Dispersal of foliar plant pathogens: mechanisms, gradients and spatial patterns

Spore dispersal

Fungi have many different methods of spore dispersal. Here we will focus on selected aspects, asking how the spores or spore-bearing structures of fungi are precisely tailored for their roles in dispersal. In doing so, we cover many topics of practical and environmental significance.

Ballistic dispersal methods of coprophilous fungi

Coprophilous (dung-loving) fungi grow on the dung of herbivores and help to recycle the vast amounts of plant material that are deposited annually by grazing animals. The spore dispersal mechanisms of these fungi are highly attuned (agreed) to their specific lifestyles - their function is to ensure that the spores are propelled from the dung onto the surrounding vegetation, where they will be ingested and pass through an animal gut to repeat the cycle. In several cases this is achieved by ballistic mechanisms of spore discharge.

In the case of *Pilobolus* (Fig. 10.7) each spore-bearing structure consists of a large black sporangium, mounted on a swollen vesicle which is part of the sporangiophore. At maturity the sporangiophore develops a high turgor pressure, the wall that encloses both the sporangium and the vesicle breaks down locally by enzymic means, and the vesicle suddenly ruptures, squirting its contents forwards and propelling the sporangium for 2 meters or more. Mucilage released from the base of the sporangium during this process serves to stick the sporangium to any plant surface on which it lands; then the spores are released from the sporangium and can be spread by water or other agencies. As a further adaptation for dispersal, the

sporangiophore is phototropic, ensuring that the sporangium is shot free from any crevices (narrow opening) in the dung. The light signal is perceived by a band of orange carotenoid pigment at the base of the vesicle, and the vesicle itself acts as a lens that focuses light on the pigment. A unilateral light signal is thereby translated into differential growth of the sporangiophore stalk, aligning the sporangium towards the light source.

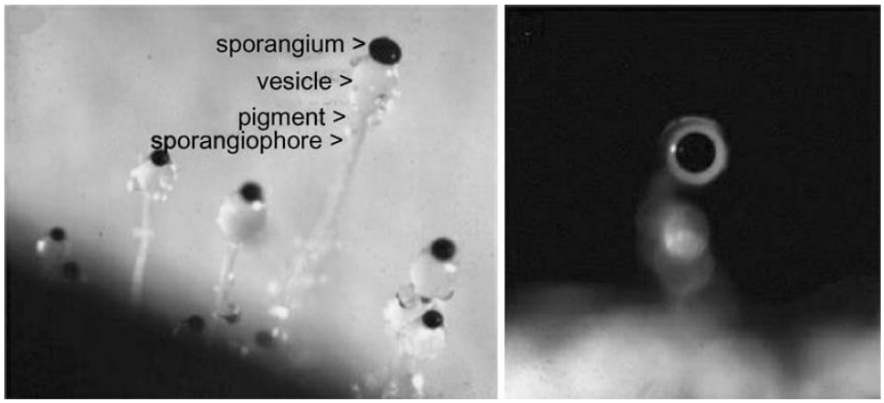

Fig. 10.7 *Pilobolus* (Zygomycota), a fungus with a ballistic method of spore discharge. (a) Several sporangia on a dung pellet. (b) A sporangium orientated towards a light source, showing how the subsporangial vesicle acts as a lens.

Insect-dispersed fungi

Insects and other small arthropods can disperse several types of fungi, including spores that are produced in sticky, mucilaginous masses. This form of dispersal can be highly efficient because the fungus takes advantage of the searching behavior of the vector to reach a new site. There are many of these fungus–vector associations, ranging from cases where the association is almost incidental to cases of highly evolved mutualism. Here we consider one classic example – the dispersal of Dutch elm disease by a bark-beetle vector.

Dutch elm disease

Dutch elm disease is caused by two closely related fungi, *Ophiostoma ulmi* and *O. novo-ulmi* (Ascomycota). These fungi enter the plant through wounds made by bark beetles, then spread in the water-conducting xylem vessels by growing in a yeastlike budding phase. This causes reactions in the xylem vessels, leading to blockage and death of all or part of the xylem. In many respects, the symptoms and host reactions in Dutch elm disease resemble those caused by other vascular wilt pathogens. But bark beetles of the *Scolytus* and *Hylurgopinus* are specialized vectors of Dutch elm disease.

The disease cycle starts when young, contaminated beetles emerge from the bark of dead or dying elm trees in early spring, fly to neighboring healthy trees, and feed on the bark of the young twigs. During feeding, the beetles cause incidental damage to the xylem, thereby introducing the fungus into the tree. The fungus then spreads in the xylem, killing the whole tree or some of its major branches, and the bark of the newly killed trees is then used by the female beetles for egg laying. The female beetle tunnels into the inner bark and eats out a channel, depositing eggs along its length – the "brood gallery." The eggs hatch and the young larvae eat out a series of radiating channels before they pupate for overwintering. Meanwhile, the fungus that killed the tree grows from the xylem into the bark and sporulates in the beetle tunnels. In this way, the young adult beetles that emerge from the pupae in the following spring become contaminated with spores; they leave the bark and fly in search of new trees, repeating the disease cycle.

Dispersal of aquatic fungi: appendaged spores

Fungi that grow as saprotrophs in aquatic environments often have spores with unusual shapes and conspicuous appendages (Fig. 10.1). One of the more common types is the tetraradiate (four-armed) spore, often found in the fungi that grow on

fallen tree leaves in well-aerated, fast-flowing streams (e.g. *Alatospora*, *Tetracladium*, *Tetrachaetum*). Similar tetraradiate spores have been found in two marine fungi (Basidiomycota), while tetraradiate sporangia are produced by *Erynia conica* (Zygomycota), a fungus that parasitizes freshwater insects. There is even a yeast, *Vanrija aquatica*, which grows in mountain tarns (pools), that produces tetraradiate cells instead of the normal ovoid yeast cells. In extreme cases, aquatic spores such as *Dendrospora* (Fig. 10.1) can have up to 20 radiating arms. And other aquatic spores are curved or sigmoid – for example, *Anguillospora* (Fig. 10.1).

The tetraradiate conidia of other fungi have been shown to sediment slowly in water, at about 0.1 mm s^{-1}, although differences in sedimentation rates are unlikely to be important in the turbulent, fast-flowing streams where these spores are commonly found. Perhaps more important is the role of spore shape in entrapment (sting), because small air bubbles are often trapped between the arms of tetraradiate spores, causing the spores to accumulate in the "foam" of fast-flowing streams. In addition, these spores settle like a tripod on a natural or artificial surface, and then respond rapidly by releasing mucilage from the tips of the arms in contact with the surface, but not from the fourth (free) arm. This attachment is also followed rapidly by germination from the contact sites, so that the fungus establishes itself from three points, which is likely to increase the efficiency of colonizing a substrate in

competition with other microorganisms.

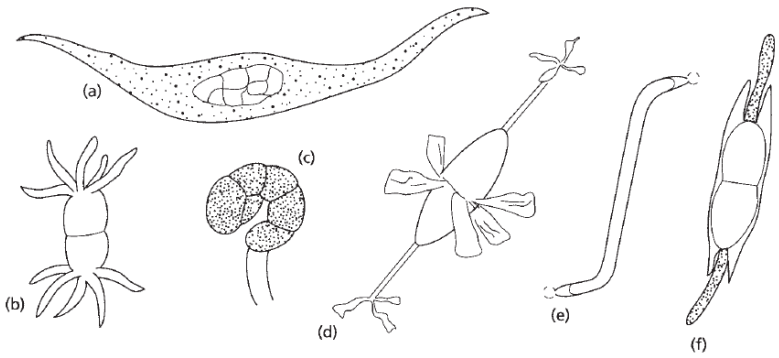

Fig. 10.11 Appendaged spores of estuarine and marine environments. (a) Ascospore of *Pleospora* with mucilaginous appendages (stippled), about 400 µm. (b) Ascospore of *Halosphaeria* with chitinous wall appendages (25 µm). (c) Conidium of *Zalerion* (25 µm). (d) Ascospore of *Corollospora* with membranous appendages (70 µm). (e) Ascospore of *Lulworthia* with terminal mucilaginous pouches (60 µm). (f) Ascospore of *Ceriosporiopsis* with mucilaginous appendages (stippled) (40 µm).

Dispersal and infection behavior of zoospores

Zoospores are motile, wall-less cells that swim by means of flagella. They are the characteristic dispersal spores of Chytridiomycota, Oomycota, and plasmodiophorids, although only the Chytridiomycota are true fungi.

Zoospores can swim for many hours using their endogenous energy reserves, and they show a remarkable degree of sensory perception, owing to the presence of receptors on the cell surface. These receptors enable zoospores to precisely locate the sites where they will encyst – whether on a host or an organic substrate. An example was shown in the nematode parasite *Catenaria anguillulae*. Other important examples include the many Oomycota (*Pythium*, *Phytophthora*, and *Aphanomyces* spp.) that cause devastating diseases of crop plants or of salmonid fish, while a wide range of saprotrophic species play important roles as primary colonizers of organic substrates in natural waters. In this section we discuss the structure and function of zoosporic fungi.

Structure and organization of zoospores

The zoospores of Chytridiomycota are small, typically 5-6 μm diameter, and tadpole-shaped (pollywog OR young frog). Except for some rumen chytrids (which have several flagella) they have a single, smooth, posterior flagellum of the whiplash type.

The plasmodiophorids also have small zoospores, about 5 μm diameter, but with two flagella – a short one directed forwards and a longer one directed backwards. By contrast, the zoospores of Oomycota (Figure) are larger, typically 10–15 μm, and kidney-shaped, with two flagella inserted in a ventral groove. The longer flagellum is whiplash type and trails behind the swimming spore; the shorter flagellum projects forwards and is tinsel-type, with short glycoprotein hairs (mastigonemes) projecting along its length.

Sensitivity of zoospores to lysis: a potential basis for disease control

Zoospores of all types, including Chytridiomycota (e.g. *Allomyces* spp.) and Oomycota (*Saprolegnia*, *Aphanomyces*, *Pythium*, and *Phytophthora*) lack a cell wall during their motile phase and the early stages of encystment. For this reason, zoospores are highly susceptible to disruption by surface-active agents (surfactants) such as rhamnolipids which are produced by the bacterium *Pseudomonas aeruginosa*. The key feature of surface-active agents is that they have both a hydrophobic and a hydrophilic domain, so they can insert into the cell membrane of wall-less cells and disrupt the cells.

The roots of oat plants (*Avena sativa*) and the closely related wild grass, *Arrhenatherum elatius*, produce soap-like compounds (saponins) from a narrow zone just behind the root tips. In this case the saponin is termed avenacin, and it naturally fluoresces bright blue when viewed under ultraviolet illumination. A similar saponin, β-aescin, is produced by the leaves of horse chestnut trees (*Aesculus*

hippocastanum). When oat roots are placed in a suspension of fungal zoospores the spores rapidly accumulate at the root tips in response to root tip nutrients and then lyse within a few minutes.

The use of surfactants such as rhamnolipids, or even crude extracts of saponin-containing tissues such as oat roots, could provide disease control in hydroponic glasshouse-cropping systems where zoosporic fungi can cause serious diseases. This is now being investigated in several laboratories, to find environmentally safe alternatives to the use of fungicides.

Zoospore motility

In appropriate conditions zoospores of Oomycota can swim for 10 hours or more, at rates of at least 100 μm s^{-1} fuelled by endogenous nutrient reserves. So they could, in theory, swim as far as 3–4 meters for dispersal to new environments. However, the zoospores make frequent random turns, and because of this the rate of dispersion by *Phytophthora* zoospores in still water has been found to be little more than the rate of diffusion of a small molecule such as HCl.

Zoospores as vectors of plant viruses

About 20 plant viruses are currently known to be transmitted by zoospores, and in some cases this is their main or only means of transmission. These zoospore vectors belong to three genera: *Olpidium* (Chytridiomycota), *Polymyxa* (plasmodiophorids), and *Spongospora* (plasmodiophorids). All are common and usually symptomless parasites of roots. The feature that makes them significant as vectors is that the zoospore encysts on a root and then germinates to release a naked protoplast into the plant. Any virus particles that bind to the surface of the swimming zoospore will therefore be introduced into the host.

Dispersal of airborne spores

Most terrestrial fungi produce airborne spores that are dispersed by wind or rain-splash. These are the spores of most significance in plant pathology and for allergies and fungal infections of humans. In this section we consider how spores become airborne (take-off), how they remain airborne (flight), and how they are finally deposited in appropriate environments for future development (landing). These are features of fundamental significance in understanding the ecology of airborne fungi.

Spore liberation – take-off

The essential feature of spore liberation is that a spore needs to break free from the boundary layer of still air that surrounds all surfaces. Above this boundary layer the air becomes progressively more turbulent in local eddies (vortexes), until there is net movement of the air mass which can carry spores to a new site. The depth of the boundary layer can vary considerably – from a fraction of a millimeter on a leaf surface on a windy day, to a meter or more on a forest floor on a perfectly calm day. So the fungi that grow in these different types of environment require different strategies for getting their spores airborne. Some of these strategies are often involve adaptations of the spore-bearing structures rather than of the spores themselves.

Fungi that grow on leaf surfaces sometimes produce chains of spores from a basal cell so that the mature spores are pushed upwards through the boundary layer as more spores are produced at the base of the chain (e.g. *Blumeria* (*Erysiphe*) *graminis* and other powdery mildew pathogens). The spores are then removed by wind or, sometimes more effectively, by mist-laden air (e.g. *Cladosporium*). Other types of spore are flung off the spore-bearing structures by hygroscopic (drying) movements that cause the spore-bearing hyphae suddenly to buckle (bend and give way under a weight or force) (e.g. *Phytophthora infestans* and downy mildew fungi such as *Peronospora*).

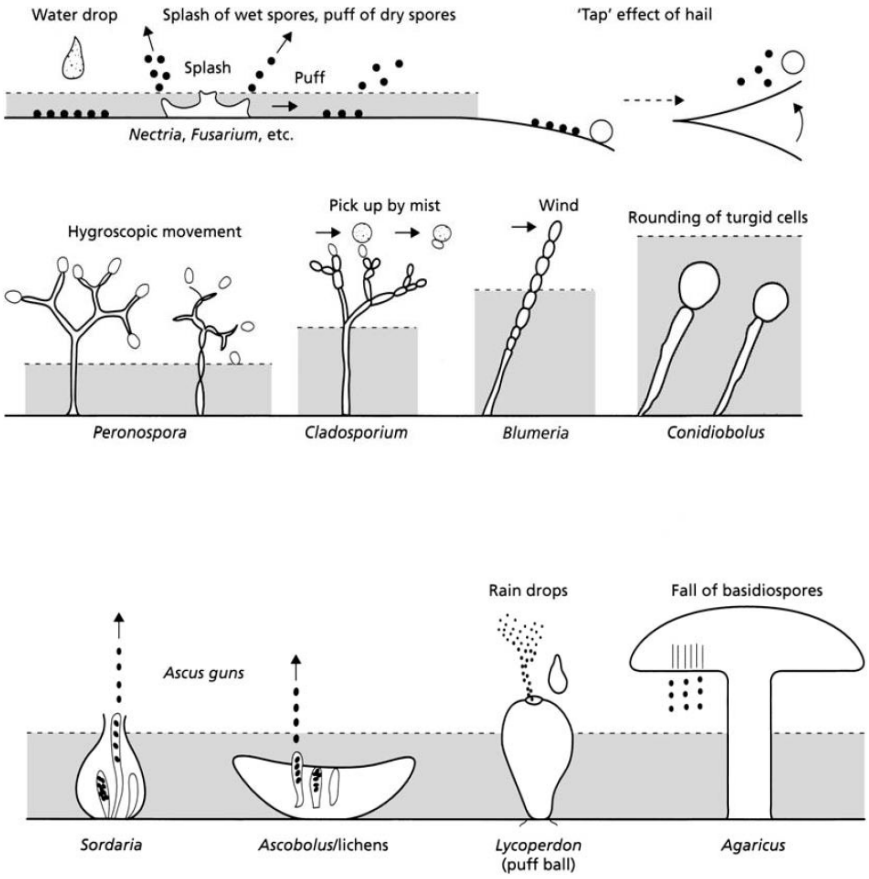

Fig. 10.23 The diversity of mechanisms of spore liberation through a boundary layer of still air (shown by shading).

Flight

The fate of spores in the air is determined largely by meteorological factors – wind speeds, rain, etc. – but at least two features of spores are significant for long-distance dispersal: their resistance to desiccation, conferred by hydrophobins in the walls, and their resistance to ultraviolet radiation, conferred by wall pigments. Thus, the hyaline (colorless), thin-walled conidia of *Blumeria graminis* (cereal powdery mildew) or the wind-borne sporangia of *Phytophthora infestans* (potato blight) remain viable for only a short time on bright, cloudless days, whereas the pigmented uredospores of rust fungi (e.g. *Puccinia graminis*) and conidia of *Cladosporium* can remain viable for days or even weeks in air.

Spore deposition – landing

Spores suspended in the air can be removed in three major ways – by sedimentation, impaction, or washout. The shape, size and surface properties of spores have major effects on these processes – even to the extent that an understanding of a spore's properties enables us to predict the circumstances in which it will be deposited.

Sedimentation

All spores settle out of the air by sedimentation in calm conditions, and the heavier (larger) spores settle faster than lighter (smaller) spores.

Impaction

Impaction is one of the major mechanisms by which large spores are removed from the air, and it has special significance for plant pathogens. When spore-laden air moves towards an object (or vice-versa), the air is deflected around the object and tends to carry spores with it. But the momentum (mass × velocity) of a spore will tend to carry it along its existing path for at least some distance.

Washout

Even light, steady rain will remove almost all suspended particles from the air. However, the spore surface properties then come into play. Wettable spores become incorporated within the raindrops and finally come to rest where the water does – spreading as a film across a wettable surface or dripping from a non-wettable one.

Mechanisms, gradients and spatial patterns of dispersal of foliar plant pathogens:

Introduction

Dispersal has long been recognised as fundamental to the development of plant disease epidemics, for without dispersal many epidemics would fail to progress. In

recent years, agriculture, especially in the developed world, has come under increasing pressure to produce crops in sustainable and environmentally friendly ways. Consequently, there is an urgent need for more efficient disease management systems. Understanding the temporal and spatial dynamics of disease epidemics is crucial to the development of such systems. For example, when developments in precision agriculture lead to spatially targeted crop spraying, there will be a need to understand and predict disease patch dynamics. The role of dispersal in gene-flow within plant pathogen populations is little understood, but may be crucial to understanding fungicide resistance breakdown or the distribution of alleles conferring virulence within populations. Knowledge of dispersal processes is also needed to understand movement of new pathogens into a landscape, for example the introduction of exotic pathogens into a country or the movement of pathogens due to climate change. Thus, knowledge of dispersal will increasingly be needed by policy makers devising plant health protection strategies. It is evident that now, perhaps more than ever, there is a need to understand the nature and scope of the dispersal of plant pathogen propagules.

Plant pathogen propagules include fungal spores, virus particles and bacteria (cells and spores) and there are many different mechanisms by which each can be dispersed from infected host plants. For example, many plant viruses are dispersed by insect vectors; insects, birds and farming activities can spread both bacteria and fungal spores. Many soil-borne pathogens can be spread in ground water or by agricultural operations. However, many economically important crop diseases are caused by foliar fungal pathogens, for which the main routes of dispersal are wind-borne or splash-borne spores. The scale of dispersal by these processes ranges from a few centimetres for some spores spread by rain-splash up to hundreds of kilometres for some spores carried by the wind. For foliar pathogens, disease spread is the direct

consequence of spore dispersal, although spatial patterns of disease may be quite different from the spore dispersal patterns which cause them. This is partly because spore dispersal is a short term phenomenon compared to most other stages of disease development. For example, conidia of *Pyrenopeziza brassicae*, the cause of light leaf spot on oilseed rape (*Brassica napus* ssp. *oleifera*), take about 18 hours to germinate under optimum conditions, while splash dispersal of conidia over typical distances of 20-30 cm takes less than one second and wind dispersal of ascospores of *P. brassicae* over 100 m takes 1-2 minutes. Even for long distance dispersal, such as for tobacco blue mould that can spread from Cuba to the southern USA or cereal rusts in the USA or India, dispersal events (hours or days) may be short compared with infection processes. Disease patterns are often the result of many individual dispersal events from many sources over periods of days or even weeks. Environmental and biological factors that affect infection and disease development can add further complications. The development of real epidemics is, therefore, a complex process.

Underlying mechanisms: spore dispersal

The mechanisms of spore dispersal will include from the point of view of the spore; from the source (lesion, pustule, fruiting body) to the new host (or loss to the ground or non-host surface). It is important to appreciate these 'primary' physical mechanisms of spore dispersal as a foundation for understanding the spread of disease epidemics.

Dispersal by wind

Winds are highly variable in both time and space. This variability or turbulence causes individual spores, released from the same source under the same wind conditions, to follow different paths and travel different distances. Therefore, as spore plumes (clouds) disperse downwind from sources their concentrations in the

air decrease. The decreases in concentration are frequently referred to as 'concentration gradients'. Wind speeds increase with height depending on the nature of the crop (height, architecture, density) and the stability of the atmosphere (temperature profile). For example, in neutrally stratified (in a stable layers) atmospheres when buoyancy (resistance) effects can be neglected, over open terrain with uniform vegetation, wind speed *u(z)* increases logarithmically with height *z*:

$$u(z) = 2.5u_* 1\mathrm{n}\left(\frac{z-d}{z_0}\right)$$

Where:

The constant u_*, the friction velocity, scales the wind speed and defines the amount of turbulence; z_0, the roughness length, scales the height and *d* is a datum level (reference level) less than the crop height called the zero plane displacement. This equation predicts that $u = 0$ at height *d* but the equation is not valid within a crop. For most crops, z_0 is an order of magnitude smaller than the height of the crop *h*, and *d* is between 0.6 and 0.8*h*. On sunny days when there is convective activity (unstable temperature lapse rate) or in the evening when atmospheric mixing is suppressed (stable temperature lapse rate), the wind profile deviates from the equation. The wind profile is logarithmic only with well-formed surface boundary layers over large uniform areas. Wind profiles near obstructions such as hedges or near changes in terrain, for example woodland boundaries, may be more complex than suggested by the equation. Mean wind profiles within crops depend greatly on crop architecture, particularly the vertical distribution of foliage and the size, shape and density of leaves. For crops where the leaves are relatively uniformly distributed with height, such as cereals, wind speed profiles can often be estimated using the equation:

$$u(z) = u(h)exp\left[a\left(\frac{z}{h}-1\right)\right]$$

where *h* is the crop height and the attenuation (decrease) coefficient *a* has a value between 0.3 and 3, depending upon crop type and leaf area density. In crops with a 'canopy and stem' structure (e.g. orchards), the wind speed profile may be S-shaped, with wind speeds greater in the 'stem' layer than the 'canopy' layer. It has been suggested that turbulent diffusivity is more or less constant within the upper two-thirds of crop canopies and decreases towards the ground level. However, wind speeds within crops are highly intermittent, with air flow at relatively low speeds interspersed with sporadic bursts at high speeds with increased turbulence. This produces highly skewed distributions of wind speed, with a long 'tail' of high wind speeds (gusts) occurring at low frequencies. These high wind speeds are caused by relatively large eddies (wind currents) which penetrate the canopy. The corollary (result) of gust penetration is the gradual ejection of air from the canopy after gusts, which can be responsible for the upward transport of spores. In another phenomenon, called outward interactions, air moves upwards at speeds greater than the local average wind speed. These complex flow patterns have consequences for spore removal and transport within and out of the canopy.

Wind not only transports spores but also removes them from infected plants. Although many fungi have evolved active spore release mechanisms to eject spores directly into the air, spores of a large number of foliar pathogens are simply passively blown or shaken off their hosts. To remove spores, the aerodynamic or mechanical forces generated by wind must overcome the forces holding the spore to the host surface. The wind speeds needed to remove spores are not known for many fungi but can be relatively large. Conidia of *Blumeria* (*Erysiphe*) *graminis* f.sp. *hordei* (cause of barley powdery mildew), which form in chains above the leaf surface, were released by wind speeds greater than 0.5 m s^{-1} and conidia of *Drechslera maydis* (cause of southern leaf blight of maize) were removed only by wind speeds of more

than 5 m s^{-1}. The wind intermittency observed in crop canopies probably plays an important role in spore removal because it is only in gusts that wind speeds are large enough to remove spores. The importance of gusts in the removal of conidia of *Passalora personata* (cause of late leaf spot of groundnut) has been demonstrated in wind tunnel experiments. Wind gusts can also remove bacterial cells (*Pseudomonas syringae*) and spores (*Bacillus subtilis*) from leaves on which they had previously been sprayed. Spores can also be dislodged by shaking; thus wind gusts may indirectly remove spores by moving the crop canopy. The removal of spores by gusts of wind has important implications for dispersal, particularly within crop canopies.

The turbulent nature of wind causes a dilution in the concentration of a spore plume as it moves down-wind. Within crops, concentrations are also depleted by deposition of spores on the crop and the ground. Spores can be deposited by gravitational settling and inertial impaction. The rate at which spores settle onto surfaces, is proportional to the spore fall speed, and the spore concentration above the surface. The rate at which spores settle onto surfaces is generally in the range 0.1 to 3 cm s^{-1} for most fungal spores, as below:

$S = CVs$

Where: S is the rate at which spores settle onto surfaces, Vs is the spore fall speed, and C is the spore concentration.

Deposition by impaction I is dependent on spore concentration (C) and wind speed (u):

$I = CuE$

The constant of proportionality (E) (the impaction efficiency), increases with increasing spore size and wind speed but decreases with increasing width of the impaction surface.

Fungal spores have been found in the atmosphere at heights of 500 -1 000 m above the North Sea many kilometres from any potential sources and spores and pollen from South America have been found in air samples taken in Antarctica. Long distance aerial transport of inoculum has been cited as the probable mechanism for the invasion of disease into new territories, although such events are rare. Long-distance transport of particles may be enhanced by natural events such as bush fires, which were implicated in spread of viable bacteria and fungal spores over 1450 km from Yucatan to Texas and from south east Asia to Hawaii.

Three dimensional time-averaged spore concentration or deposition patterns round a point source (infected plant) are complex. However, average concentrations measured in one direction away from the source decrease monotonically (not changing – staying on the same manner) with distance. These dispersion gradients have been described by a number of different equations. Two of the most commonly used are a negative exponential equation:

$C = C_0 \exp(-\alpha x)$ …… (1)

And an inverse power law equation:

$C = Ax^{-\beta}$ ……..… (2)

where C is the concentration or deposition rate, x is the distance from the source and $C0$, α, A and β are constants. The coefficients α and β determine the rate of decrease in spore concentration (or deposition) with distance.

Several studies of potential long distance aerial transport of plant pathogens have used air parcel trajectory analysis to establish links between source and receptor regions. Trajectory (route) analysis is a standard tool in the study of air pollutant movement and it tracks the movement of air parcels using information on wind fields and atmospheric temperature structures. It is widely used in air pollution studies and computational methods. Back-trajectory analysis of wind contributed to evidence for long distance dispersal of exotic *Bacillus* bacteria 1800 km from the black sea to Sweden, where the species was isolated from red-pigmented snow.

Atmospheric dispersal models are becoming more sophisticated as understanding of mechanisms of atmospheric flow increases. Atmospheric dispersal models are being developed that can account for not only dispersal processes but also changes in topography and surface characteristics. Such models, although complex, have the potential to enhance understanding of spore and pollen dispersal within realistic landscapes. This information is needed to understand gene flow in fungal pathogen communities, for example, the movement of fungicide resistance or virulence genes. Computational fluid dynamic systems, developed to calculate air flows in complex terrains such as around buildings, are now being coupled with dispersal models to investigate pollutant dispersal in urban or industrial landscapes. Such techniques can potentially help in the understanding of inoculum dispersal within plant communities, particularly at crop boundaries.

References of this lecture

- Biology of fungi, by Jim Deacon, 4th edition, 2006, p 193 - 221.
- The Epidemiology of Plant Diseases. Edited by B.M. COOKE, and B. KAYE, Second Edition, 2006.