CHAPTER 2

The Aerobiology Pathway

1. Introduction

The aerobiology pathway shown in Fig. 1.6 gives the different stages in the movement of particles such as spores or pollen from their source to the effect they cause when they land. A combination of more than one process is often studied rather than each process individually. Sampling the air for certain particles (usually spores or pollens) can be useful for monitoring climate change, estimating or forecasting dispersal of pathogens or allergens or species colonising new habitats, genetic diversity of a spore-producing organism, detection of pathogens or allergens, and risk assessment of GM pollen spread or cross-pollination of plant varieties. For example, air sampling is a valuable tool in the study of crop disease epidemiology and has enabled a better understanding of many crop diseases, leading to disease forecasting, changes in cropping practice to escape disease and optimised fungicide use. It has also enabled sources of hay fever to be identified and warnings of hay fever given during the year based on allergen detection. Indoors it has identified sources of microbial contamination in medical and food processing situations. This chapter considers the different processes in the aerobiology pathway and explains ways to interpret spore trap data. Since much of the early work was done by Gregory and his collaborators, many of the examples given below are from work at Rothamsted Research (Hirst, 1994).

2. Take-off (release)

Aerobiological particles can be considered to originate from point, line or area sources depending on the scale under consideration or type of sampling used. As microorganisms may be widespread while others are confined to rare niche microenvironments, this affects the numbers and distribution of their propagules. Bacteria generally lack mechanisms to become airborne, occurring opportunistically in aerosol generated by rain splash, bubble burst, animal or mechanical activity, and often as aggregations of many viable units on plant and animal debris. Viruses, like bacteria, often become airborne opportunistically from animal, fungal or plant sources and usually as aggregations of particles.

2.1. Spore release

Fungal spores however, vary greatly in size, shape, colour and method of release (Ingold, 1971). These various release mechanisms are essential for spores to escape the laminar boundary layer of still air to be dispersed in the turbulent boundary layer Fig. 1.1, (Gregory, 1973). Passive release of spores occurs, particularly in fungi growing on raised structures e.g. powdery mildew growing on plant leaves, where gusts of turbulence can penetrate closely enough to the substrate to detach spores. This is assisted in the case of powdery mildew by basipetal spore production, the oldest spores being raised away from the leaf on chains of progressively produced spores. However, many fungi have evolved active methods of spore liberation, some of which are illustrated in Fig. 2.1 (see also, Ingold, 1999).

The concentration of some dry airborne spores, e.g. *Cladosporium*, can increase at the start of rainfall. Hirst and Stedman (1963) showed that both rapid air movement in advance of splashes and vibration can blow or tap spores into the air. This process is most effective when large drops collide with surfaces carrying spores that are loose or raised above the surface and is different from true rain-splash dispersal in which spores mix with the water rather than remaining dry (section 7, this chapter).

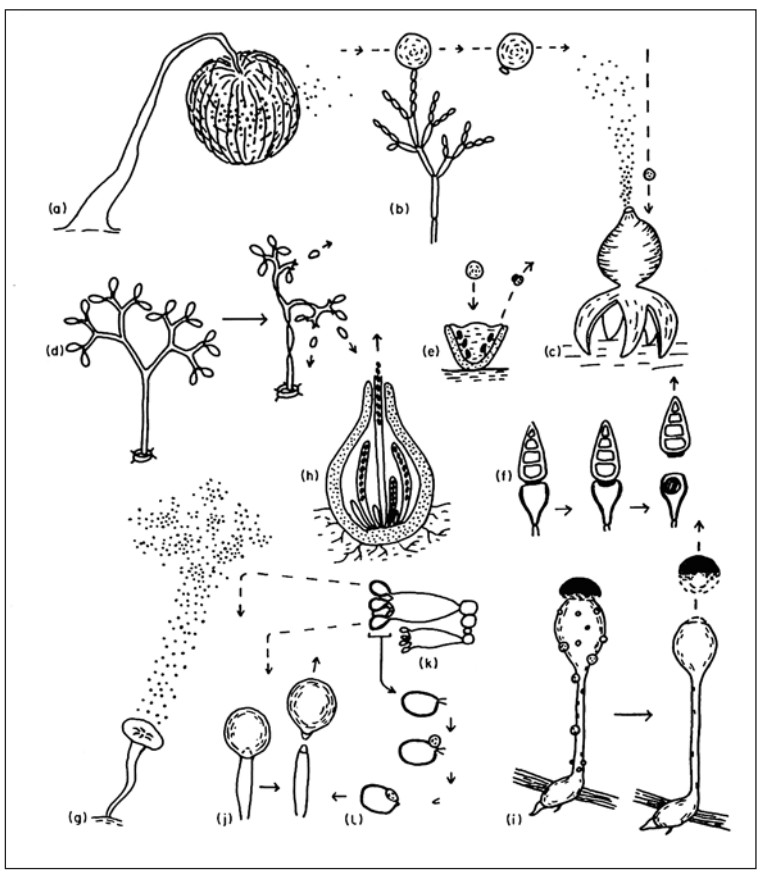

Figure 2.1
Spore liberation mechanisms: (a) deflation from raised fruiting body of *Dictydium* sp., (b) mist pick-up of *Cladosporium* sp. (Pl. 11.1), (c) bellows mechanism in *Geastrium* sp., (d) hygroscopic movements in *Peronospora* sp. (Pl. 9.68), (e) splash cup in *Crucibulum vulgare,* (f) water rupture in *Deightoniella torulosa,* (g) squirt gun (discomycete type) in *Sclerotinia sclerotiorum* (Pl. 8.19), (h) squirt gun (Pyrenomycete type) in *Sordaria fimicola* (Pl. 8.28), (i) squirting mechanism in *Pilobolus kleinii*, (j) rounding of turgid cells in *Entomophthora* sp. (Pl. 9.73), (k. l) ballistospore discharge in *Agaricus* sp. (Pl. 9.1). (Lacey, J., 1996, with permission from *Mycological Research*).

Spores of certain fungi are released seasonally rather than throughout the year and the timing of spore release can be monitored and ideally predicted if a good understanding of climatic effects on fruiting body development and sporulation is established. Often seasonal release of fungal plant pathogen spores is synchronised finely to coincide with a particular growth stage of the host plant e.g. spores of *Claviceps purpurea* (Pl. 8.15), (which causes ergot of cereals and grasses) and *Venturia inaequalis* (Pl. 8. 16), (which causes apple scab) are both released around the time of flowering of their hosts. Studies of spores by Last (1955) within wheat and barley crops infected by mildew *Blumeria graminis* (*Erysiphe graminis)*, revealed a daily periodicity in spore release. Air at different levels above the ground and at different times of the day was sampled with a portable, manually operated volumetric spore trap (Gregory, 1954). The most abundant fungal spores in the air on a dry day were *Blumeria*, (Pl. 10.30), *Cladosporium* (Pl. 11.1-2), and *Alternaria* (Pl. 11.3-6), with a peak in numbers at 16.00 GMT, while *Sporobolomyces* (Pl. 10.5) and *Tilletiopsis* (Pl. 10.4) were most numerous at 04.00 GMT. After rain, as well as *Sporobolomyces* and *Teletiopsis*, there were many spores tentatively identified as ascospores. It is thought that these periodic differences in the air spora profile reflect different mechanisms of spore release with maximal numbers of dry-spores and pollen released in late afternoon.

Other studies have since confirmed that ascospores are usually released after wetting by rain or dew, the water creating turgor pressure to force the ascospores from the ascus individually in some species or otherwise in one go. Although associated with rain, spore trapping experiments showed that most ascospores were released after rainfall, for up to five days, while the crop debris bearing apothecia was still wet. 'Leaf' wetness and 'Debris' wetness sensors were used to monitor the crop and debris. Tests in a miniature wind tunnel showed that under wet-dry cycles, spores could be produced for as long as 21 days, the largest numbers ejected whilst the debris was drying (McCartney and Lacey, M., 1990). Similarly, ascospores of *Leptosphaeria maculans* (phoma stem canker, Pl. 8.3) were released after rain, and on wet debris had a diurnal periodicity (possibly due to changes in relative humidity), most spores being released around 10 am -12 midday (West *et al*. 2002a).

2.2. Pollen release

In gymnosperms and angiosperms, pollen release is passive, with the flower parts raised into more turbulent air and anthers of anemophilous angiosperms often extended on long filaments into the airflow. Compared to insect-pollinated plants, large numbers of relatively small pollen grains are produced by anemophilous plants to ensure that some pollen will reach the intended target. Pollen from plants considered to be insectpollinated can in some cases become airborne, e.g. oilseed rape pollen and may lead to allergy problems, but generally, pollen of entomophilous plants represent a low proportion of airborne pollen e.g. <2% of pollen trapped in Cardiff (Mullins and Emberlin, 1997).

As with the release of spores of many fungal species, the release of pollen is seasonal (Fig. 2.6) and varies according to species and geographical location. The source strength for a particular species varies regionally due to differences in habitat and timing of flo-

wering (Spieksma *et al.*, 2003). This can be seen by clear differences in the start of the grass pollen season throughout the UK (Emberlin *et al.* 1994), regional variations in *Betula* pollen production in the UK (Corden *et al.*, 2000), the grass pollen season in the UK and Spain (Sánchez Mesa *et al.*, 2003) or distribution of Japanese Cedar pollen production (Kawashima and Takahashi, 1999)

Pollen production by crops also varies considerably with time of day, stage of flowering and weather events. Some crops produce large quantities of airborne pollen e.g. above a sugar beet crop, the maximum daily average concentrations reported was 12400 m^{-3}, while for oilseed rape it was 5295 m^{-3} (Scott, 1970). Free *et al.* (1975) measured maximum hourly counts at 46714 pollen grains m^{-3} of air for sugar beet and 2273 m^{-3} for oilseed rape crops. However, these measurements really include a component of dispersal with particle numbers decreasing with height above the source.

2.3. Release from lower plants, animals, etc.

Algae and diatoms can become airborne via sea-foam and bursting bubbles (Schlichting, 1971; 1974) and aerosol formation by waves, rough water (rapids, waterfalls, etc). In some mosses such as *Sphagnum*, release of spores is explosive, as drying of the spore capsule increases the internal air pressure until an operculum in the top of the capsule ruptures. Similarly spores of many homosporous ferns are released actively following dehiscence of sporangia which curve back on themselves due to an annulus of thickened cells, but spring forwards again, releasing spores as water in the annular cells turns to vapour (Ingold, 1939). In the horsetails (*Equisetum*, Pl. 7.12) spores are wrapped by four arms (part of the spore coat), called elaters, which in dry conditions, spring open to assist spore release.

In the animal world, protozoa, nematodes, mites and small insects can become airborne by wind action on water, soil, or plants or by mechanical activity (rain-splash, shaking clothing, etc). A special case is certain spiders, which 'balloon' by deliberately extending relatively long silk filaments to catch on the wind (Weyman *et al.*, 2002).

3. Dispersal

Once particles have been launched into the air they disperse, their concentration per unit volume of air decreasing with increasing distance from the point of liberation (Gregory, 1973). This is illustrated by the appearance of smoke billowing from a chimney, which disperses, often showing effects of air turbulence (Fig. 2.2). Expansion of the cloud of particles occurs due to eddy currents, causing dilution of the particle cloud as it moves in the general wind direction.

Dispersal within and above crops is difficult to measure alone as air movement affects the release, dispersal and deposition of fungal spores (Legg and Bainbridge, 1978; Legg, 1983) and pollen. Gust penetration into crop canopies is important for liberation and deposition of spores (Aylor *et al.*, 1981; Shaw and McCartney, 1985), an important consideration in the development of a spore dispersal model (McCartney

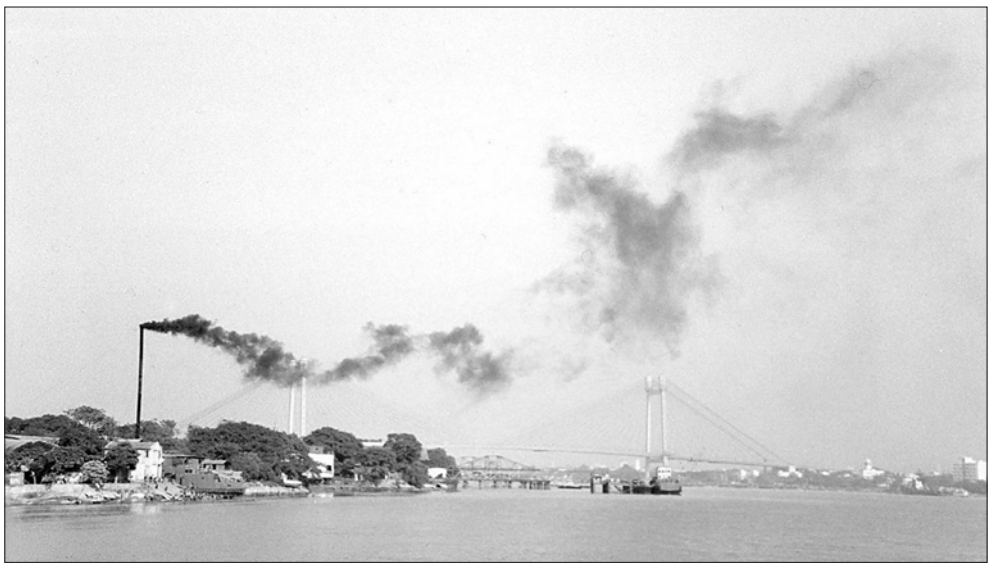

Figure 2.2
Smoke dispersal from a chimney in Calcutta, 1997.

and Fitt, 1985; Fitt and McCartney, 1986). However, it also increases dispersal. Last (1955) showed that when spores were formed in the crop the spore concentration was always greater within than above the crop, and also near the ground than at the top of the crop. This is not only due to dispersal but also due to deposition on leaves by the filtering effect of the crop canopy.

Particle dispersal is largely dependent on air mass movement, turbulence and thermal convection. Characteristics of particles such as size, shape, density and surface texture affect dispersal only very subtly, by affecting aerodynamics such as the particle's terminal velocity.

Recently, attention to the dispersal of pollen has heightened due to concerns over the possible spread of genetically modified material. Prior to the development of GM crops, as now, information on pollen dispersal was important to calculate suitable separation distances for seed-production crops so that cross-pollination is minimised. For sugar beet, Chamberlain (1967) suggested that the then recommended minimum spacing of 1000 m from a 20 acre (8.1 hectare) source to a seed-production plot, would result in the proportion of cross pollination to within-plot pollination to be 4×10^{-3} with 1×10^{-3} pollinated from the regional background (long distance) pollen. He suggested that increasing the separation distance to 2000 m would reduce the proportion of cross pollination from the source area to that of cross pollination from the background pollen. The concentration of pollen or other particles is affected by the height above ground (dispersal from the source). Hart *et al.* (1994) described concentrations of grass and nettle pollen trapped using Burkard traps simultaneously at three heights (12, 24 and 30 m) at Leicester, England. They found that pollen concentrations were generally (but not always) lower in the 30 m trap and this was thought to be due to locally produced pollens not mixing enough to reach 30 m. Peaks of pollen grains trapped were later for the higher traps than the 12m trap and this could represent pollen production from distant sources rather than local sources. McCartney and Lacey, M. (1991b) also found a decrease in pollen numbers with

height above the source and predicted that more than 60% of oilseed rape (*Brassica napus* Pl. 5.19) pollen lost from a crop would still be airborne at 100 m downwind, but that the concentration at the ground (i.e. available for pollinating a neighbouring crop) would be between 2 and 10% of that at the edge of the crop. Similarly, Jarosz *et al.* (2003) have reported the dispersal gradient of conventional maize pollen, which produced between 2 x 10^4 and 2 x 10^6 grains per day per plant. Pollen concentrations decreased by two thirds within 10 m of the source (a 20 x 20 m plot), while deposition at 30 m was <10% that at 1 m. Numerous dispersal models have been developed. Empirical models may describe gradients without explaining the causative processes and include the power law model and the simple exponential model, while physical models consider underlying principles of dispersal and include the gradient transfer theory (K theory), statistical or Gaussian plume models, and random walk models, reviewed by McCartney and Fitt (1985).

3.1. Terminal velocity

The principal aerodynamic property affecting the dispersal and deposition of a particle is its terminal velocity (or fall speed), which is the maximum speed to which a body falling through the air under gravity will reach. The speed of fall is prevented from increasing due to air resistance (drag). The terminal velocity of an object with a smooth surface is largely determined by its size and density. Hence opening a skydiver's parachute increases the surface area for air resistance, while the total weight remains the same, and so the terminal velocity slows considerably. This feature is used by some flowering plants, e.g. willow herb (*Chamaenerion angustifolium*), old man's beard (*Clematis vitalba*), and dandelion (*Taraxacum officinale*, Pl. 6.29), whose seeds have a fringe of hairs, a pappus, increasing drag considerably with little increase in weight and therefore decreasing terminal velocity. It is also used by some spiders, which can be blown considerable distances using long silk threads as balloons or parachutes (Schneider *et al.*, 2001). For pollens and spores, particle shape and surface texture can also affect terminal velocity. Many species of gymnosperm have winged pollen grains, for example the pollen of *Pinus* (Pl. 7.1) has two outer air-sacs, which increase buoyancy in air.

The relative effect of air resistance is greatest on objects with very small aerodynamic diameter; hence small objects reach relatively low maximum fall speeds or terminal velocities. Fall speeds of spores can generally be estimated only within ± 20% due to natural variation in spore sizes of a particular species, and hydration level as affected by the ambient relative humidity. Estimates range from 40 mm s^{-1} for large pollen grains (≈ 50 µm diameter) to 0.04 mm s^{-1} for actinomycete spores (≈ 1 µm diameter) (Gregory, 1976; Gregory and Henden, 1976). While terminal velocity is of high importance in very still air, normally effects of turbulence, convection or air mass movement far outweighs movements of spores at terminal velocities under the influence of gravity.

3.2. Aerodynamic diameter

The terminal velocity of a particle depends on its mass, which determines the force of gravity acting upon it, and size and shape, which together determine the drag forces

opposing gravity. Many fungal spores and pollen can be approximated to spheres in shape, but some are elliptical, elongated into thin rods or fibres, or even take more complex shapes e.g. spiral, club-shaped or with radiating 'arms'. Others may be released in chains (e.g. *Cladosporium* spp. or *Blumeria graminis*) or clumps (e.g. ascospores of *Pyranopeziza brassicae* often occur in groups of four, or the rust fungus *Puccinia striiformis* may clump in humid weather into groups of seven or more spores). In order to estimate the terminal velocity and therefore the dispersal characteristics of such particles, their size and shape can be considered in terms of aerodynamic diameter, i.e. the size of a spherical object (with, for most spores and pollen, the same density as water) that would have the same terminal velocity in air. In air at 20°C, the aerodynamic diameter d (in µm) for a spore of terminal velocity v_t is:

$$d = 18.02\sqrt{v_t}$$

when v_t is measured in cm s^{-1}. The aerodynamic diameter also affects efficiency of impaction.

Shape factors have been estimated for simple shapes such as ellipsoids and rods (Mercer 1973; Chamberlain, 1975) and can be used to estimate the terminal velocity of a non spherical spore, by dividing the terminal velocity of a spherical spore of the same volume by the shape factor (McCartney *et. al.*, 1993).

4. Deposition – sedimentation and impaction

Particles in the air descend due to gravity, eventually recrossing the laminar boundary layer and coming to rest in the still air on a solid or liquid surface (Gregory, 1973). This can be by sedimentation (passively settling onto a surface), or by impaction (the sticking of airborne particles onto a surface following an active collision) on an object's surface, e.g. a leaf or stigma of a flower because the particle's momentum may be too great to allow it to change direction and flow with much lighter air molecules around the object (McCartney and Aylor, 1987). Sedimentation can be used for trapping air particles using passive traps. Impaction is the basic principle behind many air-sampling devices such as the Andersen sampler, Hirst or Burkard spore traps, whirling arm traps (rotating arm traps or rotorods), air-filtering systems and even sticky rods. A special form of impaction, can be considered as that in which spores in the air can be removed by the action of rain. In this case, the particles impact the surface of falling rain drops, to be deposited within or on the surface of water films, depending on the particle's hydrophobicity.

5. Impact

Air-borne particles can have many effects including plant, animal and human diseases, allergies, plant pollination and colonisation of new habitats. To have an effect, particles

need to have survived the airborne phase and be viable for growth, infection or pollination. In cases of allergy, however, the particle need not be viable to cause a reaction. Viability of particles in air usually decreases exponentially with time due to mortality caused by such stresses as desiccation, uv-light, starvation and extremes of temperature. The half-life of spores varies greatly from species to species according to their size, energy reserves, metabolic rate, hydration level and spore wall characteristics (e.g. pigmentation, thickness, permeability). Furthermore, having settled or impacted on a surface, there may be biochemical signalling between particle and the surface, leading to growth (i.e. germination of a spore or pollen grain) or further dormancy (and the chance of re-dispersal).

5.1. Plant disease

Plant pathogens include known species of virus, mycoplasmas, bacteria and fungi. Whilst many (virus and mycoplasmas) require an insect vector, and some others are soil-borne or water-borne, many important bacterial and fungal plant pathogens are dispersed by wind or rain-splash, and are capable of causing severe losses in susceptible crops with important economic or social consequences e.g. *Blumeria graminis* (powdery mildew of cereals, Pl. 10.30), *Puccinia striiformis* (stripe or yellow rust of wheat, Pl. 8.48-49), *Phytophthora infestans* (late blight of potato, Pl. 9.69), *Heterobasidion annosum* (conifer polypore root and butt rot, Pl. 9.43), *Mycosphaerella musicola* (Sigatoka of banana) and *Xanthomonas axonopodis* pv. *citri* (citrus canker). Aspects of the aerobiology and epidemiology of plant pathogens are investigated by plant pathologists in an effort to devise or improve disease control methods.

5.1.1. Plant disease symptom distribution

Stem and head rot of sunflowers is caused by infection by airborne ascospores of *Sclerotinia sclerotiorum* (Pl. 8.19). The number of plants infected is related to the concentration of ascospores in the air released from the apothecia on the soil (McCartney and Lacey, M., 1991a). Further work showed that the timing of the ascospore release determined the type of disease that developed (Fig. 2.3). Stem rot developed when ascospores were present before flowering and head rot when the spores were present during flowering (McCartney and Lacey, M., 1999).

5.1.2. Timing of spore release and disease control

Epidemics of phoma stem canker are initiated by airborne ascospores of *Leptosphaeria maculans* (Pl. 8.3) produced in apothecia (pseudothecia) on crop debris in the autumn. The ascospores infect leaves, leading to phoma leaf spot and eventually stem canker. In some regions, e.g. Australia, the ascospore release is well synchronised with crop emergence (a very vulnerable crop stage). By sowing the crop late, growers in some regions of Australia are able to escape the effects of canker as the spores have been released before the new crop is present. Alternatively in Europe, where the spore release is spread

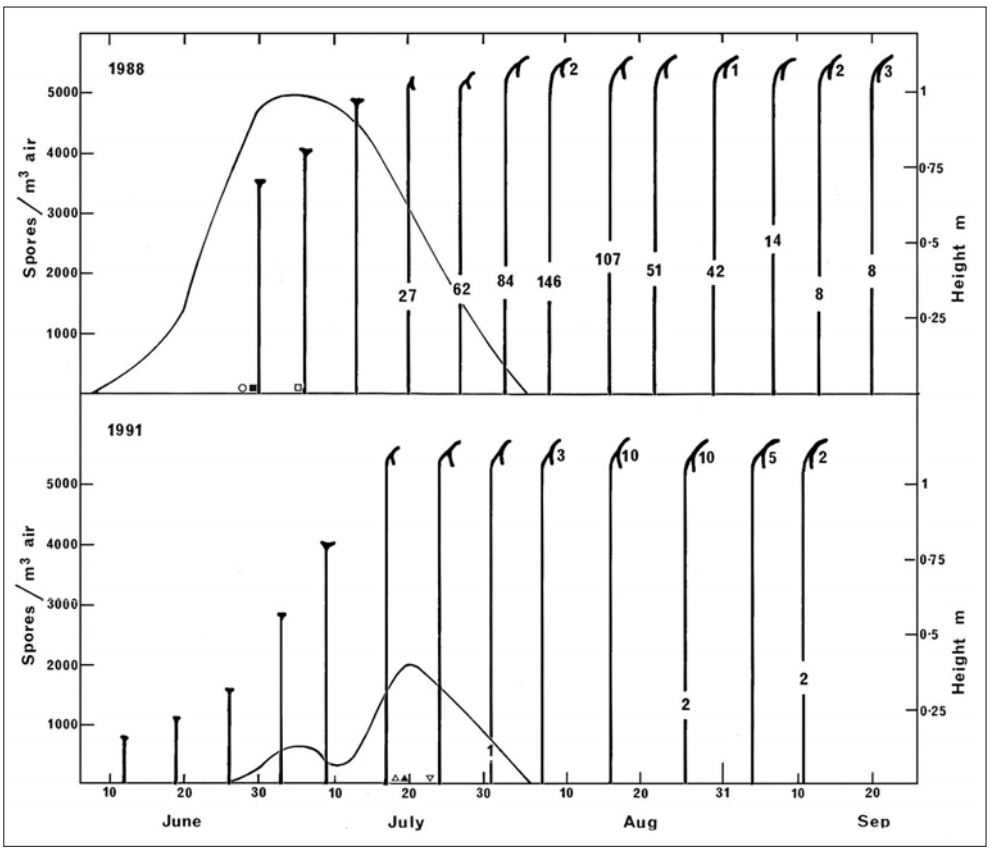

Figure 2.3
Ascospore release (line) and disease symptoms in sunflowers in 1988 and 1991. The presence of apothecia is shown by small symbols on the soil surface. Figures show numbers of new infection on the stems (at the average height) and on the seed heads.

throughout the autumn, monitoring spore release can help to target fungicide applications (West *et al.*, 2002b).

5.1.3. Disease gradients

Disease gradients can occur across fields due to spores arriving predominantly at one side of the field from a nearby inoculum source e.g. an adjacent field. Alternatively, individual disease foci can occur at random across a field when spores arrive from a distant source, producing separate patches of disease. In favourable conditions, disease severity spreads out, moving from areas of the crop with high severity to areas with low severity. Fig. 2.4 shows a near-Infra-Red image of a potato field, which shows foci of potato late blight (*Phytophthora infestans*, Pl. 8.69) as dark areas of the crop due to reduced green tissue. If favourable conditions persist, the disease patches would expand as spores from the disease foci infect the surrounding healthy areas of the crop. Due to the incubation period between infection and symptom development, an invisible zone of infection is normally already present around the visible disease foci (West *et al.*, 2003). Disease gradients are not necessarily the same as spore dispersal gradients because disease gradients are the result of many different spore production and release events over many days, each event affected by climatic factors such as wind speed and direction and incidence of rain. As a disease patch expands, the disease gradient often decreases, beco-

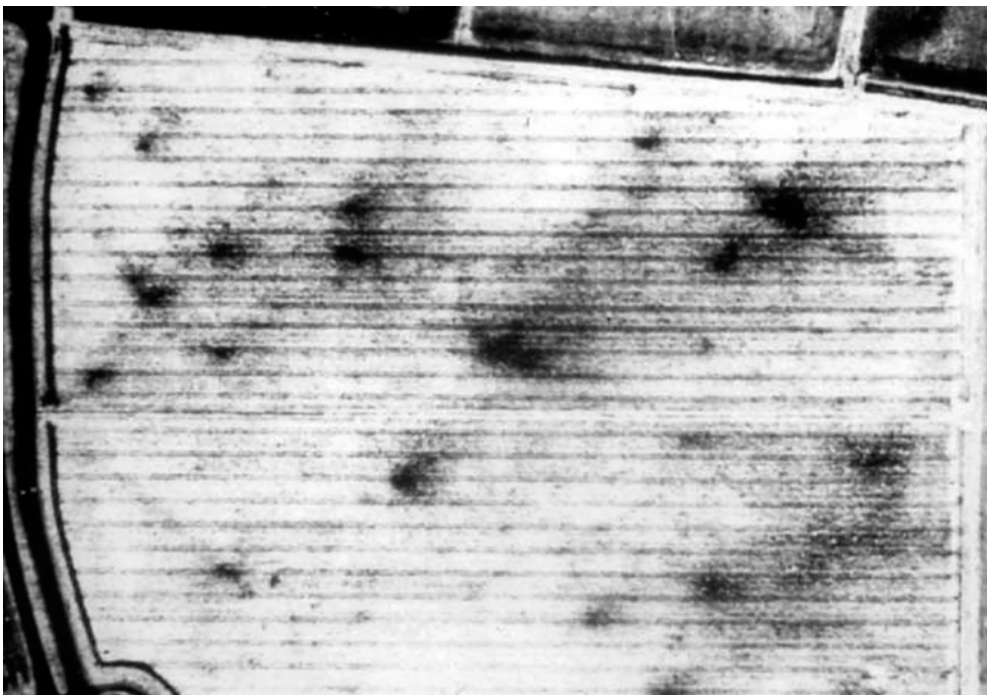

Figure 2.4
Aerial infra-red photograph of potato late blight (*Phytophthora infestans)*, disease gradients from primary foci in a potato crop (Lacey, J. *et al*., 1997).

ming less steep. Gregory explained that area sources of disease usually have shallower disease gradients than point sources because lateral diluting eddies would themselves contain spores rather than being spore-free (Gregory, (1976).

5.2. Health hazards

Many airborne fungal, actinomycete and bacterial spores are capable of causing disease in man and animals by direct infection (living tissue is invaded by the microbe), by toxicoses (ingestion of toxic metabolites of microbes), or by allergy (sensitivity to microbial proteins and polysaccharides). Respiratory allergy in man may develop immediately as in hay fever or asthma, or it can be delayed as in Farmer's Lung. Potential sources of hazardous airborne spores are many stored products including hay, straw, grain, wood chips and composts. Spore laden dust is also released into the air in many ways including distributing hay to animals, spreading out bedding and moving stored grain.

Pollen and spores are nearly always present in air but their number and type depend on the time of day, weather, season and local source. Indoors the diversity of airborne particles is usually lower than outdoors, and numbers of particles lower, unless there is a source of contamination within the building. The use of the cascade impactor and Andersen sampler together enable the different size fractions of the air spora to be monitored for both visual counts and the number of viable units. Fig. 2.5 is a very simplified diagram showing how far spores of different sizes can penetrate into the lungs and the resulting type of illness that can follow in susceptible people (Lacey, J., *et al.,* 1972).

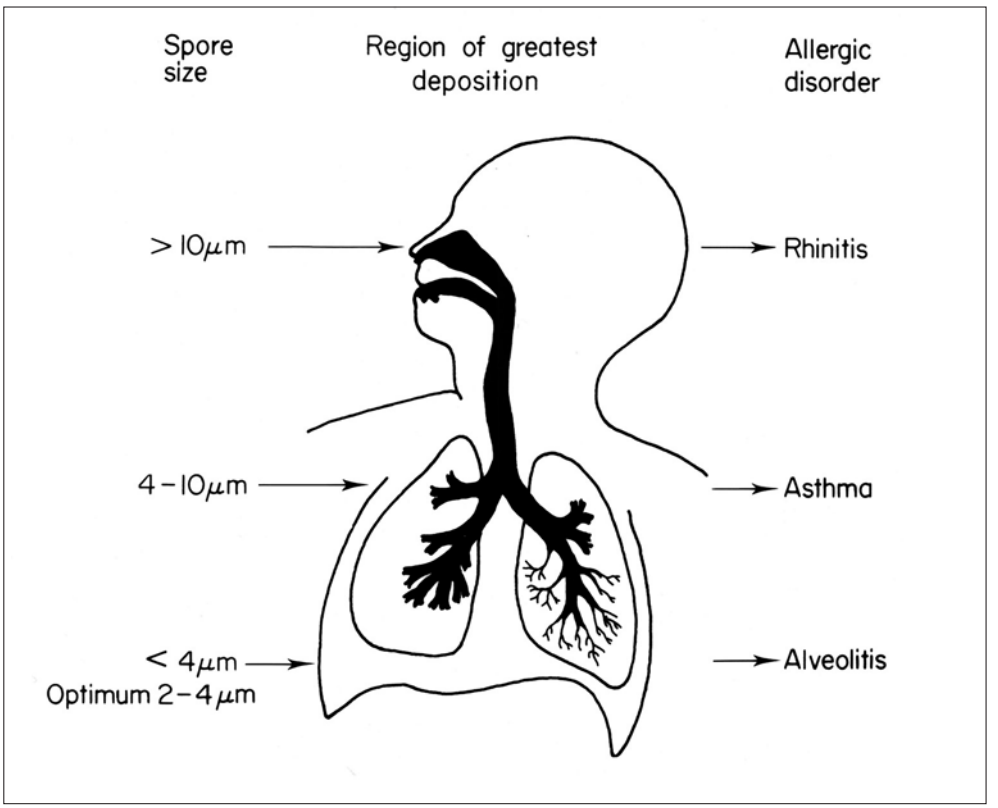

Figure 2.5
Spore size, lung penetration and type of allergic disease (Lacey, J., *et al.*, 1972, with permission from Elsevier).

5.2.1. Allergy

The increasing incidence of both pollinosis and asthma in the population at large has involved pollen and spore data being included in publications emanating from respiratory diseases, community health and medical practices (D'Amanto *et al.*, 1991; Spiewak, 1995; Emberlin, 1997; Newson *et al.*, 2000; Corden and Millington, 2001 Corden *et al.*, 2003). Pollen counts are regularly broadcast on the media and this enables sufferers have some knowledge of the presence of allergens in the air. Figure. 2.6 shows when the most common allergenic pollen is likely to be released. One of the earlier British studies investigating the relationship between pollen and spores and allergy was published by Hyde (1972). Pollen has been associated with the prevalence of allergic rhinoconjunctivitis, asthma and atopic eczema in children (Burr *et al.*, 2002). Mackay *el al.* (1992) undertook a study involving medical application of data at the Scottish Centre for Pollen Studies. The ever increasing attention to and research into the application of aerobiology to medicine is exemplified by publications involving the relationship between aerobiology and allergology (Morrow-Brown, 1994) and the airborne fungal populations in British homes and the health implications (Hunter and Lea, 1994). Between ten and twenty per cent of the world's population is considered to be city dwellers (Hunter and Lea, 1994). The changing health patterns reflect this shift from the rural environment no more so than in the increase in allergies recorded inclu-

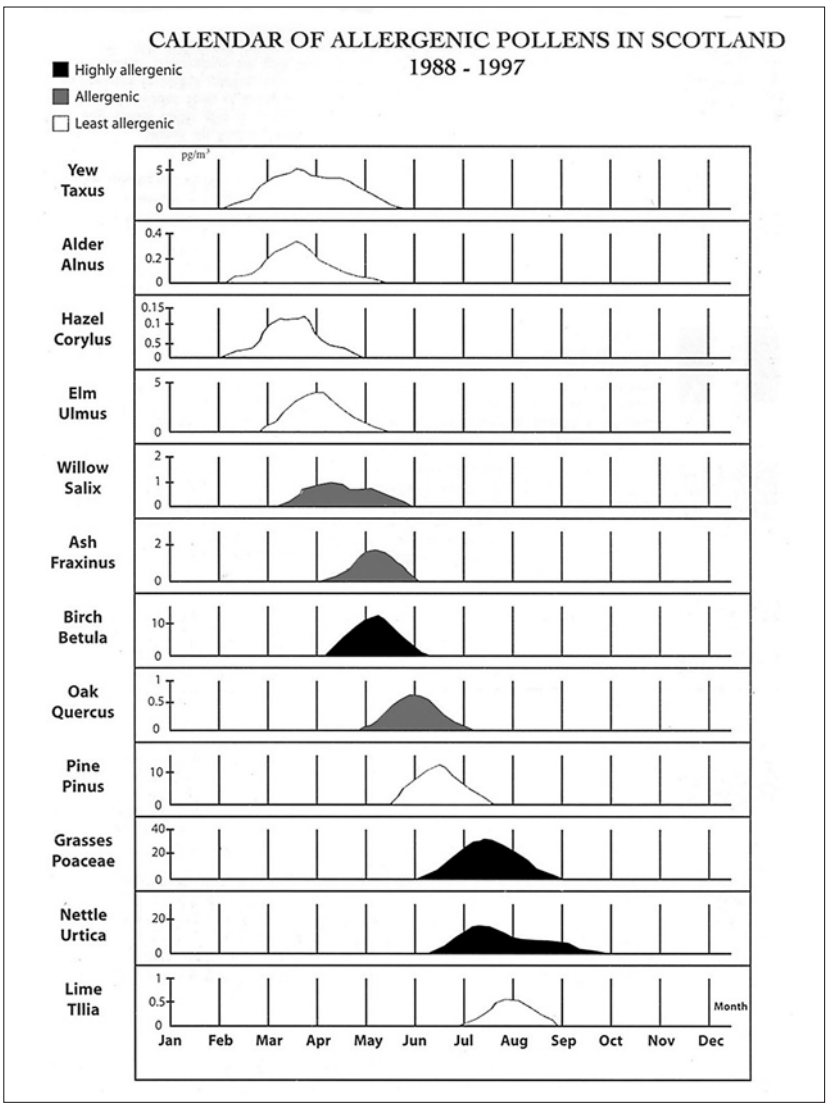

Figure 2.6
Pollen calendar showing periods when the pollen of different temperate wind-pollinated plants is likely to be in the air in Scotland. (Caulton *et al.*, 1997, with permission from The Scottish Centre for Pollen Studies, Edinburgh).

ding pollinosis, seasonal rhinitis and asthma. City dwellers spend the major part of their lives indoors working, at leisure, eating and sleeping. Public transport, schools, offices, hospitals, restaurants, libraries, community and leisure centres can all harbour pollen, fungal spores, bacteria, house dust mites, dander and other biological agents (Ranito-Lehtimaki, 1991; Reponen, 1994; Verhoeff, 1994; Nikkels *et al.*, 1996; Garrett *et al.*, 1997; Stern *et al.*, 1999 and Flannigan *et al.*, 2001).

Allergic response to allergenic pollens (Pollinosis) is not confined to humans, but also occurs in animals. Studies have been undertaken in horses (Dixen *et al.*, 1992) and dogs (Fraser et al., 2001) to identify causes of pollenosis. The methodology of these veterinary studies followed that described by Caulton (1988).

5.2.2. Late summer asthma

Many people suffer from asthma at harvest time and on dry days many spores of *Cladosporium* and *Alternaria* are in the air and can cause allergic reactions. Some asthmatic patients associated their attacks with the proximity of ripening barley. During the summer of 1972 Frankland and Gregory (1973) had a Burkard trap running in the garden of a patient in Dorset whose asthma attacks seemed to be so triggered. Large numbers of two-celled ascospores were liberated at night, similar to those seen by Last (1955), and identified as spores of *Didymella exitialis* (Pl. 8.12 and 13) produced on barley. A scientist at Rothamsted observed that his asthma usually occurred in the late summer, particularly after rain. He responded to inhalation testing with *D. exitialis* extract with an asthmatic reaction and to a skin test with an immediate reaction. Other research workers and patients were tested and it was reported in *The Lancet* that *D. exitialis* seemed to be the cause of late summer asthma (Harries *et al*, 1985). Corden and Millington (1994) confirmed that *Didymella* spores can be found in the air after rain in the summer even in an urban area.

5.2.3. Farmer's Lung

With the reduced risk of spontaneous fire in hay stacks following the widespread use of pickup balers, farmers were less cautious about making hay when it was too moist. Consequently many bales became very mouldy, increasing the problem of 'Farmer's Lung', an allergic alveolitis disease. To identify the causal agent, Gregory assembled an interdisciplinary team, including a medical team at the Institute of diseases of the Chest, Brompton Hospital, funded by the Agricultural Research Fund (Hirst, 1990). Many types of hay were examined by tumbling samples in a perforated drum at the intake end of a small wind tunnel (Fig. 2.7) and the dust caught at the other end by a cascade impactor and Andersen sampler. Visual counts from the four traces of the cascade impactor gave up to 102 million fungal spores and 1200 million actinomycete spores per g (dry weight) of hays associated with Farmer's Lung. The Andersen sampler (Andersen, 1958) allowed viable organisms to be collected dry and grown on different media at different temperatures. Predominantly thermophilic and mesophilic fungi and actinomycetes as well as bacteria were identified and counted (Gregory and Lacey, M., 1963a).

Experimental batches of hay were baled at different moisture contents and monitored for temperature, biochemical changes and mould growth (Gregory *et al*., 1963). Hay baled at 40 % moisture heated to over 60°C and contained a large flora of thermophilic fungi, particularly *Aspergillus fumigatus* (Pl. 10.16), *Absidia* spp. (Pl. 9.76-77), *Mucor pusillus* (Pl. 9.78), *Humicola lanuginose* (Pl. 10.43) and actinomycetes (Pl. 12.2-4). Extracts of the moulding hay were tested against serum from affected farmers, yielding positive reactions (Gregory *et al.*, 1964). The actinomycetes *Thermopolyspora polyspora* and *Micromonospora vulgaris* were found to be a rich source of the Farmer's Lung antigen (Pepys *et. al*., 1963). Consequently Farmer's Lung disease was able to be registered as a prescribed disease under the National Insurance (Industrial Injuries) Act, 1964.

Spores produced from mouldy hay, shaken in a perforated drum in a wind tunnel (Fig. 2.7) at wind speeds of 0.6–4.9 m s^{-1} were sampled periodically during one hour (Gregory and Lacey, M., 1963b). The number of spores released per minute decreased rapidly from the start with two-thirds removed in the first 3 minutes. The total number released was higher with faster wind speeds. 50 million spores were released after hay was blown for 31 min at 1.2 m s^{-1}, blowing for a further 31 min at 4.9 m s^{-1} released another 55 million spores.

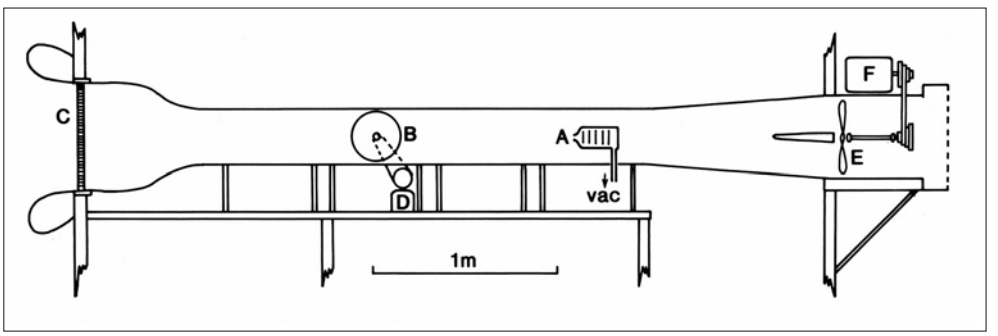

Figure 2.7
Diagram of wind tunnel showing position of collecting apparatus for studying the spore content of stored products. A, Andersen sampler or position for cascade impactor or other sampling device, B, perforated zinc drum, C, paper honeycomb, D, motor for drum, E, fan, F, motor for fan, vac, line to vacuum pump. (Lacey, J., 1990, with permission from the McGraw-Hill Companies).

Concentrations of up to 1600 million spores m^{-3} air were recorded in farm buildings while hay associated with Farmer's Lung was being shaken for animal feed (Lacey, J. and Lacey, M., 1964). Actinomycete spores were 98% of the air spora, and as they range in size from 0.5-1.3 µm in diameter, they can penetrate deeply into the lungs (Fig. 2.5).

5.2.4. Other aerobiological hazards in the work place and home

In addition to Farmer's Lung, there are many examples of occupational lung diseases caused by fungal and actinomycete spores (Crook and Swan, 2001; Hodgson and Flannigan, 2001). *Thermoactinomyces sacchari* was implicated in bagassosis (Lacey, J., 1971b) and *Penicillium frequentens* (Pl. 10.17) in suberosis (Ávila and Lacey, J., 1974). Further studies of the aerobiology of environments associated with occupational disease have allowed environments associated with occupational asthma and allergic alveolitis to be characterized (Lacey, J. and Crook, 1988; Lacey J. and Dutkiewicz, 1994; Crook and Swan, 2001).

An early example of research into spore or dust hazards in the work place is that for threshers during harvesting and grain storage. In the early 1970s many farm workers suffered respiratory symptoms caused by dust during harvesting of grain. Air which was being inhaled by workers on combine harvesters was sampled on farms in Lincolnshire.

The airborne dust around combine harvesters contained up to 200 million fungus spores per m^3 air while drivers were exposed to up to 20 million spores per m^3 air. The workers affected had an immediate hypersensitivity reaction to the spores. It was suggested that drivers could be protected by cabs ventilated with filtered air (Darke *et al.*, 1976), this is now standard practice. However, attention needs to be paid to the effectiveness of the air filters used in combine harvester cabs. Studies have shown that well fitted filters provide good protection against airborne spores, but aerosols can easily by-pass damaged or poorly maintained filters. Also, opening the cab door or window in a contaminated environment can negate the protective effect of a cab air filter within 3 minutes (Thorpe *et al.*, 1997).

The air in grain silos, sampled using a cascade sampler and an Andersen sampler while the grain was being unloaded, produced huge concentrations of bacteria, actinomycete spores and fungal spores. Many of these were viable and some were potentially pathogenic e.g. *Aspergillus fumigatus* (Pl. 10.16). It was recommended that workers should use efficient dust respirators inside silos when handling grain (Lacey, J., 1971a).

5.2.5. Compost handling and locating refuse or composting facilities

Different types of materials used for producing compost affect the type and numbers of spores released during the composting process, there can also be seasonal as well as daily changes in the number and types of spore released from composting sites. Domestic waste composts can produce high numbers of airborne bacteria (Lacey, J., *et al.*, 1992). EU legislative targets to reduce landfill disposal of waste and encourage recycling has led to a large increase in the number of green waste composting sites. However, public concern about exposure to the potentially high numbers of spores released means that the location of composting sites requires careful consideration (Lacey J, 1997). The potential for exposure to airborne spores, including *Aspergillus fumigatus*, and hazards to respiratory health associated with waste composting have been reviewed by Swan *et al.* (2002). Refuse dumps and landfill sites pose a further risk of release of potential allergens and pathogens through dry release and rain-splashed aerosols.

Mushroom compost is traditionally made from wetted straw and horse manure, which heats up during composting as many thermophilic actinomycetes grow. When moved into the growing sheds many more spores are emitted than at picking of the mushroom crop (Crook and Lacey, J., 1991).

5.2.6. Respiratory infections

In addition to allergic reactions or irritation, some airborne microbes (other than causal agents of illnesses such as colds, influenza and pneumonia) can cause respiratory system infection in humans (Campbell *et al.*, 1996; Samson *et al.*, 2001). An example, of a fungus capable of causing disease following inhalation is *Aspergillus fumigatus*, which normally grows on grain or compost. The fungus can grow saprophytically on mucus in the airways to cause bronchopulmonary aspergillosis, occasionally producing a ball of fungal growth or aspergilloma. Serious problems can occur in subjects that have suppressed

immuno-systems, due to disease, immunosuppressive drugs or radiation therapy, allowing the fungus to become invasive.

Legionnaire's disease is caused by the bacterium *Legionella*, which occurs naturally in fresh water bodies such as rivers and lakes (Postgate, 1986). However, infection of the lungs, leading to serious illness, occurs if the bacterium becomes suspended in aerosol and is inhaled. While this is rare in natural systems, aerosols in buildings produced from poorly maintained cooling systems or showers pose a serious health hazard. With their growing popularity, poorly maintained spa pools are an increasing source of this respiratory pathogen. It is likely that the original outbreak of this disease in Philadelphia in 1976 was caused by infected water in the air-conditioning system resulting in an aerosol containing *Legionella* being blown into the conference hall. Other bacterial diseases that may be dispersed by air include *Bordetella pertussis* (whooping cough), *Streptococcus* species (causing sore throats, tonsillitis and pneumonia) and *Mycobacterium tuberculosis* (tuberculosis). The disease Q fever, caused by the bacterium *Coxiella burnetii*, is an example of a zoonotic disease spread from animals to humans via the aerobiological pathway. Infection can be spread from direct contact with animals or with infected bedding straw. This was thought to be the source of a recent cluster of infections in Wales (van Woerden *et al.*, 2004).

Many of the most harmful diseases, and often the most difficult to treat, are caused by viruses. Those for which inhalation is a potential route of infection include the common cold, mumps and influenza. Animal reservoirs represent a potential source and means of spread, as shown by the outbreak of the H5N1 strain of avian 'flu in the Far East (Chen *et al.*, 2004; Guan et al, 2004), while person to person spread via aerosol and droplets was an important factor in the newly emergent viral infection severe acute respiratory syndrome (SARS) (Yu *et al.*, 2004; Wang *et al.*, 2005). Foot and mouth disease virus, although not a serious human pathogen, can cause heavy economic losses through livestock infection and the disease agent is readily disseminated over long distances via the airborne route (Donaldson and Alexandersen, 2002; Gloster *et al.*, 2005).

5.2.7. Aerobiological hazards in natural environments

The impact of Pteridophyte spores inhaled in quantity constituting a heath hazard has been investigated by Siman (1999). Bracken (*Pteridium aquilinum*, Pl. 7.6) is a fern that occurs worldwide. It reproduces vegetatively but also produces a large number of spores which Evans (1987) demonstrated could be carcinogenic to mice. A ten-year study of the incidence of airborne bracken spores on an urban roof in South-east Scotland showed that they are widely present in the air (Caulton *et al.*, 1999). A Burkard trap monitored the production of spores from a small stand of bracken at Rothamsted Research from August to October in 1990 and 1991. The daily average spore content often exceeded 750 spores m^{-3}, with a maximum of 1,750 spores m^{-3}. The spore release showed a marked periodicity with most being released between 0900 and 1000 GMT (Lacey, M. and McCartney, 1994). With the large area of bracken in the UK and other countries there is potential for vast numbers of airborne spores to be present in the air and the carcinogenic properties of which cause concern to rural workers and visitors to the countryside.

6. Interpreting spore trap data

6.1. Clumping of particles

Conidia of *Blumeria graminis* (*Erysiphe graminis*, Pl. 10.30) in a mildewed barley crop were trapped on horizontal slides, vertical sticky cylinders and in suction traps. Spores were more often removed in clumps than singly and clumps were more efficiently deposited than single spores (McCartney and Bainbridge, 1987; McCartney, 1987). This clumping is thought to be due to the hydrophobic surfaces on the spores. Spores of *Puccinia striiformis* (yellow rust, Pl. 8.48-49) also clump together, but this is due to a mucilage surrounding them, causing the dispersal unit to be more than one spore in humid weather, while in dry conditions, individual spores are released (Sache, 2000).

In ascomycete fungi, eight ascospores are produced per ascus. In some species, groups of ascospores stick together and are captured as one particle. For example, in 1985 many hyaline cylindrical spores caught using a Burkard spore trap in a field of oilseed rape infected by *Pyrenopeziza brassicae* (light leaf spot, Pl. 8.20) were identified as ascospores rather than rain-splashed conidia because they were often in groups of four. A vertical mast with 5 whirling arm traps, and also a series of 4 traps down wind (Figs. 3.5 and 6) showed that the spores were in the air after rain and behaved as airborne spores rather than splashed spores (McCartney *et al*, 1986). In May 1986 apothecia producing ascospores were discovered on decaying leaf debris. This was the first record of the teleomorph of *Pyrenopeziza brassicae* in England, which proved important in the epidemiology of the spread of the disease (Lacey, M., *et al*, 1987).

Clumping of particles has implications for particle dispersal compared to individual spores and can affect the likely amount of disease or number of colonies formed compared to estimations based on actual spore numbers. Furthermore, different results can be obtained using different air sampling techniques due to clumping of cells e.g. an Andersen sampler measures colonies formed from particles (including clumps of cells) while other techniques such as liquid impinging or use of aerosol monitors (particles trapped on filters and subsequently suspended in liquid) may separate the clumps into individual spores resulting in higher apparent numbers of colony forming units (Crook and Lacey, J., 1988).

6.2. Backtracking wind dispersal

Spores of *Pithomyces chartarum* (Pl. 10.47) were first identified in Britain on spore trap slides exposed in 1958 but counted in 1960. The fungus had just been implicated in facial eczema of sheep in New Zealand and a spore had just been painted for Gregory's book. A whirling arm trap mounted in a plastic lattice-work shopping basket was carried by Gregory during a British Mycological Society fungus foray, and spores were found. Further searching up the concentration gradient over parkland enabled the fungus to be found growing on debris of *Holcus lanatus* (Gregory and Lacey, M., 1964). Backtracking of air movements is of considerable interest when considering long distance transport (see below).

6.3. Long distance dispersal

Long-distance transport of fungal spores has been demonstrated by sampling air in regions that do not produce the spores e.g. the Arctic (Meier, 1935) and over the sea (Hirst and Hurst, 1967: Hovmøller *et al.*, 2002). Hirst investigated the distribution of spores as affected by air mass movement in westerly winds. Spores were collected, using a volumetric suction trap fitted to an aircraft, during flights made from England, over the North Sea to the east. With distance, spore numbers were diluted and spores were found generally at progressively higher altitudes. It was possible to distinguish a time scale for spore dispersal due to the distribution of spore types released predominantly at night or predominantly during daylight (Fig. 2.8). Areas with a high density of spores of *Sporobolomyces* (Pl. 10.5) indicated the dispersal of night-time released spores, while pollens and *Cladosporium* spores (Pl. 11.1-2) indicated daytime discharge events (see Hirst *et al.* 1967a; Hirst, *et al.* 1967b; Hirst and Hurst 1967). Long distance transport of plant pathogens has been reviewed by Brown and Hovmøller (2002).

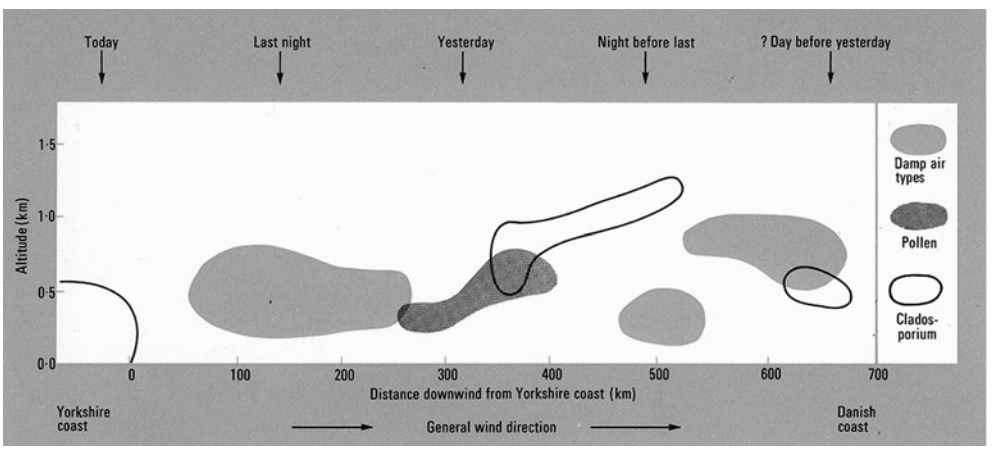

Figure 2.8
Changes in peak concentrations of pollen, *Cladosporium* spores and 'damp-air type' spores at various altitudes over the North Sea downwind of the English coast, 16th July 1964 (Gregory, 1976: from Hirst and Hurst, 1967).

Natural events that enhance long-distance transport of particles include biomass fires, which were reported by Mims and Mims (2003) to spread viable bacteria and fungal spores (*Alternaria* Pl. 11.3-6, *Cladosporium* Pl. 11.1 and 2, *Fusariella* and *Curvularia* Pl. 11.16-17) large distances e.g. over 1450 km from Yucatan to Texas. The spores were associated with coarse carbon particles collected on microscope slides (e.g. Pl. 12.29) and to eliminate contamination by local spores, a passive air sampler was flown from a kite at a Texas Gulf Coast beach. Back-trajectory analysis of the wind showed that air was travelling from Yucatan, where numerous bush fires were in progress. The authors also reported collecting spores and carbon particles at Mauna Loa Observatory, Hawaii (elevation 3400 m) on 6 July 2003, when a large fire in S. E. Asia was in progress. They speculate that convection from burning sugarcane at harvest may have helped to spread sugarcane rust (*Puccinia melanocephala*) from West Africa to the Dominican Republic in July 1978.

Turbulent weather events are thought to assist in biological particles spreading long

distances. Marshall (1996) showed that cyclones moving around Antarctica were associated with a dramatic influx of airborne biological material into the South Orkney Islands from South America. The occurrence of exotic pollen trapped in moss cushions in Antarctica (Linskens *et al.*, 1993) and exotic plants found on volcanically warmed soils in Antarctica (Bargagli *et al.*, 1996; Convey *et al.*, 2000) also act as indicators of long distance propagule transfer. Similarly, turbulent weather contributed to evidence for long distance dispersal of bacteria in Sweden, where exotic *Bacillus* species were isolated from red-pigmented snow (Bovallius *et al.*, 1978). Back-trajectory analysis of wind and analysis of associated clay, fungal and pollen particulates, indicated an origin near the Black Sea, 1800 km distant, where a sandstorm had occurred 36 hours earlier.

7. Dispersal by Rain-splash and aerosol

Many plant diseases are spread during rainfall by splash and by run-off water falling to lower parts of the crop. Generally splash borne spores or bacteria are produced in mucilage which prevents dispersal by wind alone (Gregory, 1973). The mucilage dissolves on wetting to give a suspension of spores in a thin film of water on the host surface. Rain splashed fungal spores tend to be hyaline and are often filiform in shape. Diatoms are aquatic but can be blown around in the air from dried splash droplets and bursting bubbles (Khandelwal, 1992; Marshall, 1996).

Rain consists of drops of up to 5 mm in diameter falling at terminal velocity (2-9 m s^{-1}) with the larger drops (generally over 2 mm) causing the greatest amount of splash from leaves (Fitt *et al*, 1989). The amount and type of rain-splash depends on whether raindrops fall on dry surfaces or onto relatively thin or deep liquid films. The mechanism of splash was studied initially in the laboratory under simple conditions with water drops falling from known heights on to thin films of a suspension of conidia of *Fusarium solani* spread on horizontal glass slides. One incident drop 5 mm in diameter falling on a spore suspension 0.1 mm deep produced over 5200 splash droplets of which over 2000 carried one or more spores. The number of droplets deposited per unit area on a horizontal plane decreased rapidly with increasing distance from the point of impact, and in still air few droplets travelled beyond 70 cm. The larger splash droplets contained spores if either the incident drop or the surface film was a spore suspension (Gregory *et al.* 1959).

Subsequent experiments, using a rain tower at Rothamsted Research, built in order to study the interaction of rain and wind on the dispersal of plant pathogens in controlled conditions (Fitt *et al.*, 1986), showed that droplets formed from rain-splashes comprise two types: larger ballisticly splashed droplets and smaller aerosol droplets. The incorporation of inoculum into splash droplets may be considered in three stages: removal, mixing and splash droplet formation (Fig. 2.9, Fitt *et al*, 1989).

Models have been developed to describe the incorporation of pathogen spores into rain-splash droplets (Huber *et. al.*, 1996), to describe droplet dispersal (Macdonald and McCartney, 1987) and to simulate vertical spread of plant diseases in a crop canopy by

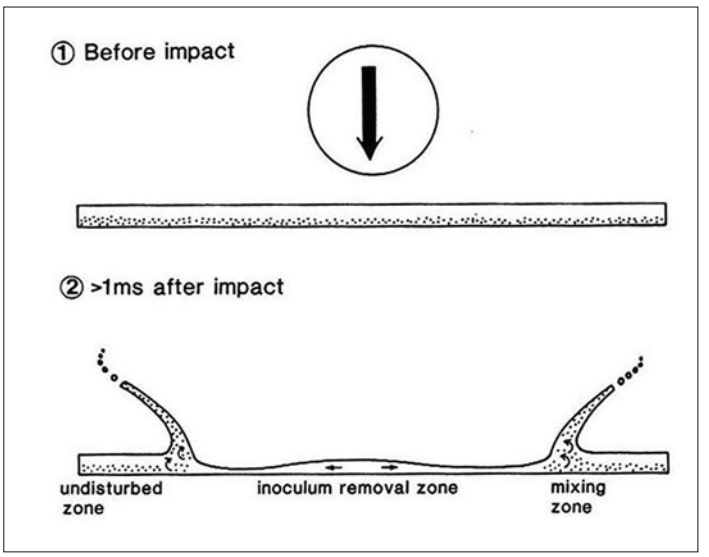

Figure 2.9
The process of inoculummdispersal in splash droplets as raindrops strike thin films of water covering host surfsce (Fitt *et al.*, 1989).

stem extension and splash dispersal (Walklate, 1999; Walklate *et al.*, 1989; Pielaat *et al.*, 2001).

Large splash droplets (ballistic drops) tend to travel relatively short distances in wind, e.g. <16 m, but even less (<3 m) if they are subject to filtering effects of crop canopies (Stedman, 1980a; 1980b). Fitt and Bainbridge (1983) showed that spores (of *Pseudocercosporella herpotricoides*; teleomorph, *Oculimacula (Tapesia) yallundae*) are dispersed mainly in relatively large drops, but small airborne droplets may carry spores much longer distances. In fierce winds, a huge amount of windborne spray or aerosol is produced after impaction of raindrops on the ground, buildings or vegetation. Particularly strong winds combined with rain in storms or hurricanes have been important in the spread of the bacterial disease, citrus canker (*Xanthomonas axonopodis* pv. *citri*), in Florida. As a result, controversial laws currently in force to limit the spread of this disease stipulate that any citrus tree within 570 m (1900 ft) of an infection site should be destroyed. Gottwald *et al.* (2002) estimated that disease spread from a source to the nearest newly diseased tree within a 30-day period was up to 3.5 km, with infections potentially extending beyond this distance.