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Spores as allergens: a problem of sensitization

The floating matter of the air

Investigation of what Tyndall in 1881 called 'the floating matter of the air' (the 'air spora' of Gregory, 1952) began in connection with the problems of putrefaction, contagion, and spontaneous generation and various sampling techniques were used in diverse situations. In the winter of 1748–9 the Berlin botanist J. G. Gleditsch [1714–86] baited the air by putting sterile pieces of ripe melon in previously sterilized vessels, covering the mouths of the vessels with muslin, and setting them in various positions in his home and garden when mould developed on most of the pieces. C. G. Ehrenberg [1795–1876] (also of Berlin) in a long series of observations between 1830 and 1847 examined thousands of raindrops, snowflakes, and dewdrops for micro-organisms as well as specimens of dust from Germany, Switzerland, Egypt, the Himalayas, and elsewhere. He also made observations on dust deposits in various buildings including hospitals filled with severe cases of cholera. In 1846 in England, Angus Smith examined water condensed from breath and on windows for microbes and also solid impurities in the air of mines. He obtained some samples by filtering the air through cotton wool and in 1866, in connexion with experiments on disinfection for the Royal Commission on the Cattle Plague, obtained others by the agitation of a large volume of air with pure water in a stoppered bottle. Dr Dundas Thompson sampled the air of cholera wards by aspiration through Woulfe's bottles containing distilled water. Pasteur (1861) adopted the filtration technique but instead of cotton wool used a plug of gun-cotton (a nitro-cellulose) which could then be dissolved in an alcohol-ether mixture leaving the catch for microscopical examination. He found that the size of the catch varied with the site and that still air, such as that of the cellars of the Paris Observatory, contained few germs. (Tyndall demonstrated the same effect by showing that particles settled out of still air so that a beam of bright light passed through it became invisible.)

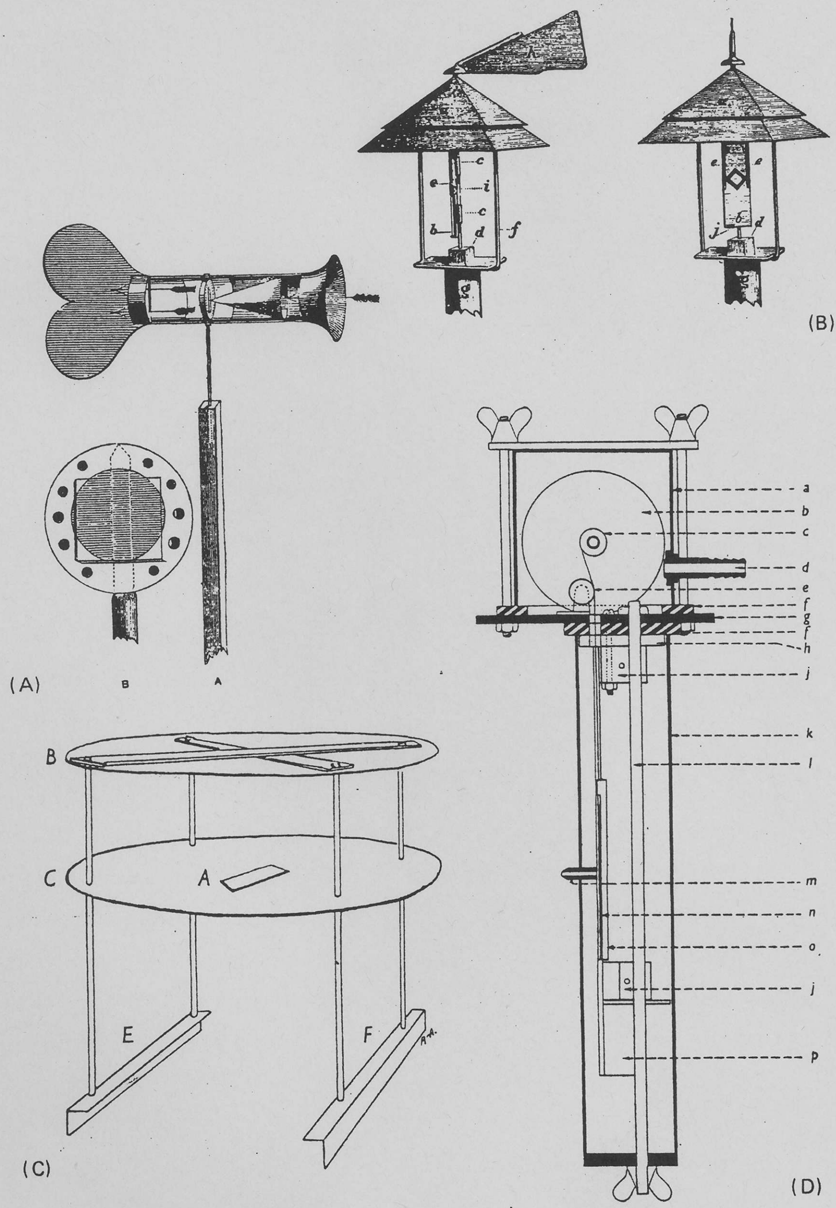


Fig. 39. Spore traps. A, Cunningham (1873); B, Blackley (1873, plate v; side and front views); C, Hyde & Williams' device for exposing gravity slides (H. A. Hyde & D. A. Williams, *New Phytologist* 43, 43: 49, fig. 1, 1944); D, Hirst's volumetric spore trap (Hirst, 1952, text-fig. 1); m, orifice; n, slide; d, outlet tube.

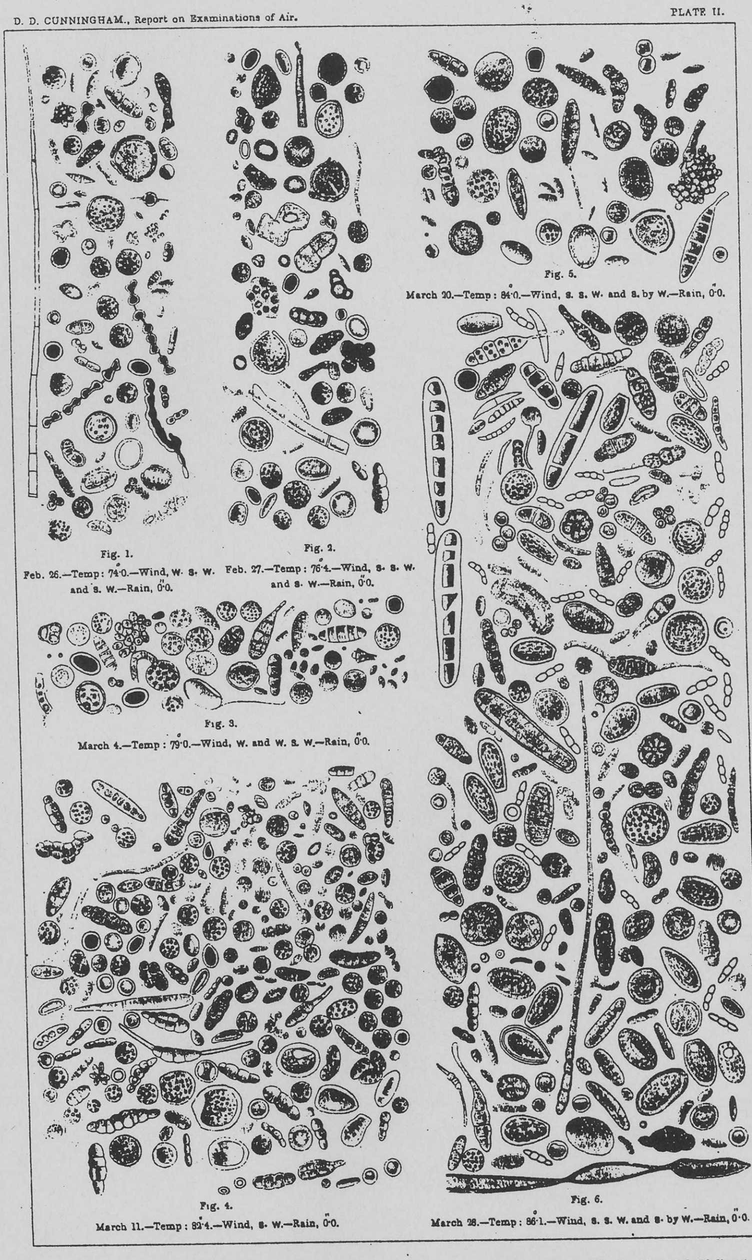
During the London cholera epidemic of 1854 the Rev. Lord Godolphin Osborne exposed slips of glass, smeared with glycerin, to the air over cesspools, gully-holes, etc. near houses in which the disease appeared and 'caught what he termed "aerozoa", chiefly minute germs and spores of fungi'. Subsequently in America in 1862 J. H. Salisbury of Newark, Ohio, suspended pieces of glass overnight over pools and swamps and examined the drops of water found adhering to their undersides in his studies on the epidemiology of malaria which occurred in the marshy valleys of the Ohio and Mississippi rivers (Salisbury, 1866).

The method of sampling the air by impaction by gravity, air currents, wind, or applied suction proved the most fruitful. M. F. Pouchet[1], in France, developed what he called an 'aeroscope' which was the basis of R. L. Maddox's 'aeroconiscope' in England[2], a modified version of which (Fig. 39, A) was used by Cunningham in India (see below). Many other types of spore trap have been used (see Fig. 39). Most gave only qualitative information about the air spora but in the currently widely used 'Hirst trap' (developed by Hirst (1952) from May's 'cascade impactor'[3]) the air spora is continuously sampled and quantified by means of suction applied by an electric pump and a moving glass slide (Fig. 39, D). Another development which greatly helped the elucidation of many problems relating to the dispersal of spores was the construction by P. H. Gregory (1951), at Rothamsted Experimental Station, of a small experimental wind tunnel which is still in use after more than thirty years of service.

A convincing early demonstration of the prevalence of fungus spores in the air was that by D. D. Cunningham, a surgeon of the Indian Medical Service on special duty attached to the Government's Sanitary Commission, who had been introduced to mycology by Berkeley, Hallier, and de Bary. Between March and September 1872, Cunningham tried to establish a correlation between the air spora and the incidence of diarrhoea, dysentery, cholera, ague, and dengue in the Presidency and Alipore Jails in Calcutta. He found no correlation, but on 59 occasions took 24-h samples at weekly intervals of the air spora with his 'aeroscope' and for each sampling published drawings of the catch (Cunningham, 1873) (Fig. 40).

From 1875 routine sampling of the air for dust, bacteria, mould, spores, etc. was undertaken at the Observatoire Montsouris in the Parc Montsouris situated in the southern outskirts of Paris. This study is particularly associated with the name of the bacteriologist Pierre Miquel [1850–1922] who was in charge of the work for 34 years and who is still remembered for his doctoral thesis *Les organismes vivant de l'atmosphère*, 1883, and for the quantitative techniques he developed for estimating the air spora.

D. D. CUNNINGHAM., Report on Examinations of Air. PLATE II.



ATMOSPHERIC ORGANISMS, &c. Collected During Periods of Twenty-four Hours in FEBRUARY and MARCH, 1872: Magnified 400 diameters.

(ALIPORE.)

Fig. 40. Spore catches collected during periods of 24 h in February and March, 1872, at Alipore. (Cunningham, 1873, plate, ii.)

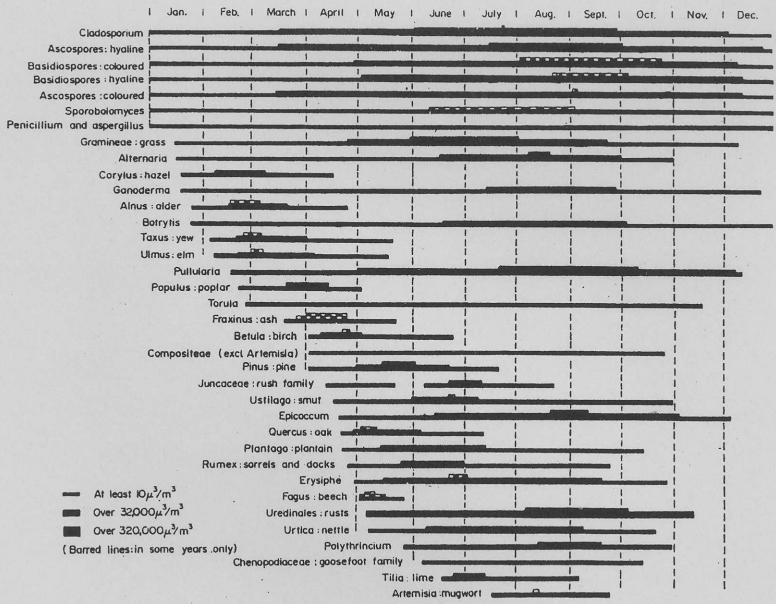


Fig. 1. Pollen and spore calendar, Cardiff.

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Fig. 41. Seasonal distribution of fungus spores (and pollen) at Cardiff. (Hyde, 1972, fig. 1).

Similar work was done concurrently in Berlin by the bacteriologist Walter Hesse [1846–1911], whose wife first suggested the use of agar-agar in place of gelatin in culture media.

Much is now known about the spores present in the air from ground level to the upper atmosphere as a result of the air sampling over both land and oceans in many parts of the world (see Gregory, 1973). These studies have shown fungus spores to be normally present in the atmosphere, often in large numbers. The pollen content of the air is naturally closely correlated with the season. Seasonal correlation is less marked for fungus spores (Fig. 41) although in the north temperate regions there is a well defined maximum spore content in the autumn and minimum during the winter months. Counts are particularly low after snow. Wet weather, which greatly reduces the number of dust particles, tends to increase the number of fungal spores and heavy rain may achieve only a temporary reduction. Another factor is the wind which is an important determinant of 'spore storms'. One of the most impressive and well documented of these storms occurred on 6–7 October 1937 throughout the eastern United States when, according to Durham (1938), the weather records showed that air masses from the north-west at an altitude of 2000–6000 feet moved rapidly east and south-east traversing Minnesota to the Atlantic seaboard in about 55 h. During this time thousands of tons of mould spores were transported several hundred miles. At nearly every station east of the Mississippi river the catch of *Alternaria* spores (up to 2027 per 1.8 cm^2 per 24 h) was the highest of the year. At 17 of the stations the average increase in spore count was a hundred times that of the daily mean for October. Mass movements of cereal rust spores regularly occur in parts of North America, and India, and from North Africa to Europe where they are responsible for initiating outbreaks of cereal rust. The spore content of indoor air is normally lower than that of outdoor air but the former may attain exceptionally high values in cow sheds or other dusty situations.

The list of identified species recorded from air is long and the list of unidentified species even longer. In North America much attention has been paid to the incidence of *Alternaria* spores. This is partly because of the ease with which *Alternaria* spores can be recognized but evidence suggests that spores of this genus, which often predominate in that region where much land is devoted to cereals, are particularly important as allergens. *Alternaria* spores regularly occur in the air of the British Isles but in smaller numbers, the most numerous spores being those of *Cladosporium*, a genus which includes ubiquitous saprobic moulds. At the Asthma & Allergy Research Unit at St David's Hospital, Cardiff, where from the early nineteen-forties H. A. Hyde and D. A. Williams made long-term investigations of atmospheric pollen and fungus spores in relation to allergy[4], in 1948 *Cladosporium* colonies accounted for half of those developing on air plates and similar results have been obtained at other localities in the United Kingdom and elsewhere.

Fungi as allergens

The first published record of an allergic reaction to fungi is possibly that 'of a remarkable instance in Bonetus', referred to by Sir John Floyer in *A treatise of the asthma*, 1698 (p. 74), 'of an asthmatic who fell into a violent fit, by going into a wine cellar, where the must was fermenting…'. In 1700 Bernardino Ramazzini in his *De morbis diatriba*, the first book on occupational diseases, devoted Chapter XXVII to the diseases of 'the Sifters and Meters of Corn' where he wrote, in the words of the anonymous English translation of 1705 (pp. 171–2):

All Grain, and especially Wheat, whether kept in Pits under Ground, as in Tuscany, or in Barns, as in Countries upon the Po, have always a very small Powder mix'd with 'em; I mean not only that which they gather upon the Barn Floor in threshing, but another worse sort of Dust that Grain is apt to throw from itself upon long keeping. For the Seeds of Corn being replenish'd with a volatile Salt, insomuch that if they are not well dry'd in the Sun before they are laid up, they heat mightily, and turn presently to a Powder; it cannot but be that some thin Particles must flie off from the Husk that surrounds 'em, over and above the Powder and rotted Dust proceeding from the Consumption made by Moths, Worms, Mites, &c and Exrements. Now their being a Necessity of Sifting and Meting Corn and other Grain, the Men imploy'd in that Service are so plagu'd with this Powder or Dust, that when the Work is done they curse their Trade with a Thousand Imprecations. The Throat, the Lungs, and the Eyes sustain no small Damage by it, for it stuffs and dries up the Throat; it lines the Pulmonary Vessels with dusty Matter that causes a dry and obstinate Cough; and it makes the Eyes red and Watery. Hence it is that a'most all who live by that Trade, are short Breath'd, and Cachetick, and seldom live to be old; nay they are very apt to be sies'd with Orthopnoea, and last with Dropsie. Besides, this Powder has such a sharpness in it, that it causes violent itching, all over the Body.

Commenting on this syndrome, which has many features in common with farmer's lung associated with mouldy hay (see below), Ramazzini perceptively speculated on a possible connexion between the grain dust and the 'little worms' – bacteria – recently described by Leeuwenhoek.

Similar distress in farm workers and others after handling, or coming into contact with, mouldy straw was reported in Ohio by J. H. Salisbury (1862 *a,b*) who attempted to verify his clinical findings by dermal testing with spore suspensions from the affected straw, a procedure which consistently gave positive results of varying degrees of severity in the patients tested.

Although much attention was paid to the microscopic content of the air it was not until the mid-nineteenth century that experimental evidence was offered, by Charles Blackley, demonstrating allergy induced by fungus spores. Blackley, a Manchester physician, suffered from hay fever and made a prolonged study of his own condition, the results of which he published in a book, *Experimental researches on the causes and nature of catarrhus aestivus* (*hay-fever or hay-asthma*), 1873, which is now a medical classic. He trapped pollen (Fig. 39, B) and tested the effects of inhaling pollen and a range of other agents, including fungi of which he wrote (pp. 57–8):

I had noticed many years ago that dust from straw sometimes brought on attacks of sneezing with me, and that this seemed to occur more frequently when we had had a long spell of wet weather. I determined to try what fungi could be generated on damp straw. For this purpose wheat straw, slightly moistened, was placed in

a closed vessel, and kept at a temperature of 100° Fahr. In about twenty-four hours a small quantity of white mycelium was seen... This, I found on examination, was the *Penicillium glaucum*. After a few days another crop of dark-coloured spots were seen... These I found to be the *bristle mould* (*Chaetomium elatum*).
The spores of these two fungi were sown again separately on straw which had been placed in separate vessels having been subjected to the action of boiling water for a short time. A separate crop of each fungus was thus obtained.
The *odour* of the *Penicillium* produced no perceptible effect upon me, but the odour of the *Chaetomium* brought on nausea, faintness, and giddiness on two separate occasions. By inhaling the spores of the Penicillium... a severe attack of hoarseness, going on to complete aphonia, was brought on. This lasted for a couple of days, and ended in a sharpish attack of bronchial catarrh, which almost unfitted me for duty for a day or two... The sensations caused by these two agents were so unpleasant that I have never cared to reproduce them.

Mould asthma

The history of attempts to correlate the incidence of asthma with season, climate, altitude, and fungus spores has been summarized and documented by Van der Werf (1958). Only during the last fifty years has the importance of mould allergy been generally recognized. Outstanding among early workers was W. Storm van Leeuwen whose investigations[5] in the Netherlands into bronchial asthma, hay fever, and related conditions did much to stimulate others both in Europe and North America including A. W. Frankland of St Mary's Hospital Medical School, London, who appointed a mycologist (C. J. La Touche) to prepare mould antigens and investigate the moulds present in the homes of asthmatics, and S. M. Feinberg (1935, 1947) in the United States. It was in 1924 that Storm van Leeuwen noted in the island of Zuid-Beveland that 0.5 to 1.0% of the inhabitants of some villages were asthmatic, an incidence greatly in excess of that prevailing in other parts of the Netherlands. He failed to associate this condition with any meteorological peculiarity and attributed the cause to 'miasmata' or 'climate allergens'. Further, he showed that sufferers were relieved by air freed from 'miasmatic substances' by filtration through cotton wool or by passage through glycerin and he suggested that these airborne substances were mainly of fungus origin. He also found that high altitude gave relief and that inhaling dust from their homes provoked an attack of asthma. It was also in 1924 that Cadam in Canada recorded three cases of agricultural workers who developed severe asthma on exposure to dust from rusted grain and infected straw. He considered the stem rust (*Puccinia graminis*) to be the causal agent and he was able to induce a paroxysm by the inhalation of a few rust spores. According to Feinberg (1947), it is possible that the rust spores were contaminated with *Alternaria* or *Cladosporium* spores because spores of most rusts are ineffective as allergens and are obtained free from other spores with difficulty. Whether this was so or not Cadam was able to afford some relief with a vaccine prepared from the rust. In 1930, in the USA, Bernton described a case of asthma associated with *Aspergillus fumigatus*. Hopkins, Benham and Kesten in the same year described a similar case caused by *Alternaria* spores derived from vegetables stored in the basement of the patient's house and Durham (1938) stated that after the storm of *Alternaria* spores referred to above 'a brief inquiry has disclosed the correlation of clinical symptoms with the advent of the storm in some of the places affected'. Cobe (1932) described a case of asthma due to tomato leaf mould (*Fulvia fulva*), the spores of which occur in high concentrations in glasshouses containing affected plants and similar cases were cited by Brown (1936) and Morrow & Lowe (1943) while Frankland & Hay (1951) reported allergy caused by spores of the dry rot fungus (*Serpula lacrimans*) and Strand *et al.* (1967) two cases of 'lycoperdonosis', one in a boy who inhaled spores of a puffball (*Lycoperdon sp.*) in an attempt to control nose-bleeding, puffball spores being traditionally used to arrest bleeding.[6]

Kern (1921) was the first to obtain positive skin reactions with house dust extracts and the next year R. A. Cooke described a similar case of a patient who gave a strong skin reaction and developed mild asthma from an extract of the dust of his own room but not other allergens. The identity of the active fraction was not established but Cooke found that 109 (or 33%) of 327 asthmatic patients gave a positive reaction to 'dust'. A low percentage of normal people react to extracts of dust and the quite extensive literature on the antigenic properties of dust is somewhat conflicting. Moulds, if certainly one of the antigenic constituents of dust, are not the only one and are of less importance than, for example, mites (*Dermatophagoides*).

Farmer's lung

Some early records of farmer's, thresher's, or harvester's lung have already been referred to. Recent interest in this syndrome may be considered to have begun when Campbell (1932) reported five cases of acute respiratory symptoms following work with hay in Cumberland. Additional cases were subsequently reported from England, North America, and Scandinavia but in 1982 Eliasson noted that the condition has been well known in Iceland since 1790 when Dr Sveinn Palsson, a country physician, in an article on Icelandic disease nomenclature stated that:

Heysott [hay sickness] is the name of a disease of those who work with badly harvested and mouldy hay in winter. This is a well-known disease in Iceland, caused by the phlogistic vapours of the hay that are inhaled, leading to cold, fever, hoarseness, cough...[7]

Other Icelandic observations made between 1794 and 1913 cited by Eliasson showed that the allergic nature of the disease was understood and that a patient suffering from *heymaedi* [literally 'hay shortness of breath'], the modern Icelandic name for the condition, improved when no longer exposed to mouldy hay dust and that medicine had very little effect on the cure. It was also observed that heymaedi occurred in horses.

In England and elsewhere there was much speculation on the cause of the disease and a number of attempts made to elucidate its aetiology. F. Fawcett in 1936 considered it to be a mycosis ('bronchomycosis feniseciorum')[8] but J. T. Duncan was not impressed by this hypothesis. E. Törnell, ten years later, categorized it as a form of moniliasis [candidosis].[9] C. J. Fuller (1953) of the Royal Devon & Exeter Hospital in the light of a series of his own cases in the west of England favoured an allergic explanation and there were unsuccessful attempts to implicate particular fungal spores. Elucidation of the problem finally resulted from the collaboration of aerobiologists at Rothamsted Experimental Station and members of the Medical Research Council Research Group in Clinical Immunology at the Brompton Hospital in London (Pepys *et al.*, 1963). Gregory at Rothamsted put samples of mouldy hay associated with cases of farmer's lung in a wind tunnel and trapped the air-borne spores. It was soon established that certain aerobic thermophilic actinomycetes – particularly *Thermopolyspora polyspora* and *Micromonospora vulgaris* (which were later more correctly classified as *Micropolyspora faeni* (Cross *et al.*, 1968) and *Thermoactinomyces vulgaris*, respectively) – induced the formation of the 'farmer's lung hay' (FLH) antigen found in mouldy hay in normal hay, and *M. faeni* also in pure culture on artificial media. FLH antigen did not develop in hay after inoculation with fungi only or with any of six other actinomycetes tested and gave positive results in agar-gel double-diffusion immunoelectrophoresis tests against sera from patients with farmer's lung. Further, inhalation of an extract of *M. faeni* by affected subjects produced features of farmer's lung.

Typical *fog fever* in cattle is a respiratory disease of animals fed on aftermath ('foggage') during the autumn in the west of England but the name had also been applied to similar diseases, also of unknown aetiology, of housed cattle. Precipitins to FLH antigen were detected in the sera from the latter and Lacey (1968) found that the hay associated with such cases was rich in *M. faeni* and *T. vulgaris*. Pirie *et al.* (1971) claimed to have recorded the first confirmed case of farmer's lung in a cow.

In 1971 Lacey reported the association of *bagassosis* – a form of extrinsic allergic alveolitis in man induced by inhaling dust from mouldy, self-heated crushed sugar cane (bagasse) – with a new species of Thermoactinomyces (*T. sacchari*) and, in smaller quantities, *T. vulgaris* isolated from bagasse. Antigens from both actinomycetes gave positive precipitin reactions with sera from bagassosis patients.

Micropolyspora faeni and other thermophilic actinomycetes (as well as various fungus spores) have been associated frequently with 'humidifier fever' which has been widely reported during recent years associated with contaminated humidifiers in both commercial premises and private houses (see Edwards, 1977).

Occupational mould allergies

A number of other mould allergies have been established. Most, like farmer's lung, are occupational hazards. In 1848 Tersáncky drew attention to severe respiratory and dermal symptoms in workers engaged in the manufacture of tinder from polypores (*Fomes fomentarius*, *F. igniarius*) which had become very mouldy in store waiting to be processed. Hirt (1871) described the same phenomenon.

Michel (1863) described the 'mal de canne de Provence' (or 'cane cutter's disease') among reed cutters exposed to mouldy reeds (*Arundo donax*) which also became prevalent in the paper mills of the Midi of France where *Arundo donax* was being used for its fibre. The disease presented as lassitude, stiffness, low fever, and slight scrotal swelling. Later a prurigenous erythema spreads upwards from the inner surface of the thighs and becomes particularly severe in the armpits. A cough develops. Work is no longer possible. The patient is obliged to rest. His condition at once improves and recovery is complete in ten to fifteen days. On re-exposure to the reeds, symptoms recur. The condition was carefully investigated by Duché (1944) who attributed the complaint to the response of a sensitized individual to *Coniosporium arundinis* [*Papularia arundinis*, the *Arthrinium* state of *Apiospora montagnei*] which occurs on the lower leaves of reeds more than one year old.

'Maple-bark disease' (or 'maple-bark stripper's lung') which was first described by Towey *et al.* (1932) in Michigan and since reported elsewhere in North America (Emanuel *et al.*, 1966) is a rather similar condition caused by *Cryptostroma corticale* (syn. *Conisporium corticale*) which forms sheets of spores under the bark of both stored logs and growing trees.

Other mould allergies include 'cheese washer's lung' (attributed to the spores of *Penicillium casei*; Schlueter, 1973) in Switzerland among those who remove the mould from the outside of mature cheese, 'malster's lung' (*Aspergillus clavatus*) in Scotland (Blyth *et al.*, 1977) and elsewhere, 'suberosis' (associated with *Penicillium frequentans*) of workers in cork-making factories in Portugal (see Lacey, 1973), and 'mushroom worker's lung'.