Lecture 6: Fungal spores, spore dormancy, and spore discharge and dispersal

Fungi are the supreme examples of spore-producing organisms. They produce millions of spores, with an astonishing variety of shapes, sizes, surface properties, and other features – all precisely matched to the specific requirements for dispersal and/or persistence in different environments. A small part of this diversity is illustrated in Fig. 10.1, for some of the more bizarrely shaped spores of the freshwater aquatic fungi that grow in fast-flowing streams. But even the common rounded spores of fungi have properties that determine whether they will be deposited on plant surfaces, or on soil, or in the human lungs, etc. In this chapter we discuss several examples of this fine-tuning, and we will see that the properties of a spore tell us much about the biology and ecology of a fungus.

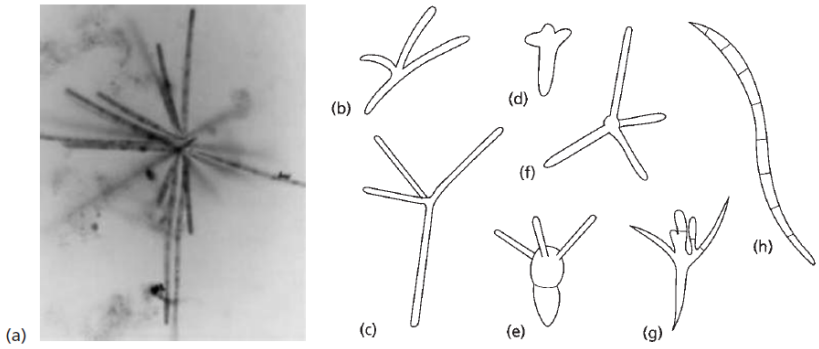

Fig. 10.1 Examples of tetraradiate, multiple-armed and sigmoid spores found in fast-flowing freshwater streams. Approximate spore lengths are shown in parentheses. (a) A single conidium of *Dendrospora* (150–200 μm); (b) conidium of *Alatospora* (30–40 μm); (c) conidium of *Tetrachaetum* (70–80 μm); (d) conidium of *Heliscus* (30 μm); (e) conidium of *Clavariopsis* (40 μm); (f) conidium of *Lemonniera* (60–70 μm); (g) conidium of *Tetracladium* (30–40 μm); (h) conidium of *Anguillospora* (150 μm).

General features of fungal spores

Because of their extreme diversity we can define fungal spores in only a general way, as microscopic propagules that lack an embryo and are specialized for dispersal or dormant survival. The spores produced by a sexual process (e.g. zygospores and ascospores) usually function in dormant survival whereas asexual spores usually serve for dispersal. However, many Basidiomycota do not produce asexual spores, or produce them only rarely, and instead the basidiospores are their main dispersal agents. Some fungi have an additional spore type, the chlamydospore. This is a thick-walled, melanized cell that develops from an existing hyphal compartment (or sometimes from a spore compartment) in conditions of nutrient stress.

The properties of these different spore types vary considerably but, in general, the spores of fungi differ from somatic cells in the following ways:

1. The wall is often thicker, with additional layers or additional pigments such as melanins.
2. The cytoplasm is dense and some of its components (e.g. endoplasmic reticulum) are poorly developed.
3. Spores have a relatively low water content, low respiration rate, and low rates of protein and nucleic acid synthesis.
4. Spores have a high content of energy-storage materials such as lipids, glycogen, or trehalose.

Spore dormancy and germination

Almost all spores are dormant, in the sense that their rate of metabolism is low. But they can be assigned to two broad categories in terms of their ability to

germinate. Sexual spores often show constitutive dormancy. They do not germinate readily when placed in conditions that are suitable for normal, somatic growth (appropriate nutrients, temperature, moisture, pH, etc.). Instead, some of them require a period of aging (postmaturation) before they will germinate, and others require a specific activation trigger such as a heat shock or chemical treatment. By contrast, nonsexual spores show exogenously imposed dormancy – they remain dormant if the environment is unsuitable for growth, but they will germinate readily in response to the presence of nutrients such as glucose.

When triggered to germinate, all spores behave in a similar way. The cell becomes hydrated, there is a marked increase in respiratory activity, followed by a progressive increase in the rates of protein and nucleic acid synthesis. An outgrowth (the germ tube) is then formed, and it either develops into a hypha or, in the case of some sexual spores, it produces an asexual sporing stage. The germination process usually takes 3–8 hours, but zoospore cysts of the Oomycota can germinate much faster (20–60 minutes) and some sexual spores can take longer (12–15 hours).

Constitutive dormancy

Constitutive dormancy has been linked to several factors but is still poorly understood. The oospores of many *Pythium* and *Phytophthora* spp. seem to need a postmaturation phase before they can germinate. Initially the oospore wall is thick, about 2 µm diameter (Fig. 10.2), but it becomes progressively thinner (about 0.5 µm) by digestion of its inner layers. This process is hastened by keeping the spores in nutrient-poor conditions, at normal temperature and moisture levels. Then, after several weeks, the spores will germinate in response to nutrients or

other environmental triggers. For example, *Pythium* oospores germinate in response to common nutrients (sugars and amino acids) or volatile metabolites (e.g. acetaldehyde) released from germinating seeds.

Ascospores can eventually become germination-competent by aging, but can be triggered to germinate at any time by specific treatments – in some cases a heat shock (e.g. 60°C for 20–30 minutes), cold shock (−3°C), or exposure to chemicals such as alcohols or furaldehyde.

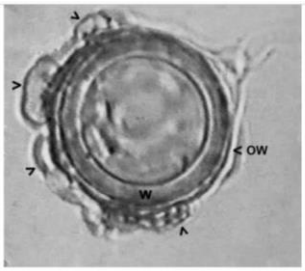

Fig. 10.2 A developing sexual spore (oospore) of Pythium mycoparasiticum (Oomycota). The spore has a very thick wall (w) and is contained in the outer wall (ow) of the oogonium (female reproductive cell). The arrows mark the positions of antheridia (male sex organs that fertilize the oogonium).

Ecological aspects of constitutive dormancy

The behavior of constitutively dormant spores often has clear ecological relevance. For example, a characteristic assemblage of fungi occurs on the dung of herbivorous animals. Their spores are ingested with the herbage, are activated during passage through the gut, and are then deposited in the dung where they germinate to initiate a new phase of growth. The spores of many of these coprophilous (dung-loving) fungi can be activated in the laboratory by treatment at 37°C in acidic conditions, simulating the gut environment. Examples include the ascospores of *Sordaria* and *Ascobolus*, the sporangiospores of several Zygomycota, and basidiospores of the toadstool-producing fungi, *Coprinus* and *Bolbitius*.

Heating to 60°C activates the spores of many thermophilic fungi of composts. It also activates the ascospores of pyrophilous (fire-loving) fungi such as *Neurospora*

tetrasperma which grows on burnt ground or charred plant remains. Most of these fungi are saprotrophs of little economic importance, but one of them, *Rhizina undulata* (Ascomycota), causes the "group dying" disease of coniferous trees in Britain and elsewhere. It infects trees replanted into clear-felled forests, and the foci of infection correspond to the sites where the trash from the felled trees was stacked and burned. The ascospores are heat-activated around or beneath the fires, then the fungus grows as a saprotroph on the stumps and dead roots and produces mycelial cords that infect the newly planted trees.

Exogenously imposed dormancy

In laboratory conditions, most asexual spores germinate readily at suitable temperature, moisture, pH and oxygen levels. Some germinate even in distilled water, although most require at least a sugar source, and a few have multiple nutrient requirements. However, in nature all these spores can be held in a dormant state by the phenomenon termed fungistasis (or mycostasis). This is very common in soil, and has also been reported on leaf surfaces.

Fungistasis is a microbially induced suppression of spore germination. For example, spores often fail to germinate in topsoil, where the level of microbial activity is high, but they germinate in sterilized soil or in subsoils of low microbial activity. The germination that occurs in sterilized soil can be prevented if the soil has been recolonized by microorganisms, and even single microorganisms – bacteria or fungi – will restore the suppression. This suggests that fungistasis is caused by nutrient competition or by general microbial metabolites (or both), but not by specific antibiotics or other inhibitors from particular microorganisms. A long history of research suggests that volatile germination inhibitors such as ethylene ($H_2C=CH_2$),

allyl alcohol ($H_2C=CH.CH_2OH$), and ammonia can play a role in fungistasis. But the strongest evidence implicates nutrient deprivation as the key cause of fungistasis. Even spores that can germinate in distilled water are inhibited in soil because they leak nutrients into their immediate surroundings, and these nutrients are continuously metabolized by other soil organisms.

Ecological implications of fungistasis

Fungistasis causes spores to remain quiescent (inactive) in soil or other natural environments until nutrients become available. Thus, saprotrophs can lie in wait for organic nutrients, and root-infecting pathogens or mycorrhizal fungi can wait for a root to pass nearby. In many cases the germination trigger is nonspecific. For example, the spores of many root pathogens can germinate in response to the root exudates of both host and nonhost plants. This can be exploited for disease control, especially in traditional crop-rotation systems (organic farming) where the spores of parasitic fungi can be induced to germinate but then die because they cannot infect a plant. This phenomenon is termed germination-lysis.

In a few cases there is evidence of host-specific triggering of germination. Perhaps the best example is the triggering of sclerotia of *Sclerotium cepivorum* (Basidiomycota) which causes the economically important “white rot” disease of onions, garlic, and closely related *Allium* spp.. These small sclerotia (about 1 mm diameter) are produced abundantly in infected onion bulbs and can survive for up to 20 years in soil until they are triggered to germinate by the host. The germination triggers are volatile sulfur-containing compounds (alkyl thiols and alkyl sulfides) such as diallyl disulfide (DADS), but the host plant releases the nonvolatile precursors of these compounds (alkyl sulfoxides and alkyl-cysteine sulfoxides) and

these are converted to the volatile germination triggers by many common soil bacteria.

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 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.

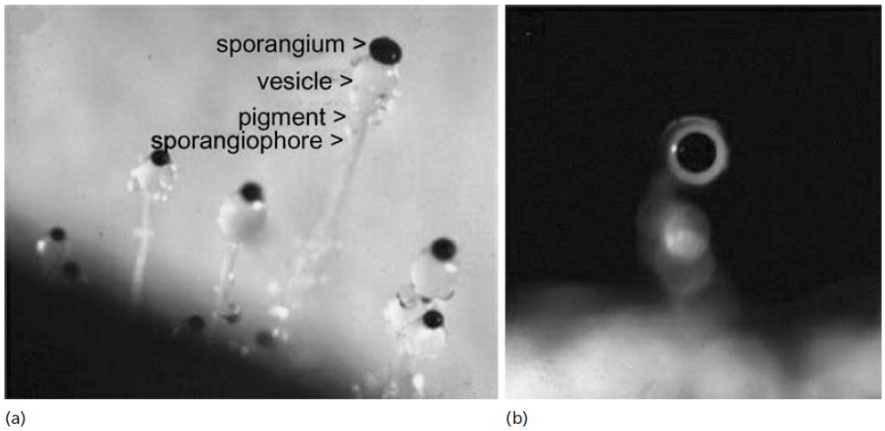

Fig. 10.7 *Pilobolus* (Zygomycota), a fungus with a ballistic method of spore discharge. (a) Several sporangia on a dung pellet. (b) A sporangium orientated towards a light source, showing how the subsporangial vesicle acts as a lens.

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, 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.

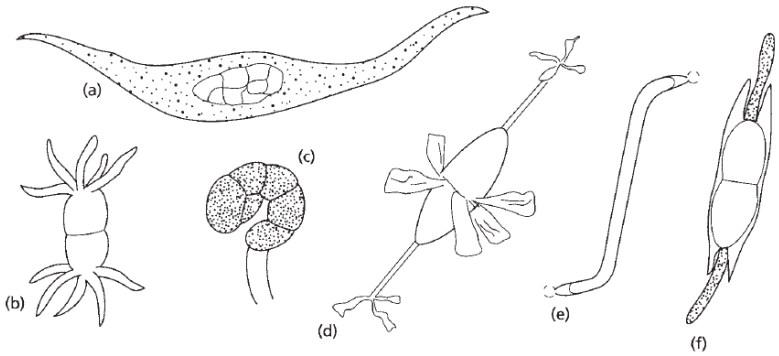

Fig. 10.11 Appendaged spores of estuarine and marine environments. (a) Ascospore of *Pleospora* with mucilaginous appendages (stippled), about 400 μm. (b) Ascospore of *Halosphaeria* with chitinous wall appendages (25 μm). (c) Conidium of *Zalerion* (25 μm). (d) Ascospore of *Corollospora* with membranous 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 disrupution 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, 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 (e.g. *Phytophthora infestans* and downy mildew fungi such as *Peronospora*).

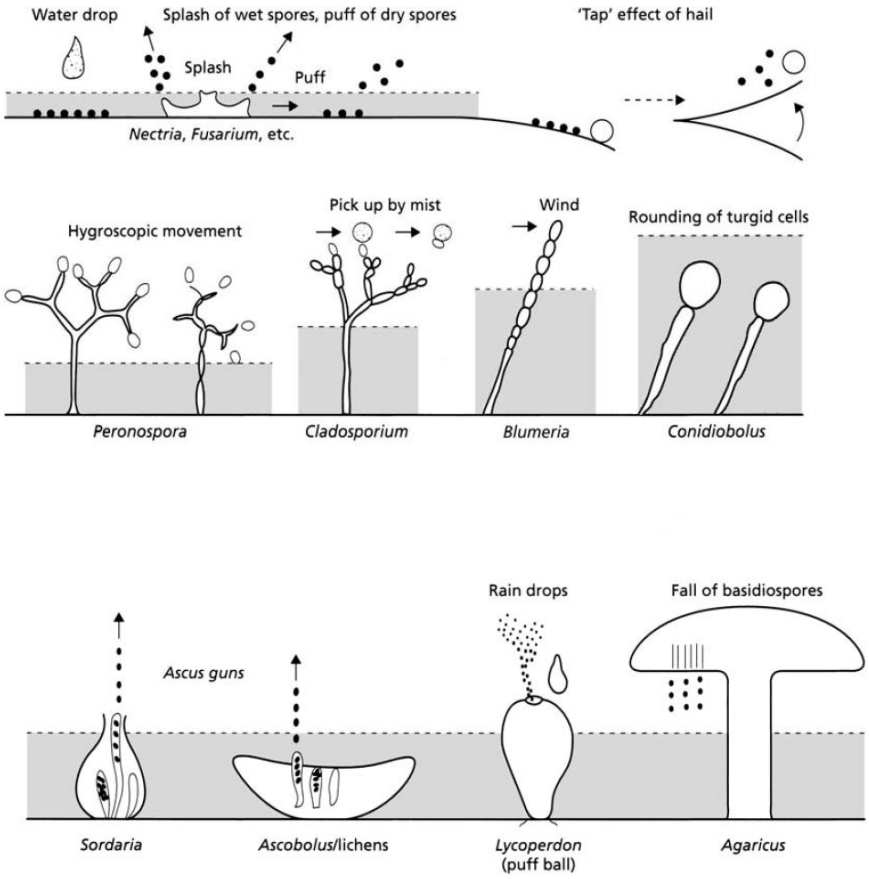

Fig. 10.23 The diversity of mechanisms of spore liberation through a boundary layer of still air (shown by shading).

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 nonwettable one.

References of this lecture

- Biology of fungi, by Jim Deacon, 4th edition, 2006, p 193 - 221.