979
20	*Cladosporium cladosporioides, Penicillium lanoso-coeruleum*	Paintings of burial vault of Saint Sebastian in Pavia, Italy	Bianchi *et al.,* 1980
	Microorganisms	Isolated from	Reference
21	*Acremonium charticola, Aspergillus versicolor, Cladosporium sphaerospermum, Engyodontium album, Penicillium brevi-compactum, P. citrinum, P. decumbens, Phoma glomerata, Micrococcus luteus, Bacillus* spp., *Pseudomonas spp., Nocardia* spp., *Streptomyces* spp.	Frescoes of the monastery of Saint Maria in Uelva, Spain	SaizJimenez and Samson, 1981
22	Micromycetes, organotrophic bacteria, *Streptomyces* spp., Proactinomycetes, *Nocardia* spp., *Micromonospora* spp.	Wall paintings of the Church of Saint Panteleymon in the village of Boyan, Bulgaria	Hadjivulcheva and Gesheva, 1982
23	*Aspergillus niger, Cladosporium cladosporioides, C. cucumerinum, Epicoccum purpurascens, Fusarium oxysporum, Penicillium frequentans, P. oxalicum*	Wall paintings of the old Churches of Pavia, Italy	Crippa, 1983
24	Nitrifying bacteria	Wall paintings of the Church of Saint Panteley-mon in the village of Boyan, Bulgaria	Hadjivulcheva and Markova, 1983
25	*Aspergillus* spp.	Wall paintings in the Swiss Baroque Monastery Church, Switzerland	Raschle, 1983
26	*Cladosporium* spp., *Doratomyces* spp., *Fusarium* spp., Mvcor spp., *Penicillium* spp., *Trichoderma spp., Verticillium* spp.	Paintings of Takamatsuzuka tumulus, Japan	Arai, 1984
	Alternaria spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Pestalotia* spp., *Trichoderma spp., Bacillus* spp., *Micrococcus* spp.	Mural paintings of Torazuka tumulus, Japan	Arai, 1984

Phialophora spp.	Nakata-Oketsu tumulus, Japan	Arai, 1984
Trichoderma spp.	Hayama-Oketsu tumulus, Japan	Arai, 1984
27 *Beauveria alba*	Wall paintings in Canterbury Cathedral, UK	Jeffries, 1986
28 Micromycetes, *Arthrobacter,* Bacilli, unidentified Gram-negative bacteria	Wall paintings of the Church of John Bogoslov, 17th century, Rostov the Great, USSR	Lyalikova and Petushkova, 1986

Microorganisms	Isolated from	Reference
29 *Alternaria spp., Asch*ersonia spp., *Aspergillus* spp., *Chaetomium* spp., *Mucor spp., Penicillium* spp., *Stemphylium* spp., *Trichoderma* spp.	Wall paintings of San-Susi palace, Germany	Hirte *et al.,* 1987
30 *Alternaria* spp., *Aureobasidium pullulans, Chaetomium* spp., *Cladosporium cladosporioides,* C. sphaerospermum, Stemphylium botryosum, organotrophic bacteria	Wall paintings of the Trinity Cathedral in Aleksandrov, USSR	Rebricova *et al.,* 1987; Rebricova, 1991
Aspergillus repens, A.versicolor, Botrytis spp., Cladosporium cladosporioides, Mycelia sterilia,	Wall paintings of the Panteleymonovsky Cathedral in New Athon, USSR	Rebricova *et al.,* 1987
Penicillium verrucosum var. *cyclopium, Stemphylium* spp., *Tritirachium album,* yeasts, organotrophic bacteria		Rebricova, 1991
Acremonium charticola, Cladosporium sphaerospermum, Sporotrichum spp., organotrophic bacteria	Wall paintings of the Georgievsky Cathedral in Novgorod, USSR	Rebricova *et al.,* 1987; Rebricova, 1991
31 *Acrothecium* spp., *Aspergillus niger, Aspergillus* spp. (3), *Nitrosomonas, Nitrobacter,* sulfur-oxidizing bacteria, Actinomycetes	Gambara's frescoes, Brescia, Italy	Sorlini *et al.,* 1987
32 *Acremonium indicum, Alternaria alternata, Aspergillus flavus, A. nidulans, A. niger, A. terreus, A. versicolor, Cladosporium cladosporioides, C. herbarum, Curvularia lunata, C. pallescens, Chaetomium globosum, Drechslera australiensis, D. hawaiiensis, Emericella nidulans, Epicoccum nigrum, Fusarium oxysporum, F. moniliforme, F. solani, Macrophomina phaseolina, Memnoniella echinata,* Mycelia sterilia, *Paecilomyces variotii, Rhizopus nigricans, Stachybotrys atra, Trichoderma harzianum,* bacteria	Wall paintings of Ajanta Caves, India	Agrawal *et al.,* 1988

Microorganisms	Isolated from	Reference
33 *Aspergillus versicolor, Beauveria* spp., *Botrytis cinerea, Chaetomium* spp., *Cladosporium sphaerospermum, Epicoccum nigrum, Geomyces pannorum, Penicillium chrysogenum, P. cyclopium, P. verrucosum, Sporotrichum* spp., *Verticillium lamellicola, V. lecanii,* Actinomycetes, organotrophic bacteria	Paintings of the 16th century Cathedral of Birth of the Holy Virgin, USSR	Rebricova and Karpovich, 1988; Rebricova, 1991

34	*Acremonium charticola, A. terricola, Alternaria* spp., *Aspergillus sydowii, A. versicolor, Aureobasidium pullulans, Candida* spp., *Cladosporium cladosporioides, C. sphaerospermum, Hyalodendron* spp., Mycelia sterilia, *Trichoderma viride, Tritirachium album,* yeasts, Actinomycetes, organotrophic bacteria, algae *(Desmococcus vulgaris)*	Paintings of the 16th century Cathedral of Birth of the Holy Virgin, Ferapontovo, USSR	Rebricova, 1988, 1991
35	Micromycetes, *Arthrobacter,* Bacilli, yeasts, Actinomycetes, Proactinomycetes, green algae	Paintings of the 16th century Cathedral of Birth of the Holy Virgin, Ferapontovo, USSR	Petushkova *et al.*, 1989
36	*Aspergillus flavus, A. sydowi, A. versicolor, Aspergillus* spp., *Botrytis cinerea, Cladosporium cladosporioides, C. sphaerospermum, Coniothyrium cerealis, Engyodontium* spp., *Oidiodendron cerealis, Penicillium brevi-compactum, P. chrysogenum, P. meleagrinum, P. notatum, P. purpurogenum, P. rugulosum, P. variabile, Trichoderma harzianum,* Mycelium sterile moniliaceum, Mycelium sterile dematiaceum, *Rhodotorula* spp., *Tilletiopsis* spp.	15th Century frescoes of Ognissanty Church, Florence, Italy	Sampo and Mosca, 1989
37	*Acrodontium crateriforme, Beauveria bassiana, Cladosporium sphaerospermum, Fusidium viride, Geomyces pannorum, Penicillium* spp., *Phialophora* spp., *Tritirachium album, Verticillium lamellicola*	Wall paintings of Cathedral of the Nativity of the Virgin, Pafnutii-Borovskii Monastery, USSR	Karpovich-Tate and Rebricova, 1991

	Microorganisms	Isolated from	Reference
38	*Acremonium* spp., *Aspergillus versicolor, Cladosporium herbarum, C. sphaerospermum, Hyalodendron* spp., *Penicillium verrucosum* var. *cyclopium, Tritirachium album*	Wall paintings of the Church of John Precursor in Yaroslavl and the Church of Assumption of the Holy Virgin in Meletovo, USSR	Rebricova, 1991

Gargani (1968) states that hyphae spreading over the surface of paintings grow through their surface layer, breaking off pigment particles. He observed vigorous attack of fungi on the surface of murals in Florence after the flood of 1966, causing powdering of the weakened paint. Tonolo and Giacobini (1963) and Kuritzyna (1968) investigated the changes in frescoes caused in part by developing hyphae. They observed that fungi formed fruit bodies of perithecial, pycnidial and stromatic types under the painting layer, giving rise to formation of small blisters and craters. On frescoes in the Castle of Karlstein in Czechoslovakia, Tonolo and Giacobini (1963) observed abundant subsurface growth of fungi which contributed greatly to loosening the paint layer from its support. Development of streptomycetes destroys and masks the painting surface with a light, powdery deposit (Bassi and Giacobini, 1973). Advanced deterioration was always found where fungi, bacteria and streptomycetes had developed on the surfaces of the paintings. A superficial encrustation of a white, and sometimes brown or black layer was found over the paintings in some caves of Ajanta (Lal, 1966; Agrawal, 1975) and it was thought that airborne microorganisms might be playing a role in this deterioration (Tilak *et al.*, 1970). The microbial deterioration of wall paintings has been widely discussed but the

problem has not been researched in depth (Tilak *et al.*, 1970; lonita, 1971; Lazar and Dumitru, 1973; Somavilla *et al.*, 1978; Saiz-Jimenez, 1981; Crippa, 1983; Torraca, 1984; Agrawal and Jain, 1984; Arai, 1984; Jeffries, 1986). Agrawal (1986) has even questioned whether microorganisms play any role in the deterioration of paintings.

Agrawal *et al.* (1988) used standard microbiological methods to isolate the microorganisms in the Ajanta caves:

1. The aerospora inside and outside the caves was sampled by Rotorod Air Sampler and Sedimentation Plates.
2. Surface microorganisms on wall paintings were sampled by non-destructive methods.
3. Soil and dust microorganisms were isolated and identified following the methods of Waksman (1922).

Numbers of visitors, temperature and relative humidity inside and outside the caves were also recorded. Twenty-five fungal species were isolated by these methods, including *Acremonium indicum, Alternaria alternata, Aspergillus flavus, A. nidulans, A. niger, A. terreus, A. versicolor, Cladosporium cladosporioides, C. herbarum, Curvularia lunata, C. pallescens, Chaetomium globosum, Drechslera hawaiiensis, D. australiensis, Macrophomina phaseolina,* Mycelia sterilia, *Paecilomyces variotii, Rhizopus nigricans, Stachybotrys atra, Trichoderma harzianum* and bacteria (Agrawal *et al.*, 1988). More fungal species could be isolated in July than in April and October, perhaps due to the high humidities during the monsoon period providing a damp substrate favourable for the development of microorganisms. The appearance of these fungi on objects is unpleasant (Plates 1 and 2). It is evident from these studies that fungi play an important role in deterioration of the Ajanta caves, although it is still difficult to quantify the extent to which they contribute to the process of deterioration.

Petushkova and Lyalikova (1986) determined the degree of contamination of murals using Koch's plating techniques. This technique yields only an estimate of the numbers of viable microorganisms contaminating the painting. A range of heterotrophic microorganisms, bacteria and fungi were isolated from the surface of murals—from 2×10^4 to 5×10^5 viable cells in 1 g of sample from deteriorated spots on the paintings. Large numbers of heterotrophic microorganisms on the surfaces of paintings may be attributed to their ability to use organic compounds contained in the binding and reinforcing glues, leading to disintegration of the pictorial layer. Organic and carbonic acids generated by microorganisms are potent destructive agents which can attack the underlying material of paintings and dissolve the pigments to form chelates with their metal pigments. Petushkova and Lyalikova (1986) found that 11 out of 18 bacterial isolates produced acids on a sucrose-containing medium giving a pH of 5.3 to 4.1.

Microorganisms can also cause discolouration of pigments. Dark brown spots on the strip of green ground in the Deesis range (as well as black spots on the original blue and white pattern, on the murals) were found by X-ray examination to result from the conversion of the green pigment to plattnerite (lead oxide, PbO_2) (Petushkova and Lyalikova, 1986), and similar conversions in murals frequently result where white lead has been used (Matteini and Moles, 1981). However, the Italian scientists failed to establish the conditions in which this process takes place or to reveal the cause of such deterioration in frescoes.

White lead (basic lead carbonate $2PbCO_3.Pb[OH]_2$), massicot (lead oxide, PbO) and minium (lead tetroxide, Pb_3O_4) are all used in the wall paintings. Bivalent lead-containing pigments are chemically stable compounds and are only converted to the tetravalent state in the presence of strong oxidants (Remy, 1960), not normally present in murals. Petushkova and Lyalikova (1986), assuming that the discolouration of leadcontaining pigments might be biological, used nutrient media of different compositions, containing minerals, proteins and/or carbohydrates to reveal the ability of the bacteria to oxidize bivalent lead to the tetroxide.

It has been reported that many microorganisms, including heterotrophic bacteria and mycoplasmas, are capable of oxidizing iron and manganese by producing hydrogen peroxide (Balashova, 1974; Dubinina, 1978). This complete oxidation of white lead may be illustrated by the following equation:

$$2PbCO_3.Pb(OH)_2+3H_2O_2=3PbO_2+2H_2CO_3+2H_2O$$

The formation of brown PbO2 by bacterial action seriously damages paintings, not only because of the considerable discolouration of the pigment, but also because lead oxide, as a strong oxidant, can damage other components of the paintings; for instance, the carbonic acid produced in oxidation of lead oxide reacts with insoluble calcium carbonate in the plaster to form soluble calcium (calcium hydrogen carbonate):

$$CaCO_3+H_2CO_3=Ca\ (HCO_3)_2$$

This process impairs the binding properties of the plaster, and allows its solubilization and partial washing out. Apart from oxidation, other conversions of lead compounds are possible. Some bacteria convert white lead, massicot and minium, as well as acetate, to black lead sulfide (Petushkova and Lyalikova, 1986). This compound results from the interaction of lead compounds with hydrogen sulfide produced by bacteria utilizing sulfur-containing amino acids. Glues of animal origin, used as a binding material in the paintings of the church in Rostov may serve as a substrate for the development of microorganisms (Bryusova, 1958).

CONTROL OF MICROBIAL ATTACK IN WALL PAINTINGS

To preserve wall paintings, control and prevention of microbial growth is essential. Modern conservation demands very high standards for all materials used to treat art objects. Conservation materials already used, or newly introduced, are critically tested to preclude any noxious effect on the object treated.

Fungicides are particularly important in the preservation of murals, which are constantly subjected to high humidities, either from the soil or from atmospheric condensation on their surface. The fungicide should fulfil the following qualities:

1. It should have high fungitoxic value enabling use at low concentrations.
2. It should not affect any component of the art material, i.e. the fastness of paints, the binders, the glues in the ground, or the plaster.
3. It should have low volatility to ensure prolonged protection of the art material.
4. It should not be liable to breakdown with the formation of noxious decay products.

5. It should not lose its biotoxic properties by combining with constituents of the objects treated.
6. It should have low toxicity to humans.

There are a few reports of the effects of fungicides on the components of paintings which include paints, binders, grounds and plaster. HueckVan-der Plas (1966) listed biocides and commercial preparations that could be used against microorganisms on paints, paper, wood and textiles. The applicability of biocides for disinfection and protection of wall paintings against the microbial attack is being studied by Strzelczyk (1981).

One of the biggest problems in biodeterioration is to kill the microorganisms without damage to the paintings when they are present in large numbers. Conventional paint fungicides do not always prove effective, and may have undesirable side-effects (Kaplan, 1968; Pauli, 1972); thus new chemicals are required. Bianchi *et al.* (1980) suggested that before applying a chemical on frescoes, it should first be tested *in vitro* on isolates of fungi growing on the object for preservation.

Only a limited choice of suitable disinfectants is available for wall paintings due to their poor persistence. Antibiotics have been discarded because of their selectivity (Gargani, 1968) and a combination of 0.3% *p*-chlor-*m*-cresol and 0.1% phenylmercuric acetate in ethanol sprayed on the object three times at 3–7 day intervals has been recommended (Strzelczyk, 1981). These chemicals have been thoroughly tested and have been found to have no noxious effect on pigments. Hueck-Van-der Plas (1966) recommended *p*-chlor-*m*-cresol for disinfecting paints, glues, binders, leather and paper. Both of these disinfectants have limited persistence (Payne, 1963), although they are superior to nystatin (an antibiotic used to destroy heterotrophic microorganisms on frescoes in Florence) (Gargani, 1968).

Murals should first be disinfected with ethylene oxide and then placed in a nitrogen atmosphere in a vacuum chamber (Tonolo and Giacobini, 1963). They should also be kept in an air-conditioned environment at constant humidity and temperature. Because of water migration from the ground and condensation on murals, however, better ways of rendering them resistant to the development of heterotrophic microorganisms are still necessary (Strzelczyk, 1981).

Products based on dichlorofluanide, quaternary ammonium compounds, organo-tin compounds and mixtures of quaternary ammonium compounds and organotin compounds have been evaluated by experts for their compatibility in the preservation of paintings and monuments (Raschle, 1983). An organo-tin compound in 70% isopropanol was chosen: it saturated the painting ground so that it shone and appeared at its best without being oiled. The moulds were stabilized in this way; it is hoped that the frescos will be protected without any negative effect until environmental measures become fully effective.

Bianchi *et al.* (1980) tested six fungicides *in vitro* but only Florasan, Benlate and Bavistin proved effective in controlling fungal growth and spore germination at a concentration of 100 ppm. Benlate also decreased fungal growth at 10 ppm. The three fungicides were also effective when applied to frescoes. Before the treatment, the fungal colonies were clearly visible on the fresco surface. A single treatment decreased the size of the colonies so markedly that they were almost invisible and have remained so for at least 6 months. The fresco was not damaged by the fungicides (Bianchi *et al.*, 1980).

Six fungicides (orthophenyl phenol, *p*-chlor-*m*-cresol, sodium pentachlorophenate, Preventol R-90, cetyl pyridinum chloride and phenylmercuric acetate) have been tested *in*

vitro against fungal isolates from Ajanta wall paintings at Maharashtra and Rajasthan, India. The isolates included *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* spp., *Fusarium* spp., *Periconia* spp., *Rhizopus* spp., *Trichoderma* spp. and Mycelia sterilia. Phenylmercuric acetate was the most effective of the fungicides, completely inhibiting fungal growth at 0.001% concentration. Orthophenyl phenol (Plate 1) and *p*-chlor-*m*-cresol were effective at 0.02% concentration and Preventol R-90 at 0.05% concentration. However, sodium pentachlorophenate and cetyl pyridinum chloride required 0.05 and 1% concentrations, respectively, to give complete inhibition (Dhawan *et al.*, 1991; Garg *et al.*, 1991).

The prevention of algal growth on mural paintings is more difficult. Their growth is promoted by light and the decay and evaporation of disinfectants from the surface of the paintings. A large number of algicides are available (Hueck-Van-der Plas, 1966; Fitzgerald, 1971; Garg *et al.*, 1988), mostly water-soluble compounds which are easily washed out of porous surfaces such as stone. It has been found that a 1% solution of Lastanox TA in ethanol sprayed at 3–7 day intervals was the most effective method of combating algae on murals, on plasters and on the surfaces of stone monuments (Czerwonka, 1976; Staszewska, 1977). This preparation caused no noxious effect on any component of the murals.

SUMMARY

Works of art and historical monuments, including wall paintings, are subject to the effects of environmental factors and microbial attack and their interactions. This chapter has described the deterioration of wall paintings by a range of microorganisms and the effects of humidity, temperature, nutrition, vegetation and visitors. Measures for the control of the deterioration of wall paintings have been reviewed and discussed. The need for a multidisciplinary approach to the preservation and restoration of wall paintings has been stressed.

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Indoor mycology: the North American experience

11

Paul Comtois and Beatriz Escamilla Garcia

INTRODUCTION

> Nobody would ever think of buying a food can without knowing its content, but we commonly breathe air of unknown biological composition.
>
> Paul Langlois

North America consists of a small number of large political units, but the continent comprises many diverse landscapes. From the tropical f orest of Mexico to the polar climate of Alaska, almost all known climatic conditions are well represented. People have adapted to different environments and many building solutions have resulted—from Hopi Indian pueblos to Eskimo igloos. In modern times, the same spectra of technological adaptions can be observed: from evaporative cooling systems in the south-west, to R-2000 houses in the north-east.

Different solutions in turn create diverse habitats for mould growth. Indeed, moulds are endowed with a miscellaneous enzymatic arsenal; as a result, there is almost no habitat that they cannot colonize (Botton *et al.,* 1990). Even in dry climates with advanced ventilation or airconditioning technologies, fungi will still find what they need to grow and reproduce.

Fungi have a high temperature tolerance, and this factor is generally not a limitation in the indoor environment. Relative humidity is more often a limiting factor to mould growth, at least in the northern part of the continent. Relative humidity only represents the water content of the air. Water activity of solid or liquid substrates, related to condensation, is probably more significant (Adam, 1990). Apart from these physical conditions, nutrient suitability (substrate type) is the only other require-

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ment for mould life, and all cellulose-derived substrates (wood, wallpapers, cotton) are generally abundant indoors.

Under normal conditions, these substrates do not allow mould growth because they decay (i.e. exchange nutrients with moulds) only when in contact with wet ground or when wetted by an external source of moisture, such as rain seepage, plumbing leaks or condensation (Levy, 1979). If the relative humidity is high enough, some surface moisture and mould growth may be expected, especially in the coldest months. Water, however, diffuses through walls to the outside during most of the year (Spolek and Oosterout, 1989). Mildew used to be especially prevalent in the south, where moulded mattresses and drapes were accepted as normal in the living environment. The introduction of modern heating systems has corrected this situation (Verrall and Amburgey, 1978).

It is important to emphasize that regular cleaning of most exposed surfaces ensures that mould does not have time to get established. However, our indoor environment is still dominated by airborne mould spores, a constantly replaced cohort of invaders, originating either from outdoors or from sources in the indoor environment. Anywhere in North America, *Cladosporium* spp. and *Alternaria* spp. are the dominant seasonal moulds. They generally show a pollen-like behaviour, i.e. their main impact will be on the upper respiratory tract. Health effects, and particularly respiratory diseases, are therefore the major consequences of over-abundance of moulds inside buildings.

This chapter reviews the available information on the presence of mould in buildings in North America. Although all North American countries have been studied, some regions have a larger database: the St Lawrence and Great Lakes lowlands, the Pacific coast and Central Mexico (Figure 11.1). Results will not be discussed from a regional perspective (except when they apply to specific indoor environments), but in relation to building types.

Domestic houses differ from high rise buildings in their relationships with the outdoor air and, consequently, in the abundance of airborne spores. Domestic houses will often yield four times as many moulds as a modern office building (400 vs 90 cfu/m^3) (Comtois, 1990; Tamblyn *et al.*, 1991), mostly because outdoor air is filtered in the ventilation systems of large buildings. However, if contamination is present in the ventilation system of a large building, microorganisms will be present in very large numbers, will be widely disseminated and will affect a large population.

HEALTH IMPLICATIONS OF FUNGI IN HOME ENVIRONMENTS

The home environment, with the varied activities that take place in this dynamic setting, offers a wide diversity of substrates for mould growth and is a constant mould producer. In North American countries, particularly in temperate regions, people spend at least 70% of their lives indoors and it has been suggested that some aspects of indoor environmental quality can be related to the frequency of respiratory disorders.

Figure 11.1 The regional distribution of aeromycological studies of buildings in North America.

During the last five decades, a number of different techniques have been developed for detecting and evaluating mould exposure. As in other countries of the world, gravitational methods have been traditionally used by allergists and clinicians interested in studying airborne mould particles in the home. Despite their simplicity, these methods have strong limitations: they give only crude information regarding airborne moulds, with no quantitative evaluation of fungal exposure. Some data often considered essential in airborne fungal surveys cannot be obtained by gravitational methods. To date, there is no simple and reliable method for evaluating airborne moulds, and any one method presently available gives only partial and biased information. Information is often required on both viable and total spores; thus, it has been recommended that volumetric collectors should be used to allow both culture and microscopic assessments of spores (Kozak *et al.*, 1980a; Solomon, 1984).

For collecting viable particles, the medium should be chosen according to the type of microorganism being studied. A suitable medium should allow growth of all the predominant airborne genera and any others that could be important in allergic reactions. The most common media used are Sabouraud dextrose agar (SDA) and malt extract agar

(MEA). By comparing results from both media, Terracina and Rogers (1982) found that malt extract agar yielded 95% of the fungi sampled, while Sabouraud dextrose agar yielded only 63%, i.e. malt extract agar has a better retrieval rate. In indoor mould surveys it is strongly recommended that samples be collected not only to detect the presence of fungi, but also to find the pathway by which fungi are affected by human activities. All important areas in a building should be sampled, particularly those where any people spend most of their time (Kozak *et al.*, 1980a; AlDoory, 1984, 1985; Solomon, 1984).

Recently, Agarwal *et al.* (1981) suggested that counts of viable airborne allergens did not adequately reflect total airborne concentrations. They therefore proposed an immunochemical method for allergen quantification in which airborne particles were collected on glass fibre sheets, through which a constant air flow was maintained. The filters collected 95% of airborne particles larger than 0.3 µm diameter. Different amounts of *Alternaria* spore extract powder were filtered by the sampler. Allergen was recovered by elution and quantified by RAST inhibition. They reported that airborne *Alternaria* allergen concentrations were highly correlated with daily *Alternaria* counts. However, they suggested that allergen activity might be enhanced through the contribution of other cross-reacting genera. This immunochemical method for the quantification of atmospheric allergens could be a valuable tool when other methods are unsatisfactory.

Whatever the method used, results of investigations in North America show that there is a typical group of microorganisms that can be isolated in houses, comprising 15 fungal and 2–3 yeast genera, present in 10–100% of samples. *Cladosporium, Penicillium, Alternaria, Streptomyces* and *Epicoccum* predominate in more than 50% of samples, while *Aspergillus, Aureobasidium, Drechslera (Helminthosporium), Cephalosporium, Acremonium, Fusarium, Botrytis, Rhizopus, Rhodotrula, Beauveria* and *Geotrichum* occur in only 10–50% of samples (Cueva and Tellez, 1958; Cueva and Castillo, 1962; Cueva *et al.*, 1967; Venegas *et al.*, 1969; Solomon, 1976; Fradkin *et al.*, 1987; Kozak *et al.*, 1980b). Table 11.1 summarizes the results of these investigations. Even though there are some differences between localities, the mean concentrations of the predominant home fungi are similar. Some genera are, however, related to particular sources.

Table 11.1 Minimum, mean and maximum airborne concentrations of the most frequently isolated fungal genera, with their main sources at home

Genera	Concentration recorded, cfu/m^3			Main sources
	Minimum	Mean	Maximum	
Frequency of isolation 50–100%				
Cladosporium	12	447	4637	Indoor plants, carpets
Penicillium	0	116	4737	Evaporative air coolers, humidifiers, basement dust
Alternaria	0	41	282	Carpets, wool materials, humidifiers
Epicoccum	0	18	153	
Streptomyces	0	28	212	
Frequency of isolation 10–<50%				

Aspergillus	0	23	306	Evaporative air cooler and climatization systems
A. niger	0	3	59	
Aureobasidium	0	6	294	HVAC systems and humidifiers
Drechslera	0	7	94	
Cephalosporium	0	97	3760	HVAC systems and humidifiers
Acremonium	0	31	188	Humidifiers
Fusarium	0	5	47	Humidifiers
Botrytis	0	3	54	
Rhizopus	0	1	24	Room's surfaces
Rhodotorula	0	88	8412	Humidifiers
Beauveria	0	1	12	
Geotrichum	1	2614	111	

From Cueva and Tellez, 1958; Cueva and Castillo, 1962; Cueva *et al.,* 1967; Venegas *et al., 1969; So*lomon, 1976; Kozak et al., 1980b; Fradkin *et al.,* 1987; Comtois, 1990.

It is commonly accepted that indoor airborne fungi come from two sources: indoors and outdoors. The numbers and types of fungi from each source will depend on the building itself (age, structure, type of furniture, cloths, toys, presence of pets, plants, etc.) as well as the occupants and their standard of hygiene (Al-Doory, 1984, 1985).

In a study of the factors determining the frequency of indoor mould, Kozak *et al.* (1979) analyzed the effect of indoor plants in 68 homes in southern California. The mean concentration measured of fungal spores in houses with 10 plants or fewer was 508 cfu/m^3, while for in those houses with more than 10 plants it was 443 cfu/m^3. They could not detect statistically significant differences in mould frequency between the two groups of homes. In a later study, Burge *et al.* (1982) reported mean concentrations of 900 cfu *Cladosporium,* 300 cfu *Penicillium,* 100 cfu *Aspergillus* and fewer than 100 cfu *Alternaria*/m^3 and concluded that indoor plants did not impose a risk for allergens such as *Aspergillus fumigatus.* Agricultural activities can disperse soil particles with adherent spores and hyphal fragments into the atmosphere, but healthy undisturbed plants do not constitute a major exposure source for airborne fungal particles.

Other factors that affect the incidence of fungal spores in the home (Kozak *et al.*, 1979) include adequate dust control and the use of central electrostatical filtration systems. Homes with poor dust control had larger mould counts (163–4955 spores/m^3, mean 822 spores/m^3) than those with more efficient systems (77–1923 spores/m^3, mean 292 spores/m^3).

Factors associated with an increase in mould concentrations were shade, high levels of organic debris and neglected natural landscaping. These factors are generally interrelated: untended landscaping tends to overgrow and produce excessive shade. Homes then tend to have less lawn care, infrequent raking of leaves and an increase in the amount of organic debris around the house.

Banerjee *et al.* (1987) also reported large numbers of mould isolates associated with high shade and high levels of organic debris, poor landscaping and landscape maintenance near homes in North Carolina. Central electrostatic filtration systems and good dust control were again associated with small concentration of spores.

Climatization systems have long been recognized as important sources of mould spores in indoor environments. Results from some investigations on these systems are shown in Table 11.2. Sneller and Pinnas (1987) suggested that evaporative air coolers could provide niches for potentially allergenic fungi that can then be introduced into the home air. Their survey showed that there were some differences between this kind of climatization system and air-conditioning. Both seem to yield a similar spectrum of mould genera, but their frequencies differed. *Aspergillus, Cephalosporium, Penicillium* and *Alternaria* species were more frequent in homes with air-conditioning than in those with evaporative air coolers. Hirsch and Sosman (1976) found that significantly more *Cladosporium* could be isolated from the air and surfaces of homes with airconditioning, than from those without a system. *Gliocladium* occurred significantly more often in the winter months in buildings with hydronic heating than in those with forced air systems. Solomon (1974) found that cold mist vaporizers could enhance yeast growth by the generation of aerosols.

Fungal cultures from central air-conditioners can also be used to evaluate their role in the transmission of allergens into the home (Sneller and Pinnas, 1987). Preliminary statistical evaluations of the effect of evaporative air coolers, air-conditioning and mould growth on asthmatics revealed more eye irritations, rhinitis and chest tightness or cough/sputum in the homes of asthmatic patients with large fungal concentrations. Species of *Alternaria* and *Bispora* were more closely related to symptoms than those of any other fungi (Lebowitz *et al.*, 1982; Kodama and McGee, 1986).

Table 11.2 Mean concentrations (cfu/m3) and isolation frequencies of major mould genera by climatization system types

Genera	Natural Ventilation	Climatization system			
		Air conditioning	Humidifier	Evaporative air cooler	Cold mist vaporizer
Aspergillus	35.4 cfu	54.3 cfu (75%)	~10–30 cfu	34%	±
Cephalosporium	0.9 cfu	0.1 cfu (13%)		3.4%	
Chrysosporium	4.5 cfu	0.5 cfu			
Cladosporium	62.3 cfu	18.2 cfu	~10–970 cfu		
Curvularia		0.2 cfu			
Fusarium	0.4 cfu	0.1 cfu	~10–590 cfu	2.3%	
Monilia	0.1 cfu	0.2 cfu			
Mucor	3.0 cfu	1, .2 cfu			
Penicillium	1.1 cfu	0.6 cfu (11.6%)	~10–20 cfu	±	±
Rhizopus	3.4 cfu	0.8 cfu			
Streptomyces	12.8 cfu	11.5 cfu			
Alternaria		4.3 cfu (38%)	~10 cfu	6.1%	+
Aureobasidium			~10 cfu		
Candida			~10 cfu		
Epicoccum			~10 cfu		
Gliocladium			~20–260 cfu		
Phialophora			~30–1260		

		cfu		
Rhodotorula		±	±	

From Solomon, 1974; Burge *et al.*, 1980; Kodama and McGee, 1986; Sneller and Pinnas, 1987; Macher and Girman, 1990.

Case reports and epidemiological studies suggest that relative humidity and humidification equipment can indirectly affect the incidence of allergies and infectious respiratory diseases through their effect on the growth and survival of allergenic fungi. The majority of fungi require relative humidities greater than 70% for growth. Actively growing fungi are usually limited to surfaces with frequent condensation as a result of locally high relative humidities. Damp organic material such as leather, cotton, paper, furniture stuffing and carpets can be contaminated with fungi (Arundel *et al.*, 1986; White, 1990).

When indoor organic materials were damp for a period longer than 2 weeks, the mean spore concentration was 4893/m^3, with a range of 83–64312 spores/m^3 (Gallup *et al.*, 1987). When damp for 3–14 days, the mean was 4893/m^3 with a range from 436 to 22682 spore/m^3; within 3 days of drying, however, no spore problems developed and spore counts ranged from 118 to 3659 spores/m^3, with a mean of 914 spores/m^3. All concentrations in problem homes were significantly larger than those out of doors.

Homes of people with allergies had higher average relative humidities and more fungal isolates per m^3 than homes of subjects without allergies (Solomon, 1976). In another study, lower respiratory illness was the prevalent respiratory disease among asthmatic and non-asthmatic children associated with the presence of dampness and moulds, while asthma was the only respiratory illness not consistently associated with dampness (Brunekreef *et al.*, 1989). In a similar investigation, Dales *et al.* (1990) noted that children showed a frequency of 19.5% for respiratory symptoms in damp homes, but only 13.2% in dry houses. The frequency of symptoms in adult smokers was 38% when dampness was present and only 27% when absent. For adult non-smokers, the frequencies were 19 and 11%, respectively.

The incidence of airborne fungi has been related not only to potential sources, but also to their distribution in different room types. Several authors have suggested that particular conditions in certain rooms support survival of some genera. The results of some of these investigations are summarized in Table 11.3. Bathrooms and bedrooms yielded the greatest diversity of fungal types. Even when genera in different rooms were similar some genera could still only be isolated in specific rooms. For example, *Amblyosporium, Monotospora, Gliocladium* and *Monilia* in bedrooms, *Heterosporium* and *Humicola* in living rooms; *Monotospora, Oospora* and *Agrocybe* in bathrooms and *Monilia* in basements (Hirsch and Sosman, 1976; Banerjee *et al.*, 1987; Kozak *et al.*, 1980b, 1990; Comtois, 1990).

The varieties of indoor airborne moulds may differ greatly from room to room, and also from season to season (Comtois, 1990). Colonies of *Penicillium, Alternaria* and *Ganoderma* spores were abundant in bedrooms during the summer, but were replaced by *Alternaria* colonies and *Penicillium/Aspergillus* spores during the winter. In the living room, mould counts were dominated by *Alternaria* and *Basidiospores* (including *Ganoderma*) in winter while *Fusarium* and *Basidiospores* were dominant in summer. In basements, *Aspergillus* spores were predominant in the air during winter, while during

the summer *Penicillium/Aspergillus* spores and *Penicillium* and *Crysonilia* colonies were prevalent. *Cladosporium* was abundant in all rooms except the basement where their contribution to total counts was negligible, particularly in summer. This study also demonstrated the importance of estimating concentrations of both total spores and colony forming units (Figure 11.2).

Table 11.3 Relative abundance of major mould genera in differents rooms of North American houses

Genera	Bedroom	Living room	Bathroom	Kitchen	Basement
Colonies					
Alternaria	30%	3.8%	4.5%	5%	4.9%
Acremonium	–	6.4%	1.1%	–	–
Amblyosporium	±	–	–	–	–
Aspergillus	27%	3.9%	24%	3%	39%
Aureobasidium	17%	1.8%	15%	5%	19%
Beauveria	–	–	1%	–	–
Botrytis	2.6%	–	–	–	–
Cephalosporium	–	3.2%	–	–	–
Chaetomium	0.5%	–	4.5%	–	–
Cladosporium	40%	41%	44%	10%	47%
Cunninghamella	±	±	–	–	–
Drechslera	–	–	1%	–	–
Epicoccum	8%	2.1%	8%	–	2%
Fusarium	3%	–	2%	±	–
Gliocladium	31%	–	11%	–	30%
Heterosporium	–	±	–	–	–
Monilia sitophila	41%	–	22%	–	31%
Monotospora	15.5%	–	25%	–	–
Mucor	±	±	–	±	–
Oospora	–	–	18%	–	21%
Pœcilomyces	15.5%	–	19%	–	32%
Penicillium	33%	27%	37%	71%	45%
Pithomyces	–	3%	–	2%	3%
Rhinocladiella	0.5%	–	1%	–	–
Rhizopus	10%	±	–	±	17%
Scopulariopsis	–	–	1%	–	–
Stachybotris	–	–	–	2%	–
Streptomyces	1%	–	–	–	14%
Trichoderma	–	–	–	–	8%
Spores	–	–	–	–	–
Alternaria	2.1%	17%	1.7%	–	2.9%
Agrocybe	6.3%	3.1%	13.1%	–	7.5%
Aspergillus/ Penicillium	21.4%	8.5%	10.1%	88%	44.1%
Basidiospores	3.1%	5.4%	3.4%	–	2.4%

Cladosporium	45%	35%	49.2%	9.4%	28.3%
Coprinus	5.1%	19.1%	7.6%	–	3%
Epicoccum	–	1.3%	–	–	–
Fusarium	–	–	–	–	–
Humicola	–	19.1%	–	–	12%
Ganoderma	21.2%	20.5%	15%	–	5.8%
Leptosphaeria	–	2.6%	–	–	–
Ulocladium	–	7.8%	–	–	–

From Hirsch and Sosman, 1976; Kozak *et al.*, 1980b, 1990; Banerjee *et al.*, 1987; Comtois, 1990.

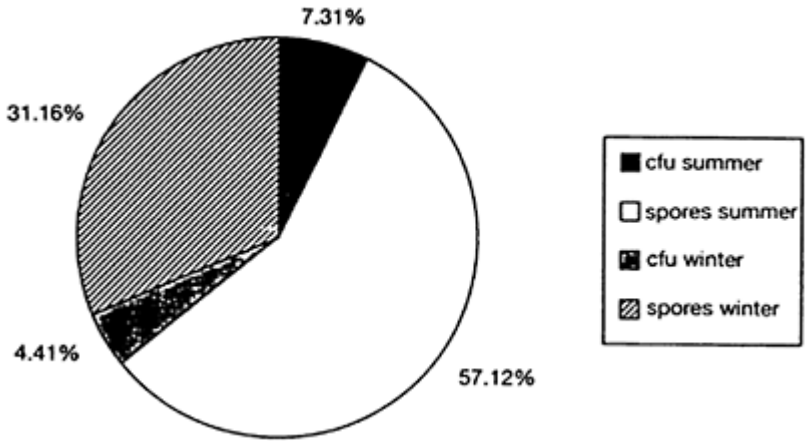

Figure 11.2 The relative abundance of biological particle types for each season in 105 homes in Montréal (Comtois, 1990).

Very few mould surveys have related mould species and concentrations to respiratory reactions. Several studies have shown that positive skin test reaction to fungi are given by 36–63% of subjects in warm regions and by 50–86% in temperate localities (Terracina and Rogers, 1982; Baeza-Bacab *et al.*, 1987; Tarlo *et al.*, 1988; Cadena Bustamante, 1990; Su *et al.*, 1990). Roby and Sneller (1979) found that in almost all instances there was a greater correlation between positive skin tests and colony forming units indoors than with those outdoors. Other investigations showed that the indoor fungi most frequently provoking skin test reactions are *Monilia sitophila, Curvularia* spp., *Cladosporium* spp., *Fusarium* spp., *Aspergillus fumigatus, Alternaria* spp., *Penicillium* spp., *Paecilomyces* spp., *Rhizopus* spp., *Candida albicans, Aspergillus niger, Mucor* spp., *Cephalosporium* spp., *Phoma* spp., *Helminthosporium* spp. and *Epicoccum* spp.

Despite the great similarity of genera between countries of North America, particular genera give a greater frequency of reactions among patients with respiratory symptoms in different localities. In the south-east of Mexico, the most frequent indoor fungi that induce positive skin reactions are *Rhizopus* spp. (50%), *Monilia sitophila* (39.9%),

Curvularia spp. (32.6%) and *Penicillium* spp. (32%). In these warm localities, the frequency of allergies induced by fungi is greater than that induced by pollen grains (Baeza-Bacab *et al.*, 1987; Cadena Bustamante, 1990). In northern countries (Roby and Sneller, 1979) the greatest frequency of positive skin reactions was given by species of *Cladosporium, Penicillium, Alternaria* and *Helminthosporium.* The results concerning *Helminthosporium* and *Mucor* were particularly interesting, because both gave a high percentage of positive skin reactions in certain age categories, notably among adults. In other studies, Chapman and Williams (1984) reported *Epicoccum* spp. as an important cause of positive skin reactions, followed by *Candida albicans,* and species of *Fusarium, Cladosporium, Curvularia, Alternaria, Phoma* and *Helminthosporium,* common to the countries mentioned above.

In almost all studies the population with the highest level of skin test reactions were children under 10 years of age. The most important allergens for patients suffering from asthma were *Monilia sitophila* (40%), *Curvularia* (33%), *Fusarium* (30%), *Penicillium* (26%); while those for patients with rhinitis were *Rhizopus* (50%), *Cladosporium* (30%), *Monilia sitophila* (34%) and *Penicillium* (32%) (Baeza-Bacab *et al.*, 1987).

All the investigations described above used commercial extracts. Recently, Tarlo *et al.* (1988) have studied the prevalence of skin test reactions related to isolates from the houses of patients. They reported that 54.7% of patients gave positive skin test reactions. They also found that the most prevalent were *Cladosporium dadosporioides* (57%), *Alternaria alternata* (50%), *C. sphaerospermum* (50%), *Fusarium* sp. (43%), *Phoma glomerata, Aspergillus fumigatus, A. flavus* (36%), and *C. herbarum* (29%). Even if there was no consistent relationship between patients with positive skin reactions and the prevalence of specific fungi in houses, patients had positive skin responses to extracts of at least one fungus predominant in their home at the time of sampling. They also compared reactions to extracts of *Alternaria* isolated from homes with those prepared commercially. All those patients who gave a definitely positive skin test response to extracts of *Alternaria* from the home also reacted to commercial extract. It was therefore concluded that the extracts were comparable in strength to the fungal allergens usually used in clinical practice.

Some cases of respiratory illness other than asthma and rhinitis have been related to an etiological source in the home. Halwing *et al.* (1985) reported a familiar occurrence of allergic bronchopulmonary aspergillosis. In their study, serological tests revealed an environmental home source of exposure because there were precipitin reactions against *Aspergillus niger* prepared from a culture of the home humidification system. In another study, a woman with antecedent of hypersensitivity pneumonitis reacted to extracts of *Cladosporium* species. Cultures of fungus isolated from a hot-tub room in her home revealed the presence of *Cladosporium*. After one week out of her home, the patient did not manifest any symptoms. Within 4 hours of re-exposure to the hot-tub room, symptoms reappeared. Bronchial provocation with a commercial extract of *C. cladosporioides* led to a similar pattern 5 hours after the initial challenge (Jacobs *et al.*, 1986). In a similar investigation, Hodgson *et al.* (1990) reported a case of allergic tracheobronchitis related to a source in an HVAC (heating, ventilation, air-conditioning) plant.

Links between mould and respiratory illness and reactions are well documented in the above studies. However, the differences in methodology make it difficult to compare

results among localities. It is therefore strongly recommended that a standardized methodology is developed to provide comparable qualitative and quantitative results in different countries.

As a first approach, a patient's immediate environment should be modified to significantly decrease his exposure to mould spores. Given the large number of materials on which fungi can grow, it is not easy to make recommendations that allow their elimination. Researchers agree that keeping areas subject to fungal growth, like window molding, plumbing fixures and shower curtains, clean and dry may be helpful. In addition, materials such as carpets and furniture padding, padded overcoats, books and magazines, and wicker baskets should be kept as clean and dry as possible.

Eliminating humidity at surfaces and providing ventilation should help to prevent mould growth. All humidification devices, dehumidifiers and air-conditioners require frequent and meticulous cleaning to prevent moulds and yeast becoming established. An efficient dust control or filtration system could be useful. Kozak *et al.* (1979) and Maloney *et al.* (1987) found that central electrostatic filtration decreased mould counts. It is important to prevent the accumulation and growth of fungal particles within the house (Kozak *et al.*, 1979; Jennings, 1982; Al-Doory, 1985; Pollart *et al.*, 1987).

More recently, mycotoxins have been implicated in indoor contamination (Croft *et al.*, 1986). These can lead to toxic effects at very low concentrations (Health and Welfare Canada, 1987). Almost all the instances reported have involved *Stachybotrys* spp.; the exact etiology has not been determined.

HEALTH IMPLICATIONS OF FUNGI IN OFFICE ENVIRONMENTS

The situation in offices is quite different, and indissociable from the sick building syndrome (Gammage and Kaye, 1985; Godish, 1989; Visher, 1989; Kay *et al.*, 1991; Brooks and Davis, 1992). Indeed, offices will generally carry a higher chemical pollutant load than houses, mostly from furniture and office hardware while biological contamination will be far greater at home. However, exceptional situations can result in the dissemination of contamination that has no parallel in domestic houses. This is caused by the presence in these buildings of a mechanical ventilation system which acts as a lung for the rooms. When properly built and managed, these systems both decrease the pollutant load from outside by filtering the incoming air, and decrease the build-up of indoor contamination by diluting it with air from outside. However, when contamination is present, these same systems will disperse pollution to all ventilated rooms. Thus, in contrast to houses, offices are less frequently contaminated by biological particles, but when they are present, a larger population is affected, frequently with serious health problems.

Most of the serious problems in offices relate to bacterial contamination of water reservoirs of either humidifers or air-conditioning units (Molina, 1986). These include humidifier fever and Legionnaire's disease. Moulds may cause pneumonitis via hypersensitivity. Workers are generally exposed to large concentrations of one or a few species of microorganisms coming from one source, the ventilation system. The causal microbiological agents of hypersensitivity pneumonitis are frequently thermophilic

actinomycetes (Banaszak *et al.*, 1971; Hodges *et al.*, 1974; Miller *et al.*, 1976; Arnow *et al.*, 1978; Fink *et al.*, 1976) but *Aspergillus* and *Penicillium* can also cause the disease (Berstein *et al.*, 1983; Woodard *et al.*, 1988), as can *Aureobasidium*, *Alternaria* and *Cladosporium*.

Hypersensitivity pneumonitis is an immunological lung disease caused by sensitization and recurrent exposure to organic dusts from ventilation systems and other sources. No clinical feature or laboratory test is diagnostic of hypersensitivity pneumonitis and diagnosis is made from a combination of features, symptoms and abnormalities (Fink, 1986). It is, by far, the most important disease caused by fungi via the office air. Exposed offices can present as many as 10000 cfu/m^3 (Berstein *et al.*, 1983). This is far fewer than has been suggested as necessary for sensitization, i.e. $10^8/m^3$. Precipitins are found in about 10% of workers, who also presented two to three times more symptoms characteristic of the sick building syndrome (Woodard *et al.*, 1988).

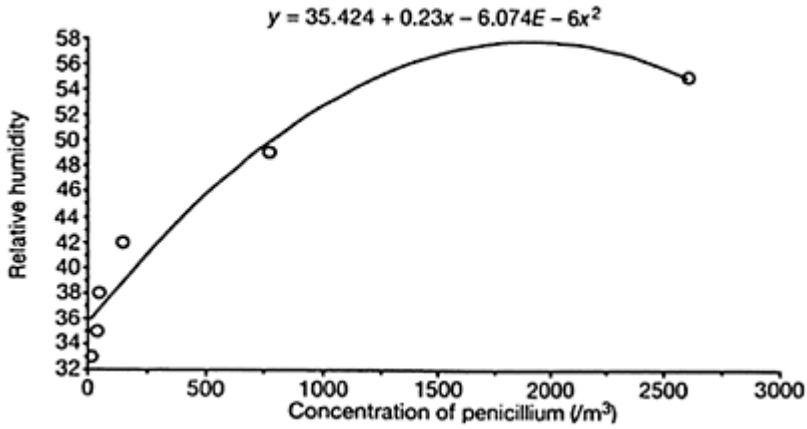

Figure 11.3 The relationship between relative humidity and *Penicillium* cfu concentrations (/m^3) in different levels of a single building in Montréal.

In a study of a multistorey college in Montréal, it was shown that a localized source of *Penicillium cyclopium* at the downward site of the ventilation system (caused by a high humidity level) would follow the same pathway as the diffusion of moisture (Figure 11.3). More problematic is the absence of generalized contamination. Indeed, in two recent studies of two other colleges in Montreal, contamination was found either in some ducts of the ventilation system, with counts up to 25000 spores/m^3 of *Penicillium cyclopium* and *P. glabrum* (Table 11.4); or, in some rooms, with counts up to 4200 spores/m^3 of *P. simplicissimum* and *P. oxalicum* (Table 11.5). In both cases, contamination was localized and only some rooms were affected.

Table 11.4 Fungal spore concentrations (/m^3) at the nodes (Figure 11.4) of the 22 ventilation systems of a single building in Montreal (Comtois, 1991)

Systems	Spores/m^3				
	Intake	Filters	Humidifiers	Cooling coils	Return
1	1037	471	565	1415	346
2	2991	1038	–	1227	94
3	2799	1038	1037	1006	1635
4	1352	659	1036	910	125
5	2234	188	1635	943	440
6	1227	2201	1098	816	565
7	879	1383	1100	785	124
8	157	407	596	943	**18774**
9	912	1384	471	188	126
10	345	1384	1131	1352	93
11	1604	1824	1887	2295	378
12	2360	533	849	628	343
13	1132	1195	1574	2802	157
14	–	1354	2266	1353	–
15	**14171**	407	535	1228	975
16	**11713**	723	1572	849	377
17	**25638**	282	723	1321	189
18	**23244**	283	503	535	943
19	219	313	345	754	534
20	2297	439	439	597	502
21	8439	755	–	314	503
22	722	219	281	408	189

Bold indicates exceptionally high figures.

Most large concentrations resulting from the misplanned management protocol of HVAC systems can be linked to an insufficient fresh air intake (allowing the build-up of indoor contaminants), condensation or water accumulation in cooling coils or humidifiers and dust (and eventually mould) accumulation on filters (Custer, 1985). However, the most serious problem is a misconceived system, with porous insulation on the inside of AHUs (Morey and Jenkins, 1989). Even when dry, these materials emit particulates, as well as moulds if they have been previously contaminated by condensation or water damage. Dry material will often increase the concentration of outdoor moulds (the epitype being *Cladosporium*) by rafting; while most material will allow the growth and sporulation of indoor moulds (*Aspergillus* spp. and *Penicillium* spp.) (Morey and Williams, 1990; Samini, 1990). Moist debris will emit up to 17000 cfu/m^3, while dry material can produce up to 41000 cfu/m^3. If heating also takes place, airborne counts can be from five to eight times greater than background concentrations. Indeed, up to 3×10^7 cfu/g of *Penidllium* have been found on filters. A source inside a ventilation system is easy to locate because of the forced flux of air at specific nodes (Figure 11.4; Table 11.4). Systematic evaluation

of the ventilation system (i.e. the sampling of the intake and return of each element) is sure to lead to the identification of contamination, if any. Efficient cleaning will decrease spore numbers by an average of 95% (Table 11.6).

Table 11.5 Fungal spore concentrations (/m^3) in rooms of a single building in Montreal, with the dominant genera

Site	Spores/m^3	cfu/m^3	Dominance
1	1520	369	*Cladosporium/Penicillium*
2	15	116	*Cladosporium/Penicillium*
3	235	163	*Penicillium*
4	230	31	*Aspergillus*
5	280	76	*Penicillium*
6	260	**1267**	*Penicillium*
7	420	299	*Penicillium*
8	90	121	*Penicillium*
9	200	71	*Penicillium*
10	**4222**	309	*Pencillium*

Bold indicates exceptionally high figures.

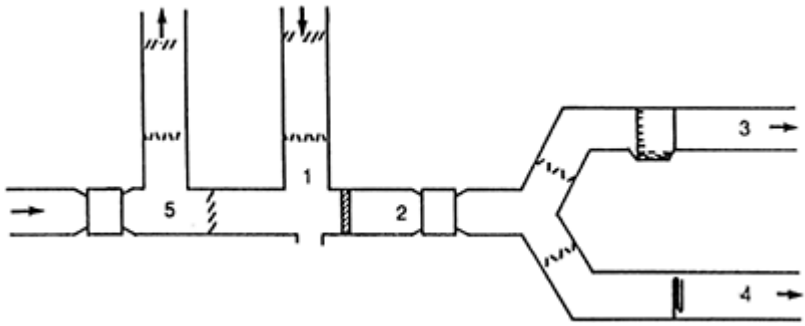

Figure 11.4 Diagram of a typical HVAC system, with all major nodes of air flux. 1, Air intake; 2, filters; 3, humidifier; 4, cooling; 5, air return.

Table 11.6 Fungal spore concentrations (/m^3) in rooms, before and after cleaning in two different building in Montreal (1) and Quebec city (2)

Spores/m^3		Decrease (%)
Before cleaning	After cleaning	
Building 1		
25638	516	98

23244	652	97
18744	573	93
Building 2		
68378	2556	97
30674	1917	94
22655	218	99

Microenvironmental contamination is much more difficult to investigate, particularly when the mould growth is not visible to the naked eye. Contamination due to general negligence in hygiene or cleaning is much more difficult to circumscribe. Most researchers will therefore refer to the outdoor air as a control in order to reach a decision. Once a ratio >1 is found between indoor and outdoor concentrations (on average the ratio is 0.33; Holt, 1990), or once species indoors differ from those out of doors (Burge *et al.*, 1987), a detailed investigation can be made of the indoor environment, including surface sampling, and remedial measures can be proposed.

As with Air Handling Units, dry materials (such as ceiling tiles, carpets, etc.), previously damaged by floods, will emit large numbers of spores (Morey and Jenkins, 1989; Morey and Williams, 1990). These situations are typical of the aerobiological pathway: a damp source is needed, but it may be separated, in space or time, from the dry environment that will allow effective dissemination of spores. The health outcome will be the same as in the house environment: rhinitis or asthma. However, a precise relationship between respiratory diseases and mould air content has not been demonstrated for offices (Menzies *et al.*, 1991).

SUMMARY

In North America, the most dramatic effect of mould in the indoor environment is on the respiratory systems of its inhabitants. Many clues to a strong relationship between symptoms and aeromycological content of the home environment exist. In the office environment, only cases of central contamination (of many tens of thousands cfu/m^3) leading to hypersensitivity pneumonitis have been demonstrated, and bacterial contamination seems to be much more prevalent causing inhalation fever. The overall influence of mould in the sick building syndrome is not known precisely, probably because of deficiencies in our evaluation of the microenvironment of individual rooms. *Penicillium* spp., is, by far, the most frequently cited contaminant of the indoor air. Moreover, decay-producing fungi that cause indoor contamination (by contrast with the outdoor contribution) must have water. All reported cases of contamination have either an over-abundance of water or humidity, or past history of water damage. Healthy homes or offices can only be achieved by a tight control of water activity. A precise determination of the clinical aerobiological thresholds of airborne mould concentrations in the etiology of respiratory diseases is still required.

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Fungal problems in buildings: the Danish experience

12

Anne Pia Koch

INTRODUCTION

Fungi can attack nearly all materials in a building with more or less severe consequences. From an economic viewpoint the most important fungal problems in Danish buildings are caused by wood decaying fungi belonging to the groups Ascomycetes and Basidiomycetes. This chapter will therefore concentrate on these organisms and their effects.

For all fungal attacks, the first and most important thing to do after identification of the problem is to identify all possible moisture sources, to eliminate them to as great an extent as possible and to evaluate the moisture conditions in the building over time. It may be necessary to design a monitoring or control program.

Factors that influence the risk of attacks are:

- choice of material;
- construction and maintenance;
- the habits of people living in the building;
- the annual fluctations in relative humidity.

Solutions can include:

- repair of roof and facade;
- elimination of excess water;
- elimination of condensation;
- change of habit regarding cleaning after bathing, washing and cooking.

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NON-DECAYING FUNGI

Mould fungi can threaten the building itself, the materials inside the building and the people who live or work in the building. Attack of mould fungi can cause discoloration of materials which can be difficult to remove. Often mould fungi have a bad smell and they can cause allergic reactions in some people.

Mould fungi in general demand a relative humidity near the substrate of at least 70% and optimally 95–100%. The moisture content of the substrate itself is less important to mould fungi than to decay fungi. A mould attack can therefore easily be caused by bad ventilation or condensation alone, whereas an attack of decay fungi, causing real damage to the substrate, most often demands a specific moisture source such as leakages in roofs or facades.

Mould on relatively non-porous materials can often be cleaned away whereas mould on porous materials like wallpaper, clothes and leather will often cause discolouration which can only be removed by bleaching. The smell of fungi can also be very difficult to remove in some circumstances. Mould fungi rarely cause physical destruction of the materials to any severe extent.

THE INFLUENCE OF BUILDING TRADITIONS ON DECAY

The commonest building materials in Denmark are bricks, tiles, mortar and timber. For centuries, timber has been used for internal constructions such as load-bearing horizontal divisions and roof constructions and

Figure 12.1 Street in Copenhagen with multistorey houses built before 1920.

for interior and exterior joinery, claddings, etc. (Figure 12.1). In recent years, concrete has to some extent excluded the use of timber for certain load-bearing purposes such as floor constructions. On the other hand, timber has been given a more visible position in advanced exterior constructions of architectural value, sometimes leading to very high risk of decay (Figure 12.2). During the 1960s and 1970s the knowledge of the natural durability of timber seemed more or less neglected. Constructional design and elaboration which did not protect timber from moisture or lack of chemical protection in constructions where moisture could get access, resulted in an extremely high incidence of decay, often within 10–15 years. Today we have learned by experience.

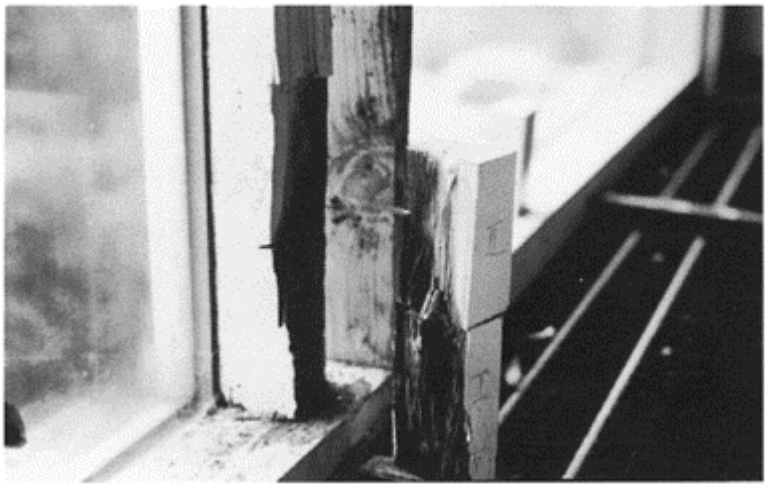

Figure 12.2 Modern window construction showing decay.

The type of decay seen in these relatively new buildings differed from that seen previously since new habitats had been created. For instance a flat roof covered by black roofing felt creates a climate with highly fluctuating temperatures and here species of the genus *Gloeophyllum* will thrive under favourable moisture conditions. Dark painted exterior constructions facing south or west will create the same good habitat for *Gloeophyllum*. In exterior constructions where moisture can get access to joinery, the conditions can also be suitable for species belonging to Corticiaceae or Dacrymycetaceae which previously were regarded as secondary invaders and not a severe risk.

Old multistorey houses are now being renovated. Decay is usually not visible until the constructions are opened, for example by taking up floorboards. The most common and most costly decay type is the dry rot fungus *Serpula lacrymans* (Fr.) Schroeter (Figure 12.3).

Figure 12.3 Fruit bodies of the dry rot fungus in a floor construction.

THE WORK OF THE DANISH TECHNOLOGICAL INSTITUTE

The Danish Technological Institute has dealt with problems concerning building mycology since 1935. At that time Danish insurance companies decided to offer insurance against decay in buildings. This soon created a need for an impartial party to investigate and identify the damage and to decide whether the damage could be regarded as an insured event or whether it was the result of bad maintenance of the construction and/or the surroundings which affected the construction.

A biologist, Louis A.Harmsen MSc, was employed in 1935. Through his work a special branch of mycology was developed: building mycology. The first step was to identify the decay fungi. Since decay fungi seldom produce fruit bodies indoors, the traditional identification keys based on descriptions of fruit bodies could not be used. Therefore the fungi from wood samples in every case had to be cultivated in the laboratory and compared with identified cultures made from fruit bodies. Through this experience the commonest decay fungi in Danish buildings could be identified from the substrate mycelium and the surface mycelium in the absence of a fruit body. Today about 40 common decay fungi can be identified to genus or species by a quick and simple microscopy technique. Further knowledge of the decay fungi and the conditions under which decay can be established in a timber construction could only be gained by surveys *in situ.* The biologists' knowledge became a supplement to that of architects and engineers and is today well respected.

A European survey of dry rot (Koch, 1990) showed, among other things, that widespread insurance against wood decay as it is known in Denmark is unique. Only in very few other European countries is it possible to insure a house against different types of decay caused by fungi (not only dry rot) and insects. Even though such insurance is

not obligatory, a private Danish house without insurance will lose value unless it is a very special building or owned by the state (which is self-insured).

SIGNIFICANCE OF THE DECAY PROBLEM

Since 1935 attempts have been made to collect information for statistical analysis of the incidence and significance of decay in Danish buildings. The information gained when samples of decayed wood are sent to DTI for analysis is most often not very detailed regarding the type of building, age of the building and the causes of decay. However some interesting trends reflecting architecture and building politics in Denmark can be seen.

Figure 12.4 shows the general distribution of the most common decay fungi found in 1991. The statistics for 1991 are based on 1976 investigated wood samples from 875 buildings. The figures are assumed to be reasonably representative of the total situation in Denmark.

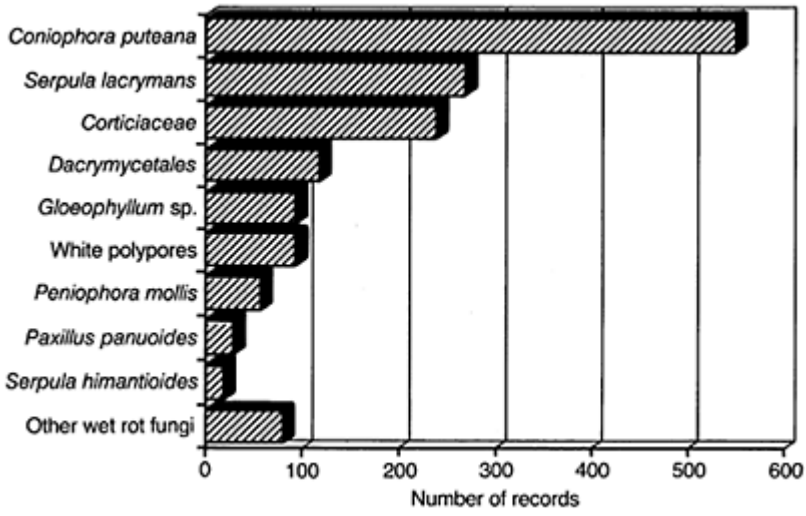

Figure 12.4 Wood decaying fungi identified in Danish buildings during 1991.

If we look at the seven most common decay types (Figure 12.5), the number of detected attacks has increased remarkably over the past 45 years. Among the reasons for that are an increase in the number of surveyors who know what to look for and where to look, and a greater awareness of the problems of decay in buildings, not only among surveyors but also among houseowners, insurance companies, those who plan or renovate the building and the building workers themselves. The increase can also be explained by the greater incidence of attacks in newer buildings, decreasing budgets for general maintenance

combined with increasing cost of maintenance and the political decision to restore old buildings (Koch, 1985).

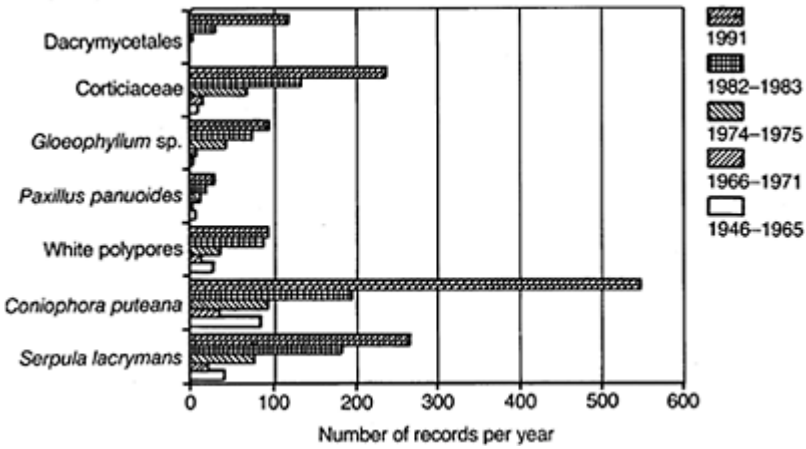

Figure 12.5 Occurrence of the seven most common decay fungi over the past 45 years.

Fungi belonging to the Dacrymycetaceae, Corticiaceae and the genus *Gloeophyllum* have only recently been identified as causing severe decay; they have been introduced by new building designs. The increase in *Coniophora* is explained by many old buildings being renovated in recent years. This fungus can be found in every timber construction in these buildings, often as a result of long-term bad maintenance. The increase in dry rot has occurred for the same reasons.

Years ago dry rot was mainly found in cellars where it most commonly attacked the lowest floor construction. Today many hidden attacks of dry rot are found at the base of roof constructions and in the horizontal divisions between the lowest and upper floor. In 1991 dry rot, with very few exceptions, was found in buildings built before 1920. (Surveys in 1991 were evenly distributed between new and old buildings (Figure 12.6)). This tendency can be regarded as general.

The relative occurrence of decay in floors, roofs and exterior timber during different periods (Figure 12.7), reflects the design and the building and housing politics in Denmark at those times. An increase in

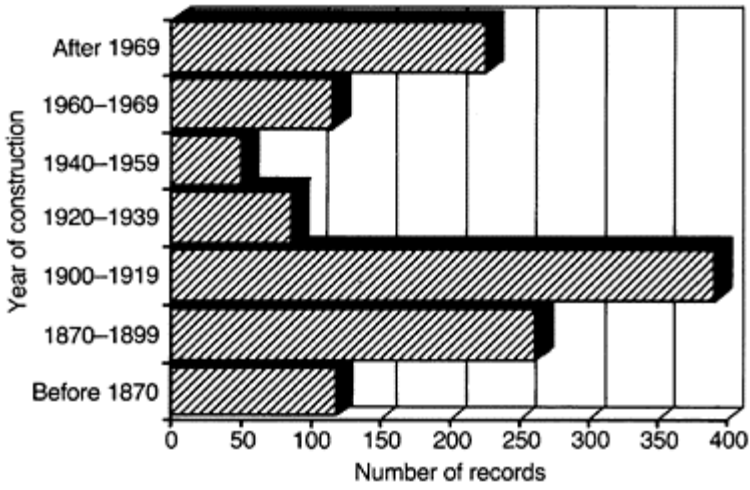

Figure 12.6 Age distribution of buildings of known age visited during 1991.

damage to exterior timber occurred during periods when many new single-storey houses and office buildings were built from very bad designs and with a degree of elaboration that did not take into account the perishable nature of wood under prevailing conditions. Most timber for exterior use is chemically treated, which has led to a decrease in decay incidence.

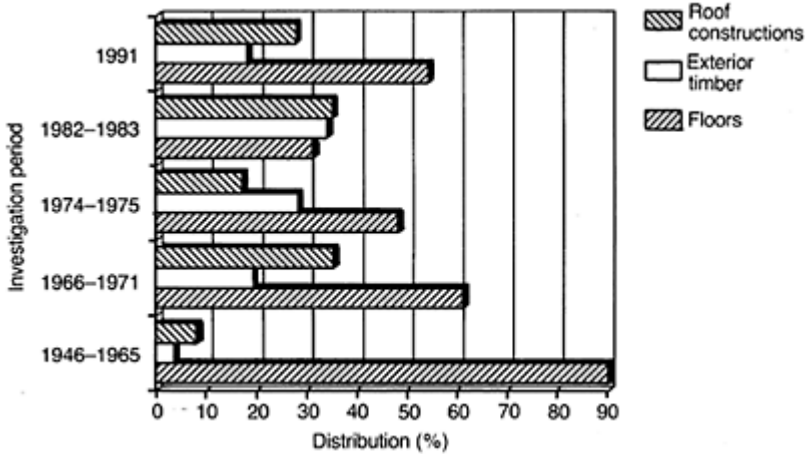

Figure 12.7 Relative occurrence of decay related to construction over the past 45 years.

Problems of maintenance also contribute to damage of exterior timber. The increased use of do-it-yourself (DIY) systems for exterior maintenance, combined with new constructions and use of new materials, has often resulted in defective and insufficient maintenance. An example of this is the lack of sealing between glass and timber in windows and doors.

The amount of damage found in floors decreased relatively during, and shortly after, periods of high building activity. This kind of damage is mainly found in old buildings. Thus an increase will occur when government decides to renovate older buildings instead of building new ones.

The increase of attacks in the horizontal construction of roofs seen from 1965 to 1971 also reflects design problems. The decrease during the period from 1971 to 1975 is due to the improvement of already built constructions and changes in new constructions. The increase from 1975 to now can mainly be explained by the increase in renovation activities during which many concealed attacks are detected. During this period the erection of new houses decreased drastically. About 60% of the decay in old Danish buildings is caused by leakages in roofs or facades. Other reasons are penetrating and rising damp or failures in plumbing systems.

The general impression is that decay in buildings has increased during recent decades. Although this is true for new buildings, decay has been going on for years in older buildings but active investigations to reveal hidden attacks have only started recently. This raises the question of how these hidden attacks in buildings can be detected.

DETECTION OF DECAY

Decay in interior timber constructions is most often hidden under floorboards and behind plaster. Even decay in exterior constructions can develop to a severe extent before it becomes visible. External surfaces of moist constructions dry quickly and conditions for decay are most suitable inside the timber. Collapse of constructions is very rare. Decay can become visible by the appearance of fruit bodies or the development of surface mycelium, but most often only cracks and dents in the timber surfaces are seen. Behind these cracks the decay is most often well established.

An experienced surveyor looking for decay searches for the abovementioned signs. A moisture-meter to detect possible decay in moist constructions can be useful, but often decayed timber is dry and it would be necessary to open constructions to reach areas where there is a risk of decay. No instruments have yet been developed to replace fully the experienced surveyor and his simple tools: a hammer, a chisel and a moisture-meter. A more thorough survey will often damage the building to some extent. Non-destructive detection methods are still under development. These will be very important tools for the surveyor, saving money by avoiding the opening and repairing of constructions which are not decayed, and so facilitating more cost-effective plans for a restoration.

DOGS

In Sweden dogs trained to detect mould fungi in buildings and decay in poles have been used for many years. Recently dogs have also been trained to detect general decay in buildings in Denmark and the UK.

In Denmark dogs have only been trained to detect dry rot since 1988 (Figure 12.8) (Chapter 7). Dry rot is the most severe fungus in old buildings and we wanted to be able to detect it in a building prior to the restoration or sale of the property without destroying any constructions, since people often have to continue to live there, sometimes for years, before restoration can take place.

Figure 12.8 A dog searching for dry rot.

On training courses, dogs are able to differentiate between the true dry rot fungus and other decay fungi. In actual buildings, however, the results were less consistent. It seems that the task is more difficult because extensive attacks can fill the whole building with the chemicals to which the dogs react. These problems can be solved. Today a welltrained dog with a good handler in cooperation with a building surveyor will be able to detect 90–100% of all existing dry rot in the building without causing material damage. Constructions still have to be exposed, however, to verify the results and determine the extent of attack.

INSTRUMENTS

Most instruments for non-destructive detection have been developed to find decay in poles and standing trees or timber prior to use. They require access from two sides which is generally not possible in a building.

The Danish Isotope Centre, in collaboration with the Danish Technological Institute, has developed a portable instrument to detect decay in buildings. The instrument (Figure 12.9) is based on collimated photon scattering whereby the density of the timber can be measured to a depth of 20 cm. The instrument contains a source of gamma rays which are collimated into a ray that penetrates the material either at right angles or at 45 degrees. The rays are then reflected at right angles back to a detector which counts the photons. The results seen on the screen depend on the photon energy, the scattering angle and the local electron density. The intensity of reflected photons is proportional to the density within the investigated area. The results have to be correlated with results from non-decayed timber of the same species. A computer program can convert the results of density to loss in strength (Madsen an

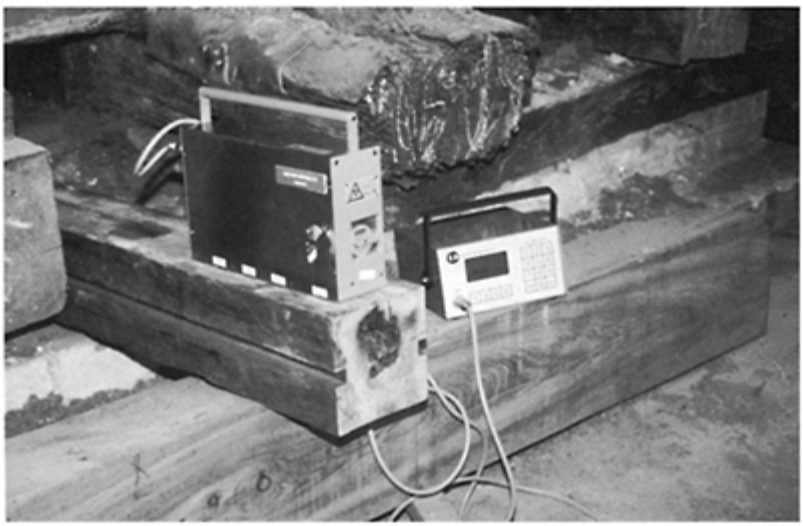

Figure 12.9 The RDS instrument for detecting the density of wood.

Adelhoej, 1989). The instrument is expensive to make and not yet commercially available.

Another possible way of detecting decay is to use ultrasound. The Medicotechnical Institute in Denmark and the Danish Technological Institute have recently started a project to develop an instrument based on this principle. In the near future, a range of methods that can be used singly or in combinations under different circumstances might be available.

IDENTIFICATION

Wood samples of decayed timber can be taken from the constructions and the decay organisms identified by experts using microscopy. Fungi are identified by the surface mycelium, substrate mycelium or fruit bodies, or by cultivation and identification of the cultures obtained. The knowledge and experience obtained in Denmark on Danish wood decaying species cannot necessarily be transferred directly to other countries. Therefore, results and information have not yet been published internationally, but the increasing interest in decay in buildings all over Europe might make it worthwhile to exchange information with experts in other countries.

Simple methods for identification, that do not require special education and experience, are not yet available. Attempts have been made to produce a simple immunological test-kit for identification of dry rot, which can be used by anybody, but non-specific reactions have made production difficult. These problems might be solved in the near future. The use of dogs trained to react to a specific fungal species is now possible but even here a handler and trained surveyor are necessary. In the future it might be possible to develop an instrument that can detect chemicals exuded from the active mycelia, but very little is yet known about which compounds fungal mycelia excrete, and whether various species found in a building excrete specific compounds. From studies of the proteins in various species of decay fungi it has been possible to identify proteins specific to some fungi and to use this knowledge in immunological studies (Chapter 8).

TREATMENT

A timber construction will only deteriorate if water gains access to the timber so that the moisture content exceeds 20% over a long period. Therefore, in a specific case of decay the most important thing is to identify the source of water and if possible eliminate it so that the moisture content can be quickly decreased to below 15%, the minimum for possible growth of decay fungi. With the possible exception of dry rot, decay fungi will then stop growing and eventually die. Some fungi can withstand dry conditions for a long time and then become active if the moisture content increases to a level suitable for growth.

If dry conditions can be established and the loss in strength does not affect the strength of the construction as a whole, no more needs to be done. If necessary, *in situ* (remedial) treatment of the decayed timber can be carried out to give additional security. This is most important if there are doubts about whether the water content can be maintained below 15%. The objective of remedial treatment will then be not only to kill the existing mycelia, but also to prevent spore germination and growth of new fungi.

If there is a loss in strength which demands replacement of timber, it is important to use treated timber particularly if a water source cannot be completely eliminated. It is also recommended that treated timber should be used where the new timber comes into contact with old infected timber.

It is traditional to distinguish between wet rot and dry rot. The former means all decay types except soft rot and dry rot. The term ‘dry rot’ is reserved solely for decay caused by *Serpula lacrymans*. In most countries dry rot is treated differently to wet rot. It is

certainly the most destructive decay fungus. No other fungus is able to spread through a building from one floor to the other and to cause such destruction. There are many opinions to the question 'how much treatment is necessary?' The first question to ask is whether we want to get rid of the fungus in the building or just to control it, knowing that if our control system fails a renewed attack will probably take place. Traditional treatment of dry rot is very destructive to the building and very expensive, with a high consumption of chemicals and much inconvenience for the residents. On the other hand, experience has shown that the method is very successful as long as the extent of the attack has been fully identified. There is a need for successful methods of controlling dry rot which are less costly, less destructive and which are more environmentally safe. The sensitivity of dry rot to high temperatures suggests two possibilities: hot air or microwaves.

HEAT TREATMENT

The temperature required to kill fungal mycelia, the lethal temperature, varies for different decay fungi. Dry rot is one of the most sensitive to high temperature with lethal temperatures below 50°C, whereas *Gloeophyllum* species are very tolerant of high temperatures (Miric and Willeitner, 1984). The time for which the fungus is exposed to a certain temperature is of course very important for the effect.

To sterilize a construction against dry rot mycelium means that all the attached timber, brickwork and other materials must be heated to at least 50°C for a defined time. For hot air treatment this has been determined to be 16 hours for practical purposes. The spores are not killed: that would require heating to 90°C for 2 hours (Hegarty *et al.*, 1986). It is also regarded as irrelevant to kill the spores inside the building since they are present in the surrounding air and will be able to infect the building again as soon as the heat treatment is finished. Instead it is important to eliminate any moisture sources that could make it possible for new spores to germinate and establish a new attack.

Table 12.1 Heat treatment—lethal dose necessary to kill *Serpula lacrymans*, as reported in the literature

Temperature	35°C	37.5°C	40°C	45°C	50°C
Lethal time			hours		
Falck (1912)			$\frac{1}{2}$		
Liese (1931)			$\frac{1}{4}$		
Montgomery (1936)			$\frac{1}{4}$		
Langvad and Goksøyr (1967)	6	4			
Savory (1971)			$\frac{1}{4}$		
Miric and Willeitner (1984)	>24		6	3	1
Danish Technological Inst. (1992)					16

The background for setting the lower limit of the lethal dose to 50°C for 16 hours is based partly on literature studies (Table 12.1), and partly on investigations in the

laboratory and *in situ* (Koch *et al.*, 1989). In practice, before a heat treatment takes place, a thorough survey of the extent of the attack must be undertaken and the dimensions and strength of the constructions involved have to be reported. The water sources must be identified and eliminated and water measurements must be recorded. The heat treatment can then be simulated. Before the actual treatment, the building or part of it is insulated on the outside (Figure 12.10) and where necessary also on the inside. Thermometers are placed throughout the construction in areas regarded as most difficult to heat. The heating system is set up and the treatment can start. During the treatment, the temperature and heat supply are controlled so that quick adjustments can be made. A final report of the treatment is drawn up by the company in charge of the treatment. These companies are under the control of an impartial institution, thereby providing the necessary assurance that the treatment is carried out responsibly according to rules set up by the control organization. A special insurance has led to the introduction of a 5-year warranty of heat treatment.

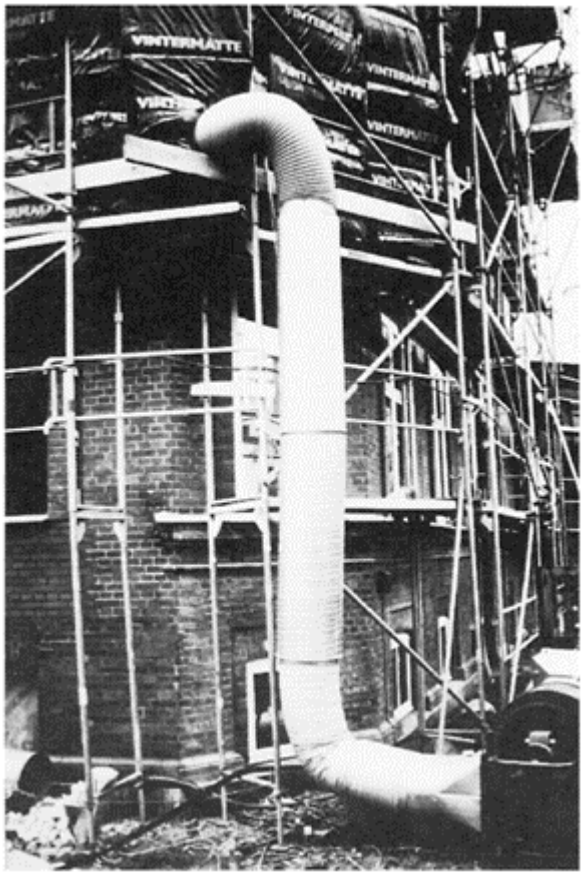

Figure 12.10 Heat treatment of a roof construction with dry rot.

The first heat treatment by hot air was carried out in 1986 and since then more than 60 buildings have been treated. In one of these treatments, the entire building was heated while the others were partial treatments. Experience shows that heat treatment of dry rot will often be cheaper than traditional repair, depending on the extent and situation of the attack. Up to 50% of the cost of repairs can be saved since less replacement of timber and no treatment of the brickwork is necessary. However, on party walls between neighbouring houses and occasionally in the ground floor construction, it might be necessary to supplement traditional treatment with chemicals. The total amount of chemicals used is decreased significantly The time needed to conduct a heat treatment is shorter than for traditional treatment.

MICROWAVE TREATMENT

Another possibility for sterilizing a construction containing mycelia of dry rot is use of microwaves. Experiments have shown that application of microwaves can lead to lethal temperatures within brickwork and timber. This method is expected to be applied in special situations once quality control issues have been addressed.

ATP TEST SYSTEM

A test system to evaluate the effect of treatments on mycelium in wood and in brickwork has been developed. This test system includes evaluation of the viability of the test mycelium by a combination of measuring ATP (adenosine triphosphate, an energy molecule) produced by the cells and assessment of their ability to grow using cultivation techniques (Hegarty and Schmitt, 1988).

SUMMARY

Decay in buildings is very costly to society and is a threat to every houseowner. The cost of good surveys and repair of attacks, and of maintenance in general, is increasing whilst budgets tend to be decreasing. Therefore it is essential that architects and engineers experiment with new designs, within the limits of the natural durability of wood and with respect for the experience of traditional constructions, and ways of protecting timber which use its own anatomy most beneficially.

Public awareness of the potential environmental hazards associated with the use of chemicals in buildings adds to the importance of this approach.

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The effect of filamentous fungi on stone monuments: the Spanish experience

13

Gonzalo Gomez-Alarcon and M.Angeles de la Torre

INTRODUCTION

Weathering of minerals in stone monuments is a continuous process that endangers the survival of the world's historical heritage. The phenomenon has been recognized and observed for many years, but it was not until this century, in fact only in the last 30 years, that conservation came to be a matter of concern. The need to preserve objects and monuments of historical value led first to a study of the causes and mechanisms involved in the deterioration, and the term **biodeterioration** came to define the role of microorganisms—mainly fungi, bacteria and lichens—in the stone (Webley *et al.*, 1963; Pochon and Jaton, 1968; Williams and Rudolph, 1974; Strzelczyk, 1981; de la Torre *et al.*, 1991b).

Stones and minerals have always been good substrates for the development of microorganisms, as is apparent in the growth of crusts and biofilms, in changes in the mineralogical and chemical composition, and in physical alterations such as changes in porosity, stability, density and colour (Krumbein, 1988). The microflora present on the affected surfaces of stone monuments is usually very diverse. Bacteria, algae, fungi, lichens and even some plants of a higher order may be found on the same stone. In favourable circumstances, fungi may penetrate and colonize rock substrates very quickly, given the capacity of expansion afforded by the hyphal system they develop. In the following paragraphs, we

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provide details of the various mechanisms such as acidolysis by microbially formed organic acids, complexation of cations, and oxidation of certain mineral cations, by which fungi contribute actively and significantly to the process of mineral weathering (Eckhart, 1985; Petersen *et al.*, 1987; Krumbein, 1988).

Spain has an immense historical heritage made up in part of important stone monuments. Many of these buildings are in urban and industrial areas, so they face serious problems of deterioration resulting from harmful environmental factors and the

lack of resources for their preservation. Until recently few studies had been made of the biological deterioration of monuments in Spain. The references available are those of Saiz-Jimenez and Samson (1981) and Saiz-Jimenez (1984) on the degradation of the limestone of the Giralda in Sevilla and the studies made by Ascaso *et al.* (1982) of lichens. In recent years, new groups are working on the recognition and the role of fungi, algae, cyanobacteria and atmospheric pollution in the deterioration of stone monuments in Spain (Saiz-Jimenez *et al.*, 1990,1991; de la Torre *et al.*, 1991a).

This work shows some experimental results that help to account for the biodegradation processes brought about by fungi, as a result of their metabolic activity, on the materials of several stone monuments. To widen the scope of the study, three monuments were chosen in three

Figure 13.1 Geographical location of the historic monuments studied.

different regions of Spain (Figure 13.1) all of them showing signs of bio-alteration, and each built of a different material (Table 13.1).

Table 13.1 Monuments chosen for study

Cathedral	Building material
Salamanca	Sandstone
Toledo	Limestone and granite
Sevilla	Limestone

FUNGI FROM WEATHERED STONES

Clear evidence of biocorrosion was found on the facades of the three cathedrals that were the subject of this study: crusts and patinas of loose stone material as well as organic material of different origin (algae, lichens, bird droppings, plant residues, etc.). In some cases, the presence of organic material was not so obvious, although the colouring and texture of the materials indicated the effects of microbiological action. Care was taken to distinguish between these biogenic crusts and those caused by the action of air pollution. The latter are very dark, almost black, and spread evenly over the zones nearest to the traffic. Certain parts of the cathedrals of Sevilla and Toledo are affected by these deposits, whereas that of Salamanca, further away from exhaust fumes, is less affected.

Samples were taken from the walls of the three cathedrals, along all the facades and at different heights from the ground where signs of biological corrosion were previously observed. Various strains of filamentous fungi, commonly found in the air and on the ground, were isolated and identified in the samples. Table 13.2 shows the common fungi isolated from the three cathedrals.

Strains of the genera *Penicillium, Phoma, Alternaria, Cladosporium* and *Aspergillus* have often been found on weathered stones (Webley *et al.,* 1963; Eckhardt, 1985; Petersen *et al.,* 1987). These authors also isolated organisms belonging to genera that were not identified in any of the materials from the three monuments from which our samples were taken. Webley *et al*. (1963), for example often found *Cephalosporium* and *Pullularia* on weathered granite, and Eckhardt (1985) found *Aerobasidium* and *Torula* in crusts from standstone.

The presence of one or other of the fungal strains in the patinas and scales formed on the materials under study depends more on environmental and geographical factors than on any specificity for the stone substrate; most of these species are commonly present on the soil and in the air, which makes for easy propagation and development in the micro-fissures and holes on the surface of the stone monuments.

Table 13.2 Fungi most commonly isolated from weathered stones taken from three cathedrals in Spain

Source	Species
Sandstone (Salamanca)	*Penicillium frequentans* Westling *Cladosporium cladosporoides* (Fres.) de Vries *Phoma* sp. *Alternaria alternata* (Fr.) Keissler *Trichoderma pseudokoningii* Rifai *Trichoderma viride* Pers. ex S.F.Gray
Limestone (Toledo)	*Alternaria tenuissima* (Fr.) Witshire *Cladosporium cladosporoides* (Fres.) de Vries *Cladosporium herbarum* (Pers.) Link ex Gray *Phoma glomerata* (Corda) Wollenw. and Hochapfel *Penicillium frequentans* Westling
Limestone (Sevilla)	*Phoma pomorum* Thum *Cladosporium cladosporoides* (Fres.) de Vries *Fusarium reticulatum* Mont. *Penicillium lanosum* Westling *Alternaria alternata* (Fr.) Keissler *Fusarium equiseti* (Corda) Sacc.
Granite (Toledo)	*Penicillium frequentans* Westling *Cladosporium herbarum* (Pers.) Link ex Gray *Alternaria alternata* (Fr.) Keissler *Epicoccum nigrum* Link ex Link

PRINCIPLES AND MECHANISMS OF BIODETERIORATION OF STONE

PRODUCTION OF ORGANIC ACIDS

The production of organic acids by fungi is one of the principal mechanisms of biodeterioration. The percentages of acidogenic strains, selected on the basis of the reduction of the pH in the culture medium, were as follows: in Salamanca, 46.5%; Toledo, 21.7%, and in Sevilla, 32.6%, percentages similar to those reported by Petersen *et al.* (1987) and Kuroczkin *et al.* (1988) for weathered sandstones.

The determination and quantification of the organic acids was carried out by high performance liquid chromatography (HPLC) (de la Torre *et al.*, 1991a). In descending order of incidence, the acids produced by the fungi were gluconic, citric, fumaric and oxalic acids, a classification identical to that made by Eckhardt (1985) using thin-layer chromatography (TLC). Kurockin *et al.* (1988) identified several organic acids by HPLC, and found that the acid most commonly excreted by the fungi they studied was gluconic acid which is consistent with our observations. Other acids, such as malic, succinic and itaconic acids were also produced, but on a smaller scale. Itaconic acid was found only in the cultures of *Penicillium steckii*, a strain that was isolated from the samples taken from Toledo. Kuroczkin *et al.* (1988) also identified this acid, but in cultures of *Penicillium roquefortii*. The strains of greatest acidogenic capacity were those of *Aspergillus niger*, *Trichoderma* spp. and most importantly those of *Penidllium*.

SOLUBILIZATION OF CATIONS

Filamentous fungi can grow well and develop on stone substrata using the cations released from the mineral as their saline support (de la Torre *et al.*, 1990). Strains of *Penicillium frequentans* isolated from samples taken from the cathedrals of Salamanca and Toledo were grown in a culture broth containing small quantities of glucose and nitrate, with powdered stone as the mineral support (sandstone, limestone and granite). In addition, parallel cultures were prepared as controls with Czapek-Dox as the saline medium. Throughout the culture period, no significant differences were observed in the levels of total proteins, reducing substances, organic acids excreted into the medium and dry weight of mycelium, between the cultures with powdered stone and the controls. Limestone was the most favourable of the three stone materials for the growth of fungi and for the assimilation of glucose (Figures 13.2 and 13.3).

Laboratory experiments to determine the corrosive capacity of the most commonly excreted organic acids (oxalic, gluconic and citric) on sandstone, limestone and granite, showed that they solubilized iron and aluminium. Citric acid was the most effective in solubilizing sodium, gluconic acid was the most effective in contact with calcium, and oxalic acid on potassium. On limestone, these acids solubilized large amounts of calcium and magnesium which was to be expected since they are the principal cations in the composition of calcium carbonate and dolomite. On sandstone and granite, the order of effectiveness in solubilizing cations was oxalic acid, followed by citric and gluconic acids, with a similar effectiveness on limestone.

The action of organic acids on stone materials was shown by X-ray diffraction and Fourier-transform infrared spectroscopy (FTIR). In the case of sandstone and granite, there was a deterioration of silicates (mainly clays), feldspars and micas, while in limestone, the deterioration was in the amount of calcium carbonate and dolomite. These results coincide with those obtained from cultures of acidogenic fungi in the presence of quarried specimens. Gluconic and citric acids were the most commonly found in the filtered liquids. Oxalic acid was not detected in the solution but its presence was shown by scanning electron microscopy (SEM) coupled with X-ray analysis system (KEVEX) since oxalate crystals were identified on the surface of the samples.

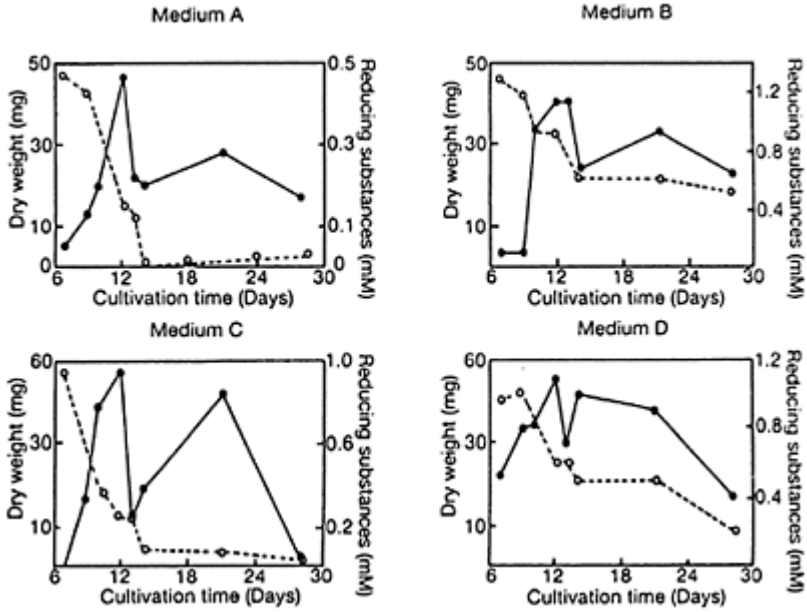

Figure 13.2 Changes in the mycelium dry weight (—) and total reducing substances (--) when *P. frequentans* is cultivated in four parallel liquid cultures which contained glucose 0.5%, $NaNO_3$ 0.2%, and as saline elements Czapek-Dox saline medium (medium A), powdered sandstone (medium B), powdered limestone (medium C) and powdered granite (medium D).

Fungi of the *Penicillium* genus were the most potent in solubilizing mineral cations, a finding that coincides with results reported by other workers (Eckhardt, 1980). *P. frequentans* was the most potent strain in this respect. Some of the fungi tested, while not acidogenic, did show a certain capacity to solubilize some cations, through metabolites such as enzymes, antibiotics, etc., which may contribute to the extraction of some cations from the stone (Jones and Pemberton, 1987).

Some studies have been published which document the solubilization of mineral cations by organic acids. Henderson and Duff (1963) observed that oxalic and citric acids were able to decompose certain silicates, releasing mainly aluminium and magnesium. Manley and Evans (1986) showed that these two acids solubilize aluminium, calcium, sodium and potassium from samples of feldspars. Eckhardt (1985) demonstrated that oxalate and citrate acids dissolved potassium, magnesium, iron, aluminium and calcium from biotite, and gluconate acid solubilized iron, magnesium, potassium and aluminium in various silicates. Silverman and Munoz (1970) found that the citric acid secreted by a strain of

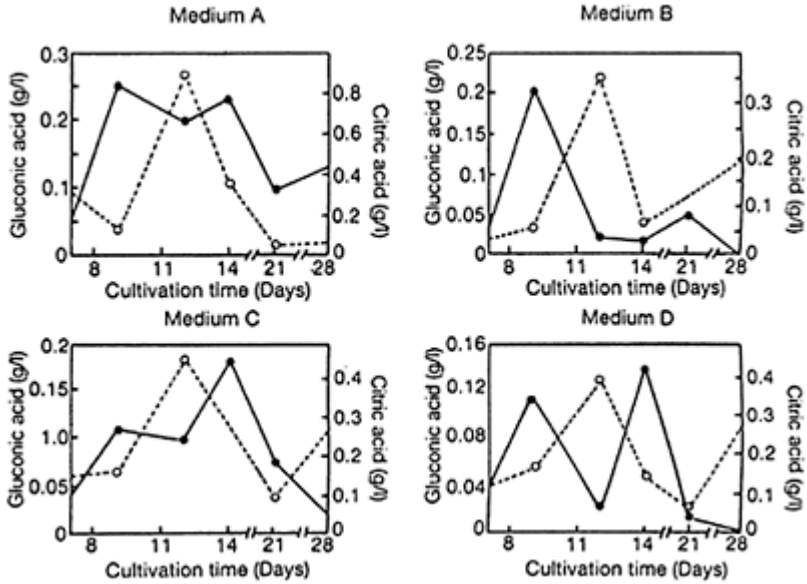

Figure 13.3 Sequential vatiation of the excretion of the gluconic (—) and citric (--) acids in the liquid cultures of media A, B, C and D (as in Figure 13.2).

Penicillium simplicissimum solubilized aluminium, iron and magnesium from granite samples.

In the case of limestone, Jones and Pemberton (1987) showed that filamentous fungi such as *Penicillium chrysogenum* and *Cladosporium herbarum* may play an important part in the dissolution of Iceland spike calcite. Ascaso *et al.* (1982) found that the oxalate secreted by certain lichens was able to solubilize calcium, iron and magnesium in limestone. In sandstone and granite, therefore, it is the iron, aluminium and magnesium cations that are most commonly solubilized by the acidogenic filamentous fungi. This suggests that silicates are the minerals most susceptible to fungal attack, and in general, those that contain iron and magnesium, such as biotite, micas, and clay minerals, while feldspars and quartz are more resistant (Silverman and Munoz, 1970). In limestone, the principal minerals, calcite and dolomite, were easily affected, as shown by the high level of magnesium and calcium, along with small quantities of iron and aluminium, which were solubilized by the acidogenic strains.

MICROBIAL PRODUCTION OF CHELATION COMPOUNDS

The formation of chelates among the mineral cations and organic acids generated by the microbial metabolism is considered an important process in the biodeterioration of stone materials (Krumbein and Petersen, 1987). Our experiments showed that in fungal cultures with a pH over 4, all iron in solution was in the oxidized form, Fe(III). Since Fe(III) is very insoluble, except in very acid media, and given this high proportion of organic acids in the medium, mainly gluconic and citric acids, it is reasonable to assume that these acids form chelates with the Fe(III), and probably also with the Al(III).

Citric acid, which is excreted by many fungi, particularly by *Penicillium*, is known to be an effective agent of chelation of Fe(III) (Silverman and Munoz, 1970; Avakyan *et al.*, 1981). Williams and Rudolf (1974) have suggested that other fungal metabolites such as antibiotics may be capable of forming chelates in the presence of trivalent cations. This is borne out by some of our results; we detected Fe(III) and Al(III) in non-acidogenic cultures growing on stone material. It would seem, therefore, that in some cases the compounding is due to some type of fungus metabolite rather than to organic acids.

FORMATION AND PRECIPITATION OF ORGANIC SALTS

Several authors have reported the presence of crystals, composed of different acids, on weathered parts of stone monuments. Alessandrini *et al.* (1989) identified whewellite (calcium oxalate monohydrate) on samples of granite and limestone, and Mariottini *et al.* (1989) isolated whewellite and weddellite (calcium oxalate dihydrate) on affected areas of limestone monuments. Crystals of whewellite, weddellite, ferric oxalate and magnesium oxalate were identified by Ascaso *et al.* (1982) on limestone samples attacked by lichens.

We studied crystal formation by acidogenic fungi grown on samples of sandstone, limestone and granite. By means of X-ray, FTIR, SEM and KEVEX we detected the presence of whewellite and weddellite crystals on the surface of the three types of stone: magnesium oxalate on granite and limestone, ferric oxalate on sandstone, and calcium citrate on sandstone and very abundantly on limestone on which magnesium citrate was also identified (Figures 13.4 and 13.5).

These findings coincide with the report of Krumbein *et al.* (1989) who stated that fungi can trigger the formation of crystals of oxalate, of various cations, on sandstone. It is not only citrate and oxalate that can form saline precipitates; gluconate is also responsible for these processes, as is indicated by Eckhardt (1985) who isolated crystals of ferric gluconate formed on sandstone by the action of an *Aspergillus* isolate.

There has been a good deal of discussion as to whether the patina known as 'scialbatura' has a biological origin. Often found on the surfaces of marble and limestone, it comprises whewellite and weddellite. The origin of these crystals is however not very clear. Some authors (del

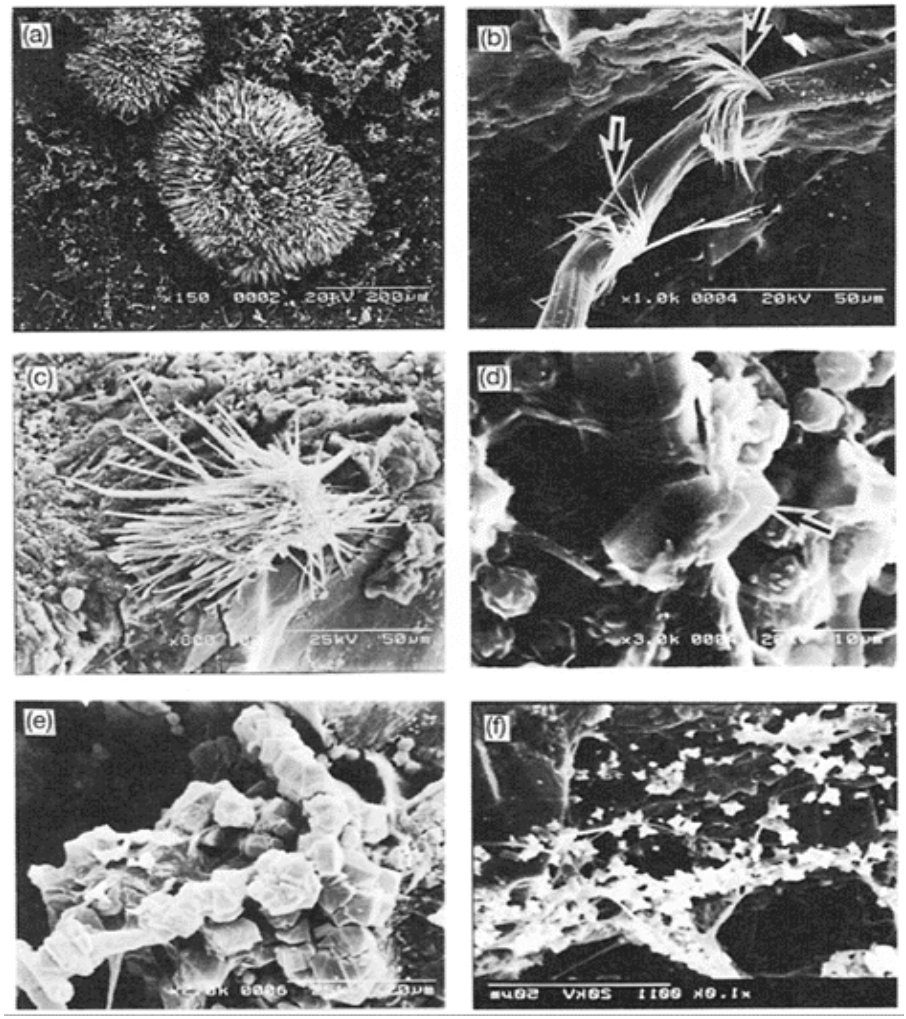

Figure 13.4 SEM micrographs of crystals on different surfaces. (a)

Calcium citrate produced by *P. frequentans* on limestone; (b) calcium citrate crystals on sandstone surface produced by *T. pseudokoningii;* (c) calcium citrate crystals on granite after the mineral treatment with citric acid; (d) weddellite crystals made up by *A. niger* above sandstone; (e) calcium oxalate and iron oxalate formed by *P. frequentans* above sandstone; (f) small pieces of calcium oxalate going around the hyphae of *P. frequentans* on granite.

Monte and Sabbioni, 1987) claim that they originate from lichens, while others (Lazzarini and Salvadori, 1989) consider the possibility of a non-biological origin. Our experiments leave no doubt as to the origin

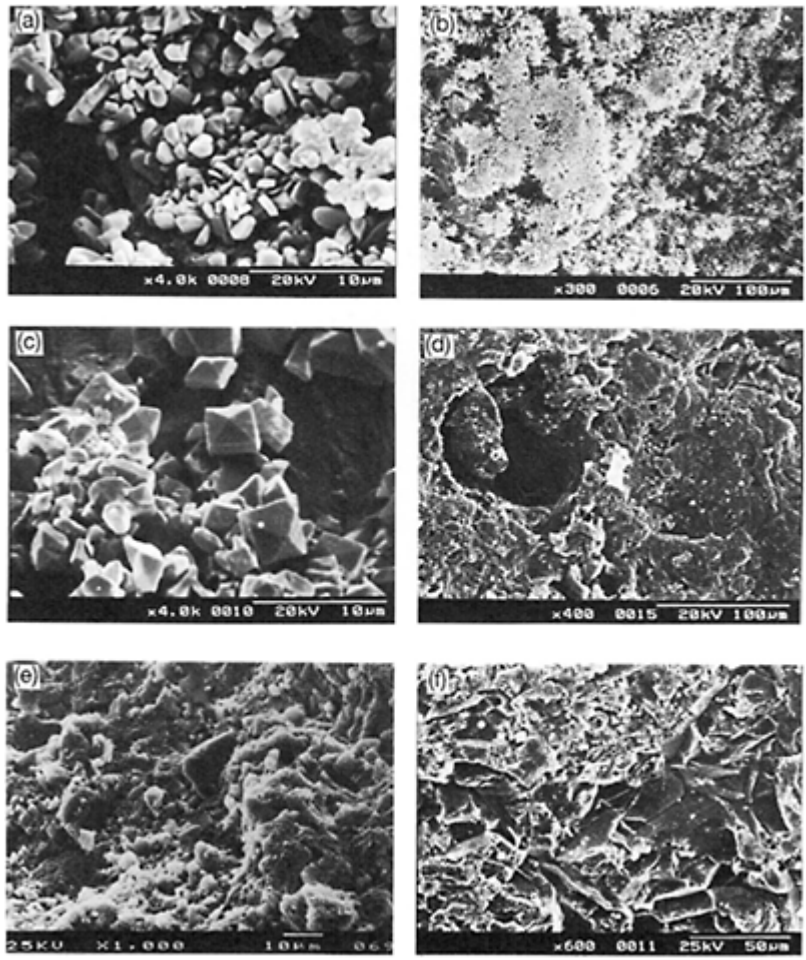

Figure 13.5 (a), (b) and (c) Calcium oxalate crystals on limestone, sandstone and granite, respectively, after treatment of the rocks with oxalic acid. (d), (e) and (f) Micrographs of control samples of sandstone, limestone and granite, respectively, which were not inoculated with fungi.

of the crystals which have been observed and identified. Organic acids from fungal metabolism were responsible for cation release from the rock material, followed by a biotransport and precipitation of the crystals onto the stone surface and the fungal mycelium.

OXIDIZATION OF MINERAL CATIONS

One of the clearest indications of biodeterioration is the presence of crusts or biofilms and exfoliations on the surface of stone facades and buildings. These biofilms can be easily lifted from the rock material.

The composition and genesis of the crusts were analysed extensively by Krumbein *et al.* (1987). According to these authors, the crust is composed of microbial communities, dust, iron and manganese oxides, sulfates and carbonates. They assume that most of the iron and manganese oxides are of biological origin, considering the enormous capacity of the fungal strains that were isolated from the rock samples to carry out the biotransfer and deposition of these cations. Also, the crusts are found most often on rocks with a higher proportion of silicates than on carbonates, the former containing a larger content of reduced metals which can be metabolized by the microorganisms.

It has been known for some time that soil fungi are able to oxidize certain mineral cations such as manganese. This was demonstrated by Bromfield and Skerman (1950) in the genera *Cladosporium, Pringsheimia* and *Pleospora* using the benzidine test. Some years later, Timonin *et al.* (1972) demonstrated, by means of visual observation of brown deposits or rings of manganese oxide, that some strains of *Phoma* mediated in the oxidation of manganese. More recently, Petersen *et al.* (1988) found that among all the strains isolated from sandstone and limestone monuments, the most effective with respect to manganese oxidation were those of the genera *Alternaria, Cladosporium* and *Phoma.*

In the course of our investigation we studied the oxidizing capacity of the strains obtained from the cathedrals of Toledo and Salamanca with regard to iron and manganese (Table 13.3). Using the leucoberbelineblue (LLB) test (Krumbein and Altman, 1973), we found that the strains best able to oxidize manganese were *Cladosporium cladosporioides, Alternaria alternata, Penicillium frequentans, P. steckii* and *Phoma glomerata.* The same strains also oxidized Fe(II) (Table 13.3).

Few studies are available to account for the mechanism by which cations are oxidized. Glenn and Gold (1985) and Glenn *et al.* (1986) demonstrated that the oxidization of manganese by *Phanerochaete chrysosporium* was the result of the action of an extracellular peroxidase dependent on manganese which would oxidize Mn(II) to Mn(III).

FUNGAL GROWTH AND PENETRATION

Studies were made of the growth and penetration of several of the strains isolated from the stone samples obtained from Salamanca and Toledo on sterile and intact blocks. In all the samples, the fungi grew well on the rock when they were given a minimum supply of glucose and nitrate. They covered the rock surface, forming a biofilm made up of the mycelial mass, crystals and salts of organic acids excreted by the fungi and precipitated on the mycelium, together with mineral particles withdrawn from the rock substratum by the fungi.

Table 13.3 Oxidation of manganese and iron by several fungal strains isolated from the Salamanca and Toledo cathedrals (Spain)

	Place of isolation*	Oxidation of Mn(II) in several culture media†	Oxidation of Fe(II)‡
Acid-producing species			
Penicillium frequentans	Sa.	MTM, MB, MZ	+
Penicillium frequentans	To.	MTM, MB, MZ	n.d.
Fusarium reticulatum	Sa.	MZ	n.d.
Penicillium steckii	To.	MTM, MBM	+
Fusarium sp.	Sa.	MTM, MBM	n.d.
Mucor hiemalis	Sa.	MTM	n.d.
Non-acid-producing species			
Alternaria alternata	Sa.	MTM, MBM, MB, MZ	+
Alternaria alternata	To.	MTM, MBM, MB, MZ	n.d.
Cladosporium cladosporoides	To.	MTM, MBM, MB, MZ	+
Phoma glomerata	Sa.	MTM, MBM, MB	+
Phoma glomerata	To.	MTM, MBM, MB	n.d.
Fusarium oxysporum	Sa.	MTM, MBM, MB	n.d.
Fusarium avenaceum	To.	MTM, MBM	n.d.
Alternaria tenuissima	To.	MTM	n.d.

* Sa., Salamanca cathedral; To, Toledo cathedral.
† MB (Bromfield, 1956); MZ (Zavarzin, 1961); MBM (Bromfield, 1956, modified with 2 g/l glucose and NaNO3 instead of $(NH_4)2SO_4$); MTM (Timonin *et al.*, 1972, modified with 1 g/l $NaNO_3$; 0.5 g/l $MnSO_4$; 0.1 g/l Ca_3 $(PO_4)_2$; 0.02 g/l $MgSO_4$; 2.5 g/l glucose.
‡ In this case we used Czapek-Dox with 30 g/l glucose. +, positive; n.d., not determined.

The film and the crust of patina differs in appearance from one genus to another. *Penicillium, Aspergillus, Trichoderma* and *Alternaria* among others, formed a homogeneous and continuous thick film, like a bed. *Cladosporium* and *Phoma* showed a dentritic and discontinuous growth all over the sample (Plate 2).

Fungal penetration was examined by the TTC (2, 3, 5-triphenyltetrazolium chloride) colorimetric method (Warchscheid *et al.*, 1990). All the strains examined were able to pass through the specimens of sandstone, limestone and granite ranging from 4.5 to 8.0 mm in depth. (Plate 3).

SUMMARY

Evidence is provided of the active role of filamentous fungi in the biodeteriorative processes occurring in many rocks and minerals. These processes may be classified as mechanical or biochemical:

1. **Mechanical processes.** These are a consequence of the growth and expansion of the mycelium on the rock. Particles of the rock are pulled out, trapped and dragged across the surface by the mycelium which also invades the pores and destabilizes the mineral structure.
2. **Biochemical processes.** These are the result of fungal metabolism.

   (a) The excretion of large amounts of organic acids onto the rock gives rise to the solubilization of the mineral cations, which in turn provokes a precipitation of organic salts, an impairment of the cementing structure and of some minerals as well as stone weight loss.
   (b) Some metabolites secreted by fungi in their secondary metabolism (organic acids, antibiotics, etc.) act as chelating agents of the mineral cations.
   (c) Certain enzymes or other metabolites produced by fungi also participate in the processes of cation oxidization (iron and manganese among others).

All these actions are carried out by the fungi, not only on the rock surface but to a depth of several millimeters, which intensifies the process of biocorrosion.

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