

Chapter 10

Fungal spores, spore dormancy, and spore dispersal

This chapter is divided into the following major sections:

- general features of fungal spores
- spore dormancy and germination
- spore dispersal
- dispersal and infection behavior of zoospores
- zoospores as vectors of plant viruses
- dispersal of airborne spores
- spore sampling devices and human health

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.

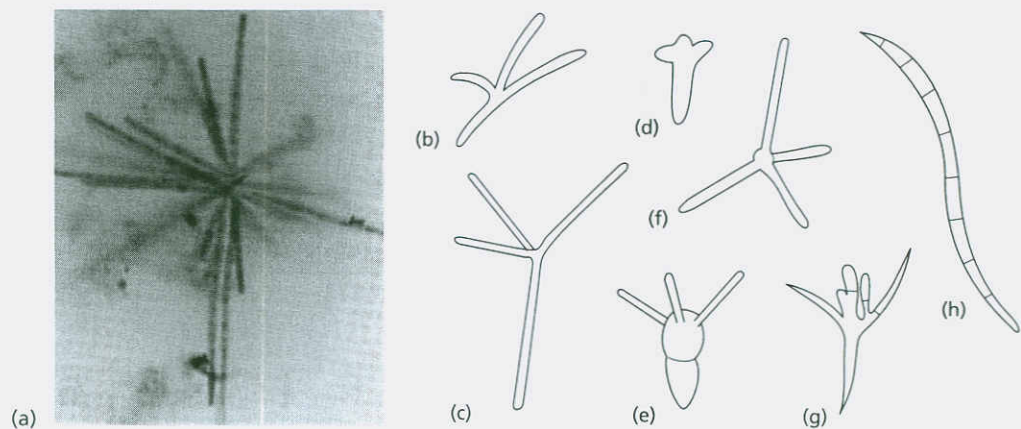


Fig. 10.1 Examples of tetra radiate, multiple-armed and sigmoid spores found in fast-flowing freshwater streams. Approximate spore lengths are shown in parentheses. (a) A single conidium of *Dendrospora* (150–200 μm); (b) conidium of *Alatospora* (30–40 μm); (c) conidium of *Tetrachaetum* (70–80 μm); (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:

- The wall is often thicker, with additional layers or additional pigments such as melanins.
- The cytoplasm is dense and some of its components (e.g. endoplasmic reticulum) are poorly developed.
- Spores have a relatively low water content, low respiration rate, and low rates of protein and nucleic acid synthesis.
- 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**

springing 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 formaldehyde. The ascospores of *Neurospora tetrasperma*

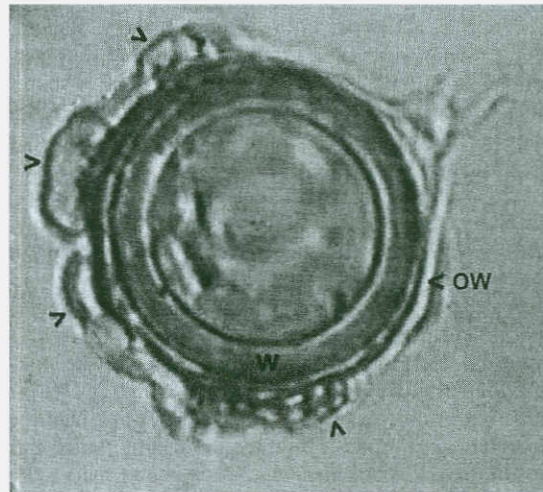


Fig. 10.2 A developing sexual spore (oospore) of *Pythium mycoparasiticum* (Oomycota). The spore has a very thick wall (w) and is contained in the outer wall (ow) of the oogonium (female reproductive cell). The arrows mark the positions of antheridia (male sex organs that fertilize the oogonium).

have been studied most thoroughly in this respect. Their dormancy cannot be explained in terms of a general permeability barrier, because they are permeable to radiolabeled oxygen, glucose, and water. Instead, their dormancy is linked to an inability to use their major storage reserve, trehalose, which is not metabolized during dormancy, but is metabolized immediately after activation. The enzyme *trehalase*, which cleaves trehalose to glucose, is found to be associated with the walls of the dormant spores, separated from its substrate. Activation somehow causes the enzyme to enter the cell, as one of the earliest detectable events in germination.

Constitutive dormancy of some other spores has been linked to endogenous inhibitors. For example, uredospores of the cereal rust fungus *Puccinia graminis* contain methyl-*cis*-ferulate, and those of bean rust, *Uromyces phaseoli*, contain methyl-*cis*-3,4-dimethoxycinnamate. Prolonged washing of spores can remove these inhibitors, and this perhaps occurs when the spores are bathed in a water film on a plant surface. At first sight it seems surprising that a spore adapted for dispersal should have an endogenous inhibitor. However, this might prevent the spores from germinating in a sporing pustule (they do not require exogenous nutrients for germination) and ensure that they germinate only after they have been dispersed.

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 (Fig. 10.8). 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 (Chapter 11). 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. Once the cause of this disease had been recognized, the problem was easily solved by abandoning the practice of burning. However this is not possible in regions where lightning-induced fires are a periodic, natural occurrence. Some of the plants in these areas have become adapted to fire – their seeds remain dormant for years until they are heat-activated. Some of the mycorrhizal fungi are similarly adapted, an example being the ascospores of the mycorrhizal *Muciturbo* spp. in Australian eucalypt forests.

Basidiospores of the common cultivated mushroom, *Agaricus bisporus*, and of several mycorrhizal fungi (see below) show constitutive dormancy, but these spores will often germinate when placed next to growing colonies of the same fungus. For example, spores of *A. bisporus* germinate in the presence of isovaleric acid and isoamyl alcohol, which may be released from the "parent" hyphae. The significance of this behavior could be to increase the gene pool of the colony.

Dormancy and germination triggers in mycorrhizal successions

Many forest trees of temperate and boreal regions (e.g. pine, oak, beech, chestnut, etc.) form mycorrhizal associations with Basidiomycota or Ascomycota (Chapter 13). These mycorrhizal fungi produce a sheath around the root tips and extend into the soil as hyphae or mycelial cords (see Fig. 7.10). Several experimental studies have shown that a succession of mycorrhizal fungi occurs on young tree seedlings that are planted in forest nurseries or in previously treeless sites. Some of these fungi establish mycorrhizas rapidly on tree seedlings from airborne spores. Classic examples of these "early (pioneer) colonizers" are *Laccaria* and *Hebeloma* spp. Other mycorrhizal fungi are slow to establish in new sites, but colonize after several years and eventually become dominant on the root systems. A classic example is the fly agaric (*Amanita muscaria*) on birch or pine trees. Figure 13.6 shows examples of some of these fungi.

These successional patterns have been studied in experimental field plots where the toadstools of mycorrhizal fungi appear above ground in autumn (Fig. 10.3) and where the range of different mycorrhizal fungi on the roots can be identified by features of the mycorrhizas themselves. When cores of soil were taken at different distances from the trees (Fig. 10.4) mycorrhizas of *Hebeloma* spp. predominated near the periphery of the tree root systems, mycorrhizas of *Lactarius* spp. predominated near the mid-zone of the root systems, and mycorrhizas of *Leccinum* (a polypore) and an unidentified mycorrhizal type predominated closest to the tree trunks. Thus, there was clear evidence of a

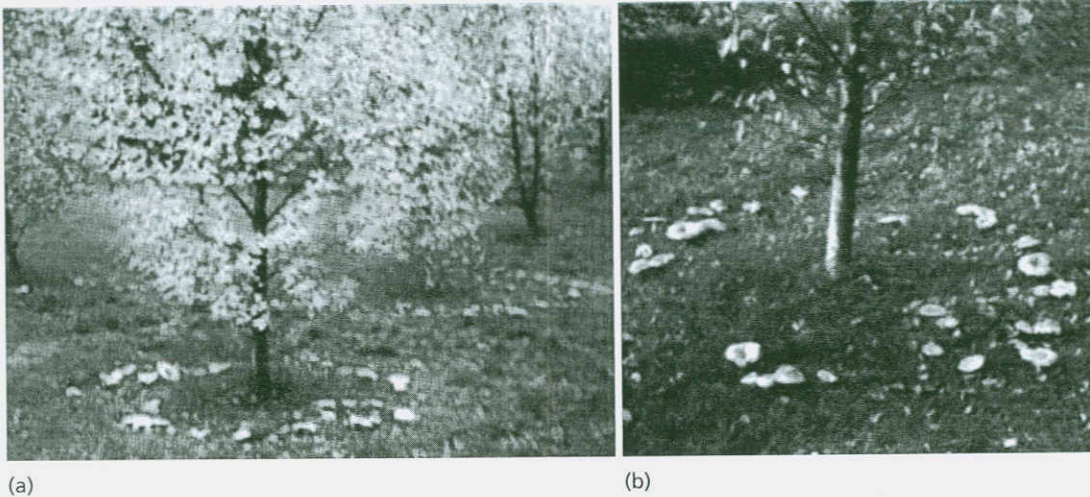


Fig. 10.3 Rings of toadstools of mycorrhizal fungi (mainly *Hebeloma* and *Lactarius* species) around young birch trees in late autumn in an experimental field plot.

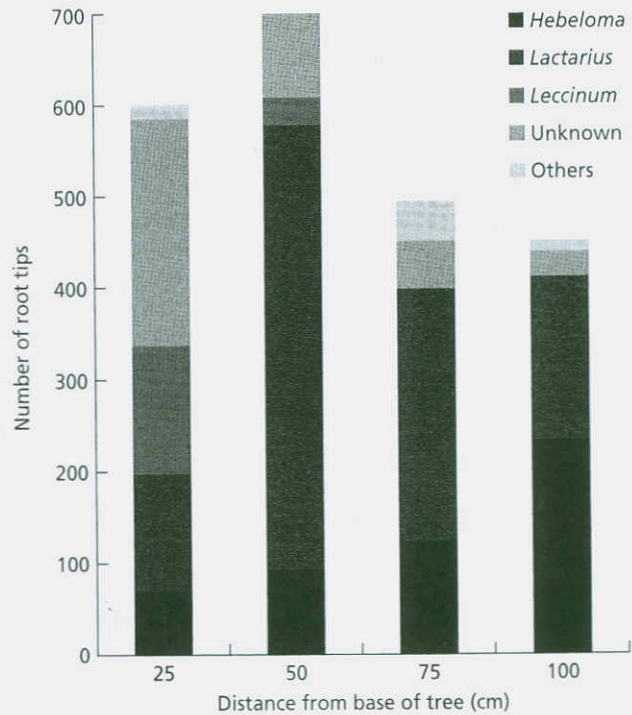


Fig. 10.4 The changing pattern of naturally occurring mycorrhizal fungi at different distances from the bases of birch trees in an experimental field plot. (Data from Deacon *et al.* 1983.)

succession, with “pioneer” mycorrhizal species colonizing the youngest parts of the root system and being replaced successively by other mycorrhizal species in the older root regions.

These distribution patterns, in which mycorrhizas of “pioneer” or “early” fungi are found at the periphery of

the root system, but are replaced by later colonizers (characteristic of older trees) near the tree base, were explained by two types of experiment.

First, basidiospores were collected from fruitbodies of either “pioneer” or “later” mycorrhizal fungi and were added to pots of nonsterile soil. Then aseptically

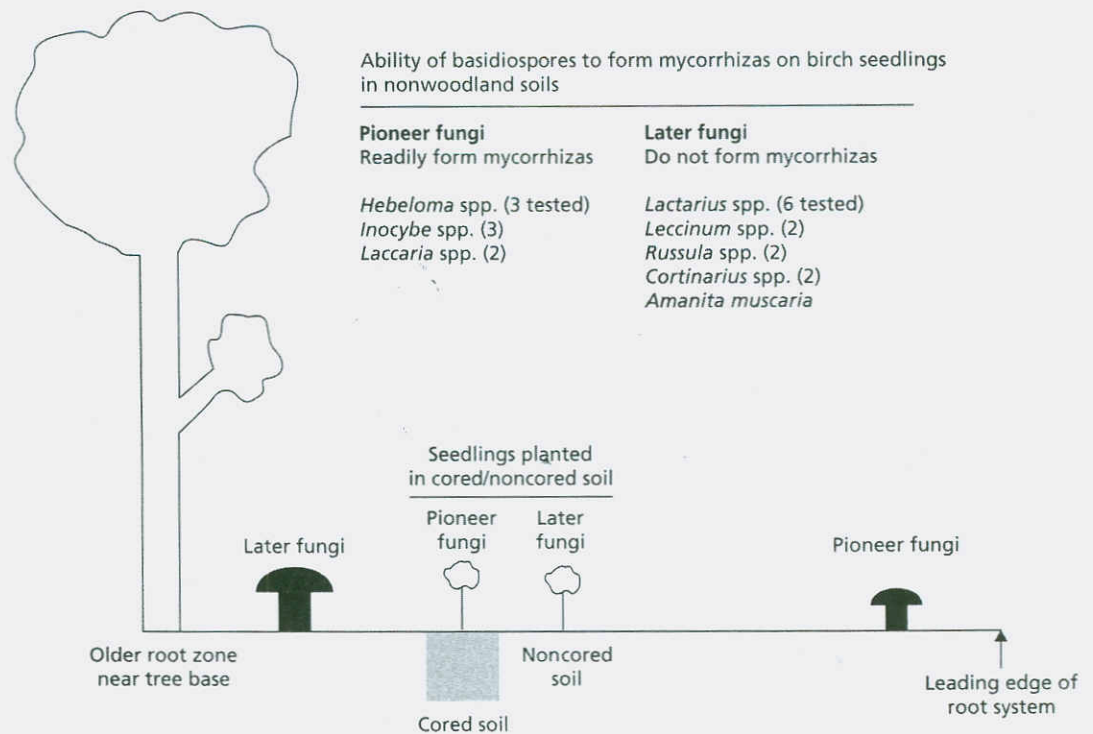


Fig. 10.5 Experimental study of mycorrhizal successions in field sites. (See Fox 1986.)

grown birch seedlings were planted in these soils. Only the pioneer fungi formed mycorrhizas in these conditions, and this was related to basidiospore germination, because only the pioneer fungi have basidiospores that germinate readily – the spores of the “later” fungi germinate extremely poorly (often less than 0.1% germination in any conditions that have been tested). Second, birch seedlings were raised aseptically (without mycorrhizas) then planted beneath older trees in a field site so that they would be infected by the fungi already established in the site. Some of these seedlings were planted directly into the undisturbed soil, but others were planted where the soil had first been removed with a corer (like the corers used for making holes in putting greens) and then replaced immediately (Fig. 10.5). All the seedlings in the cored positions became infected by pioneer fungi, presumably from spores in the soil. By contrast, all the seedlings planted in undisturbed positions were infected by the “later” fungi, presumably from hyphal networks which radiated through the soil.

So, the pattern of mycorrhizal establishment on birch in previously treeless sites can be summarized as follows. The pioneer fungi infect young seedlings in nurseries or in the field, from basidiospores that land on the soil surface and are washed into the root zone. They probably have annual cycles of infection from

basidiospores as the root system grows and expands into new soil zones. The “later” fungi cannot establish initially because their spores germinate poorly. But they germinate eventually, especially in older parts of the root zone, and become dominant by spreading as mycelial networks to infect further root tips.

In natural woodlands and forestry plantations, seedlings are likely to be infected directly by the “later” fungi, but in new sites they will initially be infected by pioneer fungi. This has practical consequences, because only the pioneer fungi are suitable for mycorrhizal inoculation programs, commonly used in land-reclamation sites. The fungi most often used for this are the puffball, *Pisolithus tinctorius*, and the toadstool-forming fungus *Paxillus involutus*. Both are pioneer colonizers that tolerate relatively high levels of toxic minerals and the low water-retention properties of mine-spoil and other land-reclamation sites.

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

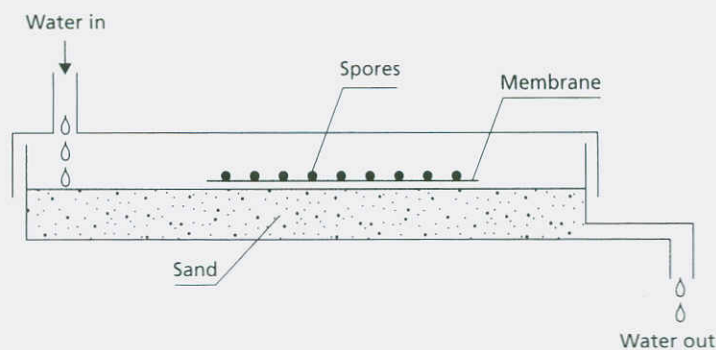


Fig. 10.6 A nutrient leaching system that mimics soil fungistasis.

nature all these spores can be held in a dormant state by the phenomenon termed **fungistasis** (or **mycostasis**). This is very common in soil (Lockwood 1977), 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.

Lockwood and his colleagues (see Hsu & Lockwood 1973) devised a simple experimental system to test whether nutrient deprivation can mimic the fungistasis observed in soil (Fig. 10.6). In completely sterile conditions, spores were placed on membrane filters over a bed of washed sand or glass beads, then sterile water was percolated slowly through the sand or beads so that any nutrients released from the spores were continuously removed. Except for the special case of “activated” (heat-treated) ascospores of *Neurospora*, which germinated in all conditions, the spores did not germinate in the “nutrient-leaching” system, but they germinated if the flow of water was stopped for 24 hours or when a flow of glucose solution was used in place of water. By using very slow rates of water percolation it was possible to simulate the fungistatic effects of natural soils. The spores of different fungi have different

fungistatic sensitivities (related to spore size, spore nutrient reserves, and speed of germination), but with few exceptions there is remarkably good agreement between the sensitivity of spores to nutrient-leaching in the model system and their sensitivity to soil fungistasis (Table 10.1).

Ecological implications of fungistasis

Fungistasis causes spores to remain quiescent 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 non-host 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. (Coley-Smith 1987). 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. Knowledge of this system has suggested some novel approaches for controlling *S. cepivorum*, but unfortunately with little success to date. One approach would be to breed

Table 10.1 Relationship between germination of fungal spores incubated on natural soil and on a nutrient-leaching system designed to mimic the continuous removal of spore nutrients by soil microorganisms. (Data from Hsu & Lockwood 1973.)

Fungus and spore type	Germination %		Leaching system	
	Distilled water	Natural soil	Water flowing	Flow stopped for 24 h
Conidia				
<i>Verticillium albo-atrum</i>	60	9	8	no data
<i>Thielaviopsis basicola</i>	89	4	5	89
<i>Fusarium culmorum</i>	94	20	9	91
<i>Curvularia lunata</i>	95	16	13	91
<i>Cochliobolus sativus</i>	97	21	19	91
<i>Alternaria tenuis</i>	95	54	71	no data
Activated ascospores				
<i>Neurospora crassa</i>	98	87	84	no data

cultivars of onions, garlic, etc. that do not produce the compounds that trigger sclerotial germination. But this is not a viable proposition because the germination triggers are important flavor and odor components of *Allium* spp. Another approach would be to trigger germination in the absence of the host crop. In this respect, artificial onion oil is used widely as a flavor component of processed foods (including cheese-and-onion flavored potato crisps) and it contains large amounts of DADS. When applied to soil, artificial onion oil triggers the germination of sclerotia, which then die by germination-lysis. Up to 95% of sclerotia can be destroyed in this way, but even the remaining few per cent can be sufficient to cause significant crop damage.

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 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.

Pilobolus, therefore exhibits three special adaptations that also are found, to different degrees, in several other coprophilous fungi (Fig. 10.8):

- 1 The spore-bearing structure is **phototropic**, an adaptation also seen in the tips of the asci of *Ascobolus* and *Sordaria*, which grow on dung.
- 2 There is an **explosive discharge** mechanism. This also is seen in the asci of *Ascobolus* and *Sordaria* because

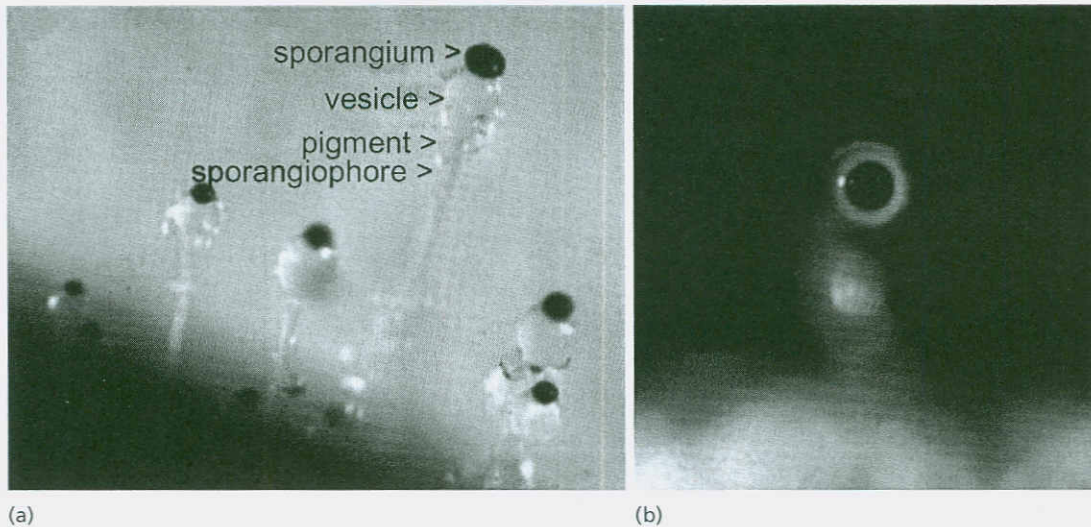


Fig. 10.7 *Pilobolus* (Zygomycota), a fungus with a ballistic method of spore discharge. (a) Several sporangia on a dung pellet. (b) A sporangium orientated towards a light source, showing how the subsporangial vesicle acts as a lens.

the asci act as guns, shooting spores up to 1–2 cm into the air. A different discharge mechanism is found in *Basidiobolus ranarum* (Chytridiomycota) which grows on the feces of lizards and frogs. In this case the sporangium is mounted on a subsporangial vesicle, as in *Pilobolus*, but the vesicle ruptures at its base, squirting the sap backwards and propelling the sporangium forwards, like a rocket. Yet another variation is found in *Sphaerobolus stellatus* (Basidiomycota) which produces basidiospores in a large ball-like structure within a cup-shaped fruitbody. At maturity, the inner layer of the cup separates from the outer layer and suddenly inverts, like a trampoline, springing the spore mass into the air.

- 3 There is a **large projectile**, based on the ballistic principle that large (heavy) objects travel further than small objects if released at the same initial velocity. *Sphaerobolus stellatus* has a spore mass about 1 mm diameter, allowing it to be thrown 2 meters vertically.

However not all coprophilous fungi employ ballistic mechanisms of spore discharge. In *Pilaira* (Zygomycota, Fig. 10.8), which is closely related to *Pilobolus* in the family Pilobolaceae, the sporangiophore merely extends several centimeters at maturity so that the sporangium collapses onto the surrounding vegetation.

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. We deal with other mutualistic associations in Chapter 13.

Dutch elm disease

Dutch elm disease (Figs 10.9, 10.10) 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 yeast-like 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

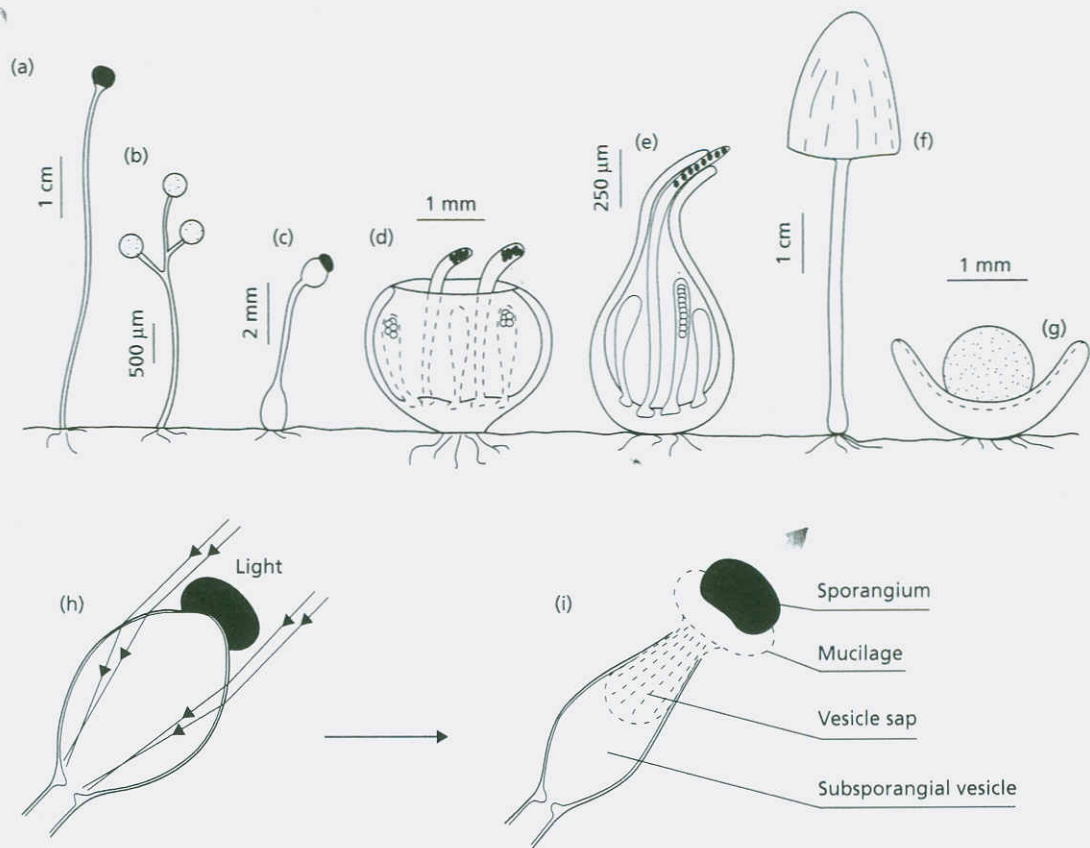


Fig. 10.8 Diagrammatic representation of some coprophilous fungi. (a) *Pilaira anomala* (Zygomycota): the sporangiophore elongates to several centimeters at maturity and the spores “flop” onto the surrounding vegetation. (b) *Mucor racemosus* (Zygomycota) with no special method of spore release. (c) *Pilobolus* (Zygomycota); see also (h) and (i). (d) *Ascobolus* sp. (Ascomycota); the tips of the mature asci project from the apothecium and are phototropic, shooting the ascospores towards a light source. (e) *Sordaria* sp. (Ascomycota); the neck of the perithecialium is phototropic and the mature asci elongate up the neck to discharge the ascospores. (f) *Coprinus* sp. (Basidiomycota). (g) *Sphaerobolus* sp. (Basidiomycota); the large spore mass is shot from the cup-shaped fruitbody when the layers of this separate and the inner layer suddenly inverts. (h,i) *Pilobolus*, showing how the terminal vesicle of the sporangiophore acts as a lens to focus light and orientate the sporangiophore, and showing also the mechanism of sporangium discharge (see also Fig. 10.7).

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.

The fungus–vector relationship clearly benefits both partners, because the beetle carries the fungus to a new host, while the beetle is ensured of a fresh supply of breeding sites in the bark of newly killed trees – it will not lay eggs in older, dead bark. As shown in Fig. 10.10b, the fungus can produce a sexual stage in the bark – a cleistothecium with a long neck which extrudes ascospores in a mucilaginous matrix. This sexual stage can ensure the generation of recombinant strains, and again the beetle is involved in this because adult beetles feed on the bark late in the season, introducing

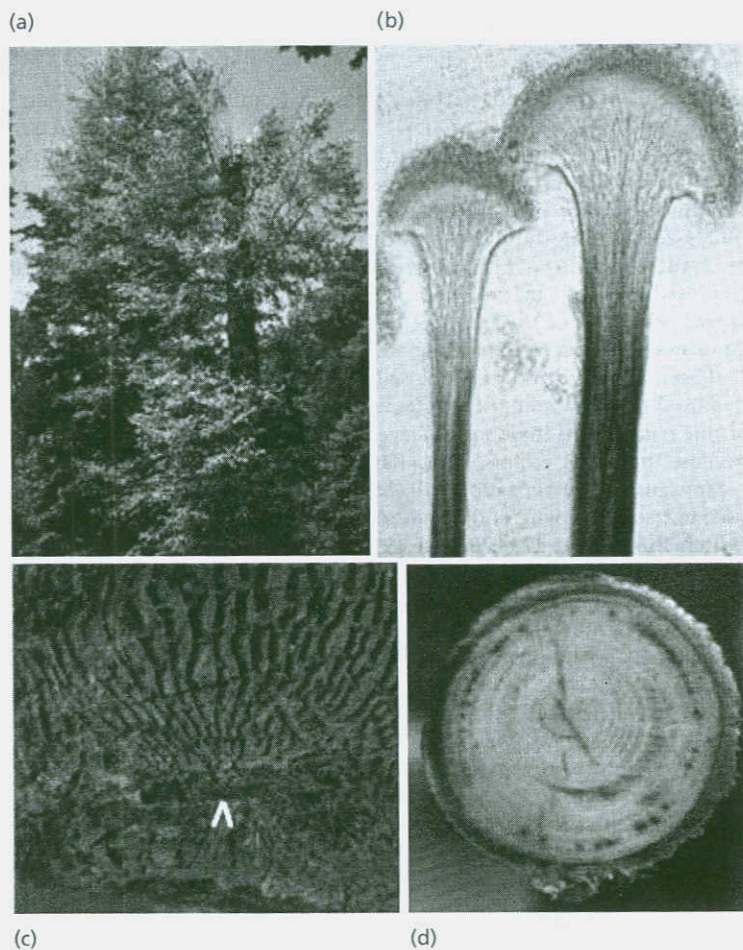


Fig. 10.9 Dutch elm disease caused by *Ophiostoma ulmi* and *O. novo-ulmi*. (a) Dying elm trees with thinning crowns. (b) Coremia of *Ophiostoma*, consisting of many aggregated conidiophores, bearing minute conidia in a large, sticky mass at the tip (much of this spore mass was lost during preparation). (c) Beetle bark galleries on the inside of the bark; the initial gallery caused by the adult female is marked, and radiating from this are galleries produced by the young larvae. (d) Section of a branch from a dying elm tree, showing a ring of blocked, discolored xylem vessels from a nonlethal attack in a previous growth season.

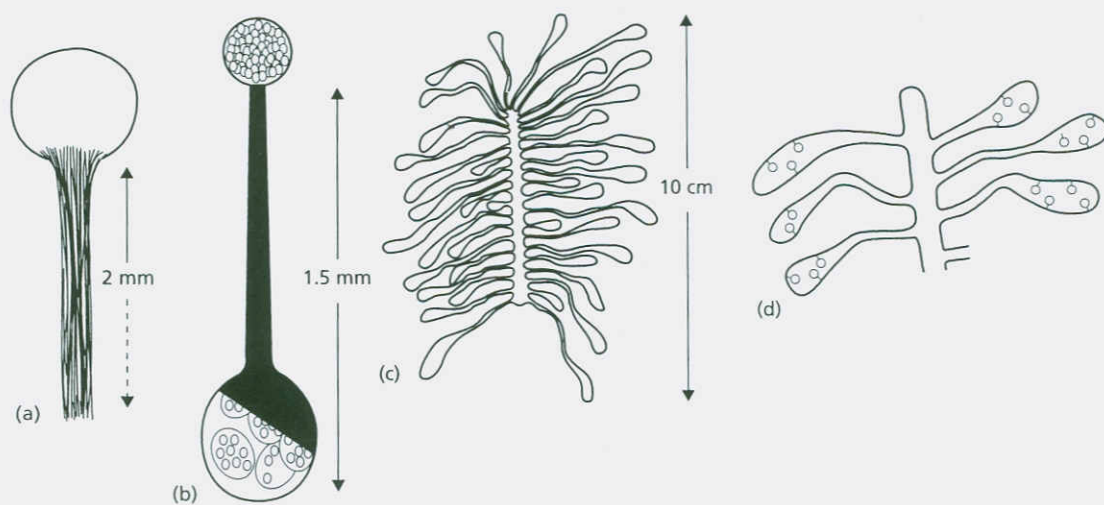


Fig. 10.10 Dutch elm disease. (a) Diagram of an intact coremium. (b) Sexual stage of *Ophiostoma* – a long-necked cleistothecium (closed fruitbody) containing oval asci each containing eight ascospores. The asci break down at maturity and the ascospores are extruded in mucilage at the tip. (c,d) Beetle brood gallery in the inner bark of a diseased tree. The adult female beetle deposits eggs along the gallery, then the emerging beetle larvae eat out a series of lateral galleries ending in chambers. The fungus sporulates in these chambers and contaminates the beetles that emerge in spring.

new strains of the pathogen, often different from the strain that killed the tree.

As testimony to the efficiency of this fungus–vector relationship, new strains of *C. novo-ulmi* swept across Britain and much of continental Europe in the late 1900s, decimating the native British elm population, after these strains were introduced on shipments of imported elm logs in the 1960s. A similar epidemic spread across the USA early in the last century. In both cases it seems that the epidemic arose from logs that had been imported and were not de-barked. If the bark had been removed from the logs (as is required by quarantine regulations) there would have been no problem because the beetle vectors would have been removed.

Molecular characterization of *Ophiostoma* has been used to trace the origins and histories of these epidemics (Mitchell & Brasier 1994; Brasier 1995). This revealed that *O. ulmi* has been present for many years in Britain and much of continental Europe, but as a heterogeneous population of nonaggressive strains comprising several vegetative compatibility (VC) groups. This population was in balance with the tree host, causing relatively little damage. The recent British epidemics have been caused by two aggressive subgroups of the fungus, one imported from North America (termed NAN) and one of Eurasian origin (EAN). These aggressive forms are sexually incompatible with the original nonaggressive population, so they have been described as a new species, *O. novo-ulmi*. At the advancing margins of the disease in Europe, the pathogen population is almost genetically pure and exists as a single VC “super group.” We could expect this from the fungus–vector relationship, because the most aggressive strain will kill most of the trees at the advancing front, and the beetle population will proliferate in these trees, carrying the strain to new trees. However, behind the disease fronts the incidence of this super group declines to only some 20–30% of the fungal population, suggesting that the population is returning to a more stable form. One of the reasons may be that *Ophiostoma*, like *Cryphonectria* discussed in Chapter 9, can harbor virulence-suppressing dsRNA. A diversity of VC groups acts as a barrier to transmission of hypovirus genes – perhaps a natural defense against these extrachromosomal elements.

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 tetra- or poly-radiate spore, often found in the fungi that grow on fallen tree leaves in well-aerated, fast-flowing streams (e.g. *Alatospora*,

Tetracladium, *Tetrachaetum*). Similar tetra- or poly-radiate spores have been found in two marine fungi (Basidiomycota), while tetra- or poly-radiate 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, that produces tetra- or poly-radiate 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).

In contrast to the freshwater fungi of Fig. 10.1, wood-rotting Ascomycota of estuarine and marine environments often have ascospores with flakes of wall material or mucilaginous appendages (Fig. 10.11). All these bizarrely shaped spores must be functionally significant, either in terms of their buoyancy or in terms of their entrapment or adherence onto substrates in aquatic environments.

Several lines of evidence suggest that the common tetra- or poly-radiate spores of freshwater fungi may serve a range of different roles. For example, the yeast *Vanrija aquatica* produces appendages in response to nutrient-poor conditions in laboratory culture, suggesting that they might increase the surface area for nutrient absorption. The tetra- or poly-radiate 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 tetra- or poly-radiate 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. A quite different role was discovered quite recently: some of the appendaged fungal spores are produced by fungi that grow on the living leaves of trees that overhang fast-flowing streams. These conidia are easily dislodged from the conidiophores by raindrops. So, the tetra- or poly-radiate or sigmoidal spore form might represent an adaptation to a multiplicity of needs in the habitats where these fungi grow.

The mucilaginous appendages of the marine Ascomycota function in attachment to surfaces. These fungi often colonize wood in estuarine environments (Moss 1986), where they have important roles as decomposers of woody materials.

Fig. 10.1 appendages of *Zaleria* with terr (40 μm).

Dispersal of zoospores

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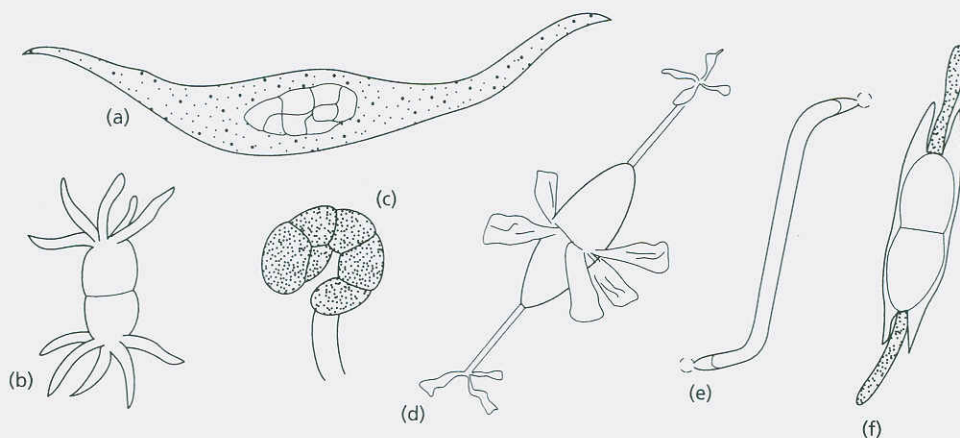


Fig. 10.11 Appendaged spores of estuarine and marine environments. (a) Ascospore of *Pleospora* with mucilaginous appendages (stippled), about 400 µm. (b) Ascospore of *Halosphaeria* with chitinous wall appendages (25 µm). (c) Conidium of *Zalerion* (25 µm). (d) Ascospore of *Corollospora* with membranous 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 Chapter 2 (see Fig. 2.2) for the nematode parasite *Catenaria anguillulae*. Other important examples include the many Oomycota (*Pythium*, *Phytophthora*, and *Aphanomyces* spp.) that cause devastating diseases of crop plants (Chapter 14) 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 zoospore fungi. Many aspects of zoospore biology and infection have been reviewed by Deacon & Donaldson (1993) and Hardham (2001).

Structure and organization of zoospores

As shown in Fig. 10.12, the zoospores of Chytridiomycota are small, typically 5–6 µm diameter, and tadpole-shaped. Except for some rumen chytrids

(which have several flagella) they have a single, smooth, posterior flagellum of the **whiplash** type. The most conspicuous feature of these zoospores is the nucleus, surmounted by a large nuclear cap, which is rich in RNA, protein, and ribosomes. The flagellar membrane is continuous with the cell membrane, and the core of the flagellum – the **axoneme** – consists of a ring of nine triplets of microtubules surrounding two central microtubules. This “9 + 2” arrangement is typical of most motile, flagellate cells. At the root of the flagellum is a **kinetosome** (a modified centriole derived from the nuclear division that preceded zoospore cleavage), and an array of tubular elements that probably provide anchorage and energy transfer to the flagellum. Surrounding the kinetosome is a large mitochondrion shaped like a doughnut ring, and a microbody–lipid complex which, presumably, supplies the energy for beating of the flagellum. When the zoospore comes to rest the flagellum is retracted into the cell by a reeling-in mechanism involving the rotation of the cell contents, then a cyst wall is formed. It is notable that, although the zoospores of Chytridiomycota are wall-less cells, they do not seem to have an osmoregulatory apparatus.

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 (Fig. 10.13) 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

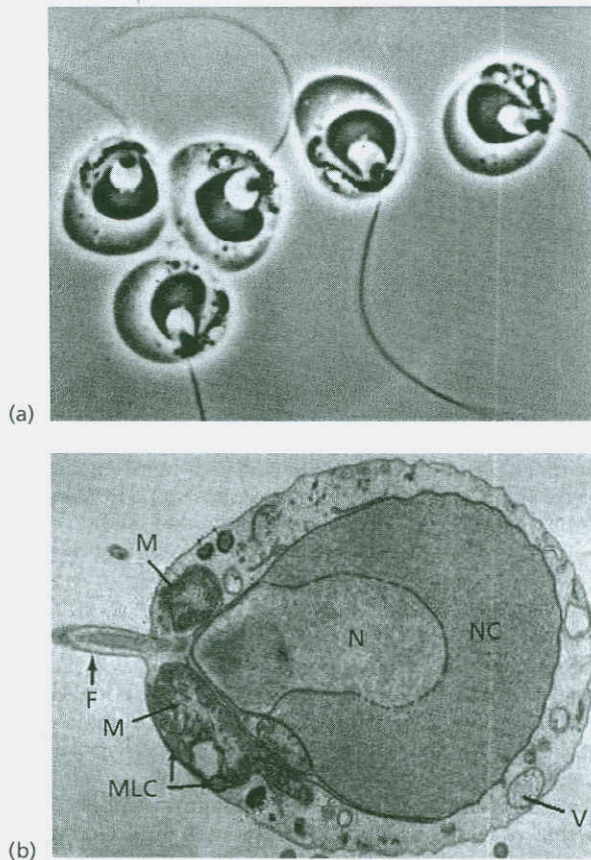


Fig. 10.12 (a) Zoospores of *Blastocladiella emersonii* (Chytridiomycota) with a smooth, posterior whiplash flagellum. (Courtesy of M. S. Fuller.) (b) Electron micrograph of a longitudinal section of the zoospore of *B. emersonii*. The zoospore plasma membrane is continuous with the flagellar membrane (F) but only part of the flagellum is seen in this section. M = mitochondrion; MLC = microbody-lipid globule complex; N = nucleus; NC = nuclear cap; V = vacuole. (Courtesy of M.S. Fuller; from Reichle & Fuller 1967.)

(mastigonemes) projecting along its length. These act like a series of oars, pulling the zoospore forwards as the anterior flagellum generates a sine wave from its base to its tip. This is estimated to account for at least 90% of the forward swimming thrust. The posterior flagellum acts like a rudder. It periodically kicks at an angle of about 90°, causing the cell to change the swimming direction.

The arrangement of organelles in zoospores of Oomycota is shown in Fig. 10.14. The top section passes through the region of flagellar insertion in the zoospore ventral groove and shows the nucleus (N)

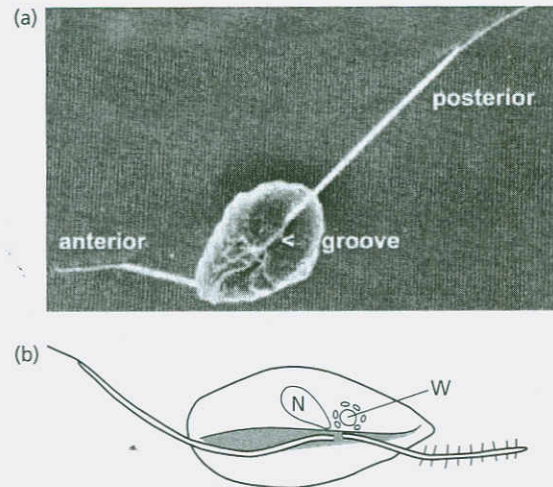


Fig. 10.13 (a) Scanning electron micrograph of a zoospore of *Phytophthora* (Oomycota) with a posterior whiplash flagellum and a shorter, anterior tinsel-type flagellum. (b) Diagrammatic representation of the zoospore, showing the insertion of the flagella in the ventral groove (shaded) and the location of the nucleus (N) and water-expulsion vacuole (W). ((a) Image courtesy of M.S. Fuller.)

extending to the base of the flagella. Beneath the plasma membrane are sheets of **peripheral cisternae** (pc), **large peripheral vesicles**, **dorsal vesicles**, and **ventral vesicles**. The cell also contains mitochondria (M) and fingerprint vacuoles (FV) which contain glucans that probably serve as carbohydrate reserves. The asterisk marks a cavity probably resulting from extraction of lipid during preparation of the specimen. The lower section passes through the ventral groove where the osmoregulatory **water expulsion vacuole** (WEV) is located. The WEV consists of a central vacuole (CT) and surrounding vacuoles (SU). It contracts and expels water regularly every few minutes.

The significance of this complex ultrastructural organization of *Phytophthora* zoospores lies in the fact that the zoospore is a transitory phase of the life cycle, specialized for dispersal. When the zoospore locates a suitable site for infection it transforms rapidly into a walled cyst – a process that takes only a few minutes. Thus, the zoospore is a pre-programmed cell, destined to undergo a rapid transition. The details of this are of much interest and can be followed by cytochemical methods.

The peripheral vesicles that lie just beneath the zoospore membrane are of at least three types, and can be distinguished by the binding of their proteinaceous contents to specific monoclonal antibodies or lectins. The **large peripheral vesicles** beneath most of the cell

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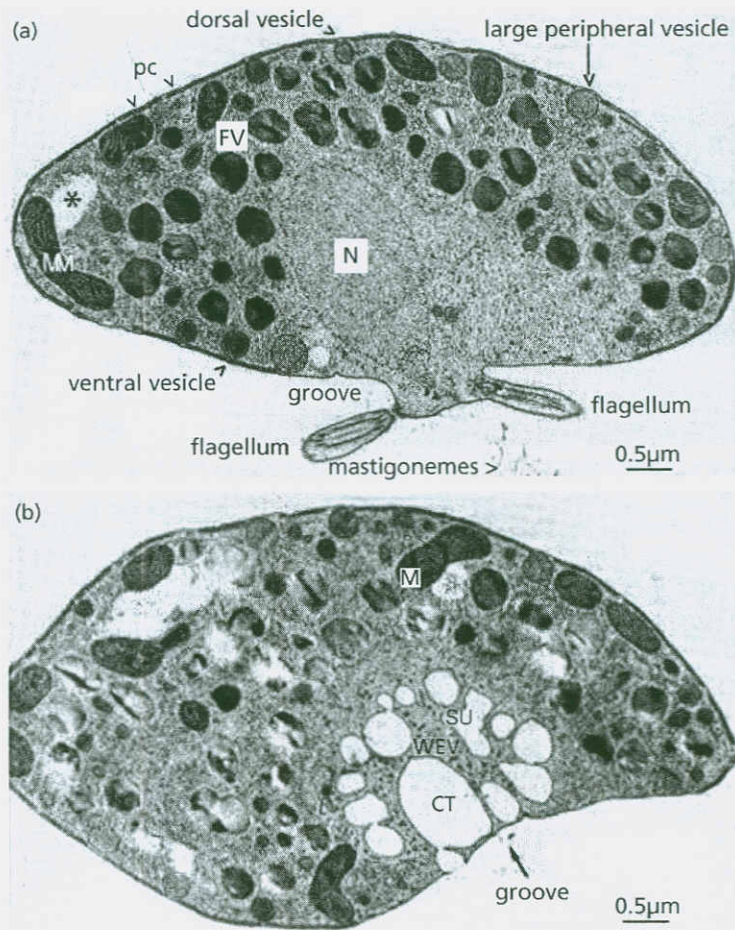


Fig. 10.14 (a,b) Two parallel cross-sections through a zoospore of *Phytophthora palmivora* (Oomycota). See text for details. (Courtesy of M.S. Fuller; from Cho & Fuller 1989.)

periphery contain a glycoprotein that is thought to serve as a protein store after encystment. These vesicles migrate towards the center of the cell when a zoospore encysts. The smaller dorsal vesicles contain a different glycoprotein which is released by exocytosis at an early stage of encystment, and it accumulates on the cell surface as a cyst coat. A third class of vesicles, the ventral vesicles, are found only around the zoospore ventral groove. They also contain a protein which is released during encystment, but it is deposited locally between the cyst and the surface on which encystment has occurred, and it acts as an adhesive.

A newly encysted zoospore has no wall, only an amorphous glycoprotein coat deposited by the dorsal vesicles. But a true wall is synthesized beneath the cyst coat in the first few minutes of encystment. This wall is derived from the peripheral cisternae (membrane lamellae) that lie immediately beneath the plasma membrane of the motile spore (Fig. 10.15). Once the

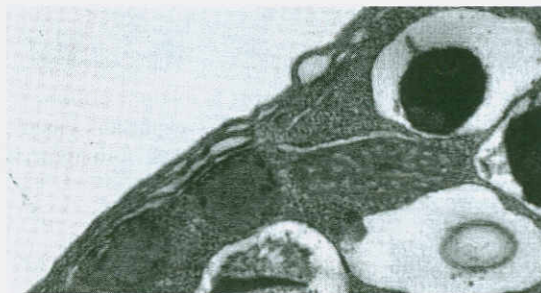


Fig. 10.15 Close-up view of a part of the zoospore surface of *Pythium aphanidermatum*, showing the laminate peripheral cisternae that will subsequently become vesicles for release of the cyst wall.

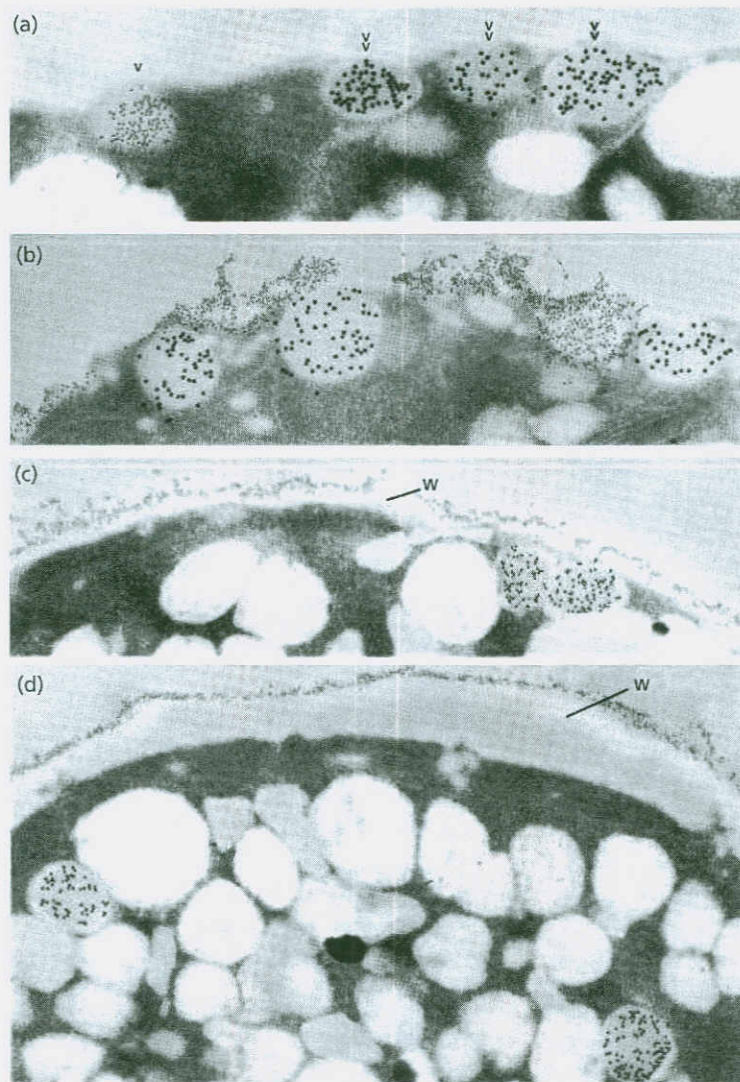


Fig. 10.16 Stages in encystment of *Phytophthora cinnamomi* zoospores (Oomycota) immunogold-labeled with monoclonal antibodies (mAbs) specific to the contents of different peripheral vesicles. (a) Outer region of a motile zoospore showing two classes of peripheral vesicles: the contents of large peripheral vesicles bind to a specific mAb that has been complexed to large (18 nm) gold particles (double arrowheads) whereas the contents of small dorsal vesicles bind to a different mAb complexed to 10 nm gold particles (single arrowhead). (b) Zoospore labeled at 1 minute after addition of pectin to induce encystment. Most of the small dorsal vesicles have released their contents onto the cell surface but the large peripheral vesicles still lie beneath the surface. (c) Spore labeled at 5 minutes after encystment. The spore has started to synthesize a cell wall (W), the surface of which is coated with material from the small dorsal vesicles. (d) Spore labeled after 10 minutes. The wall is now thicker, and the large peripheral vesicles have migrated towards the center of the cyst. They are thought to be a protein store for use during cyst germination and germ-tube growth. (Photographs courtesy of F. Gubler & A. Hardham; (a) from Hardham 1995; (b–d) from Gubler & Hardham 1988.)

cyst wall has developed, the water-expulsion vacuole disappears. The sequence of cyst wall formation is shown in Fig. 10.16.

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 pro-

duced 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 (Ron & Rosenberg 2001).

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 (see Fig. 9.12), and it naturally fluoresces bright blue when viewed under ultraviolet illumination (Fig. 10.17). 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

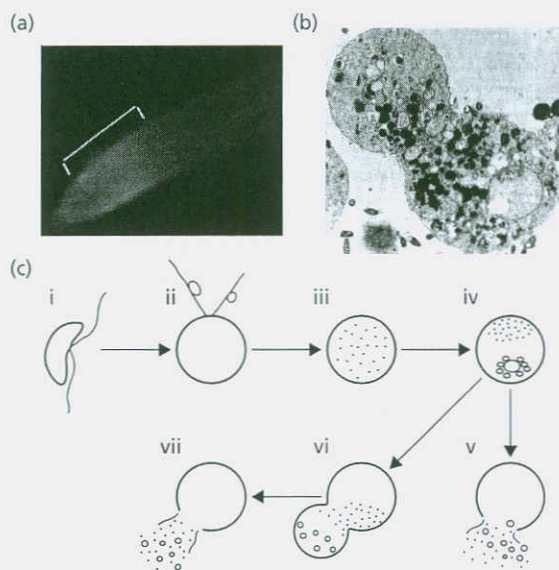


Fig. 10.17 (a) Oat root tips with natural blue autofluorescence caused by the presence of avenacin. (b) A *Pythium* zoospore undergoing disorganization and lysis in the presence of avenacin. (c) Responses of wall-less zoospores of Oomycota to saponins such as avenacin or β -aescin: (i) motile zoospore; (ii) immobilization and rounding-up; (iii) development of phase-dark granules; (iv) localization of granules and development of vacuoles; (v) lysis; (vi,vii) ballooning followed by lysis. Disruption of the zoospores usually occurs within 5–10 minutes. (From Deacon & Mitchell 1985.)

accumulate at the root tips in response to root tip nutrients and then lyse within a few minutes (Fig. 10.17).

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 $100 \mu\text{m s}^{-1}$ 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 (Fig. 10.18), and because of this the rate of dispersal 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.

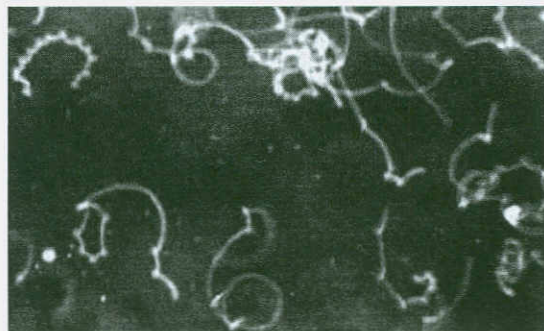


Fig. 10.18 Zoospore tracks of Oomycota, captured as negatives on photographic film during a 5-second exposure and showing frequent random turns in the absence of an attractant.

Clearly, the swimming activity of zoospores must serve other roles. One of these roles is that swimming zoospores can remain in suspension and be carried in moving water, whereas nonmotile spores tend to settle out. This has been demonstrated both in artificial soils and in field soils, where zoospores of Oomycota can escape entrapment in narrow, water-filled soil pores, so they can remain suspended and spread in surface run-off water, whereas zoospore cysts are easily trapped in soil. But the main role of zoospores is that their swimming is linked to sensory perception: they can swim towards attractants such as nutrients or oxygen (**positive chemotaxis**) or avoid unsuitable chemical environments (**negative chemotaxis**). Zoospores also can respond to pH gradients, to electrical or ionic fields (**electrotaxis**), and they can accumulate by autoaggregation. The extreme responsiveness of zoospores enables them to settle and encyst in environments that are most appropriate for subsequent development. For example, zoospores often accumulate in large numbers near root tips, at plant wound sites, or around individual stomata on a leaf surface. This “homing and docking” sequence of zoospores, discussed below, is as sophisticated and rapid as any that have been described in the biological world.

The “homing and docking” sequence of zoospores

The events in the “homing and docking” sequence of zoospores (Fig. 10.19) have been studied in detail for *Pythium* and *Phytophthora* species. The sequence begins when a zoospore detects a gradient of chemoattractant, which causes a partial suppression of random turns so that the spore tends to move up the attractant gradient. This phase of zoospore taxis, or zoospore kinesis, can

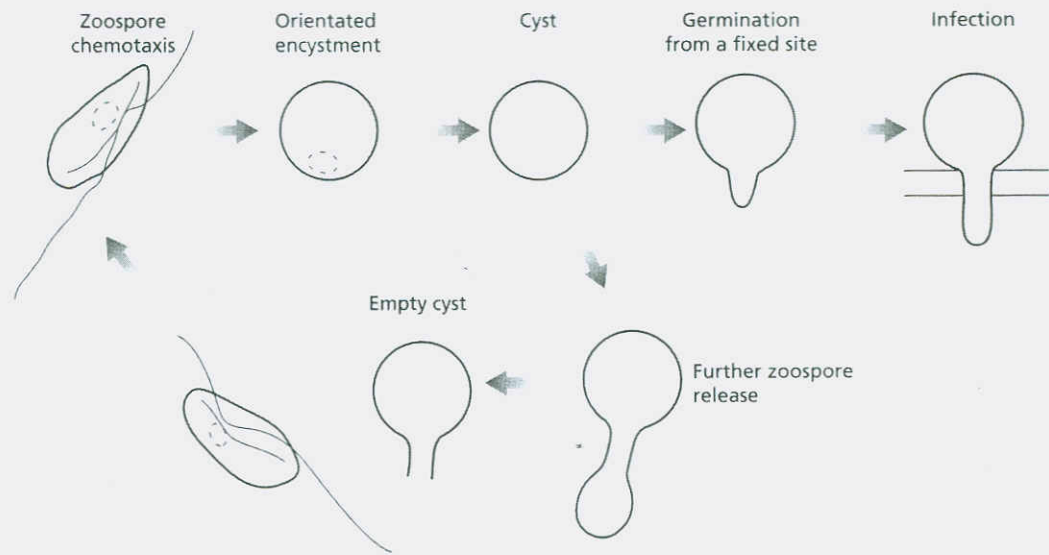


Fig. 10.19 The homing and docking sequence of zoospores.

be studied using capillaries filled with potential attractants. Often the zoospores of plant pathogens show chemotaxis to several individual sugars or amino acids, or to volatile compounds such as ethanol and aldehydes, which are likely to be released as fermentation products of roots in moist soil conditions. However, the strongest responses are usually seen with mixtures of compounds, such as seed and root exudates.

Most *Pythium* and *Phytophthora* species show taxis to the roots of both host and nonhost plants, but a few interesting examples of host-specific taxis have been reported. For example, the host-specific pathogen *Phytophthora sojae* shows chemotaxis *in vitro* to the flavonoids daidzein and genistein, which are known to be present in the soybean host. Similarly, *Aphanomyces cochlioides* shows strong chemotaxis to the flavonoid "cochliophilin A" from spinach plants. It should not be assumed that these compounds are the only factors involved in attraction to the host roots, but the

findings are notable because they parallel the behavior of *Rhizobium* spp., which show *in vitro* chemotaxis to the specific flavonoids of their hosts.

The next phase of the sequence seems to involve **recognition of a host surface component**, because the zoospores move over the host surface, with their flagella in contact with this. Some zoosporic fungi might have specific host surface requirements, but often they respond to pectin and other polyuronates (e.g. alginate) *in vitro*. For example, Fig. 10.20 shows the massing and encystment of *Pythium* zoospores on the root tip mucilage secreted by root cap cells of wheat. Similar massing and encystment is seen in the elongation zone of roots that are coated with calcium alginate gel – a treatment that masks any specific receptors on the root surface.

Recognition of a host surface component (perhaps coupled with a high concentration of root-derived nutrients) leads to **orientated encystment**, with the

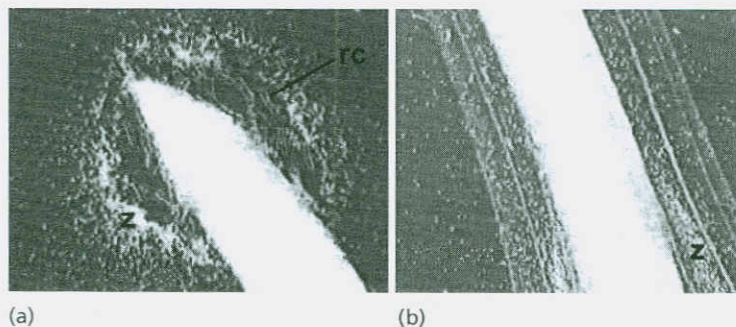


Fig. 10.20 Accumulation and encystment of *Pythium* zoospores on wheat roots. (a) Zoospores (z) accumulating in large numbers on the surface of a ball of root tip mucilage; some root cap cells are indicated (rc). (b) Zoospores (z) accumulating and encysting in the elongation zone of wheat roots. In this case the root had been coated with a double layer of calcium alginate gel to block any specific receptor compounds on the root surface.

ventral groove of the zoospore located next to the host. During this process the flagella are withdrawn but the zoospore remains adhered to the host by secretion of an adhesive. This stage of orientated encystment is important, because the zoospores of *Pythium* and *Phytophthora* spp. are known to have a fixed, predetermined point of germ-tube outgrowth. If the spore were to settle in a different orientation the germ-tube would not penetrate the host. There can also be a degree of host-specific encystment at this stage, because the *Pythium* spp. that parasitize grasses and cereals show significantly more encystment on grass roots than on the roots of dicotyledonous plants.

The precise orientation of encystment is also important because it ensures that the adhesive released from the zoospore's ventral vesicles will be deposited next

to the host surface. Receptors on the flagella probably are involved in this process, because monoclonal antibodies that bind to both flagella of *Phytophthora* zoospores cause rapid encystment *in vitro*, whereas other monoclonal antibodies that bind to only the anterior or the posterior flagellum do not cause rapid encystment.

Cyst germination can be studied *in vitro* by agitating a zoospore suspension, causing the spores to encyst, and then adding individual amino acids or sugars to trigger germination. However, cyst germination *in vivo* (on the host plant) is suggested to be an autonomous process, triggered by events in the earliest stages of encystment. Consistent with this, major transmembrane fluxes of calcium occur in the early stages of encystment, and seem to coordinate cyst germination (Fig. 10.21).

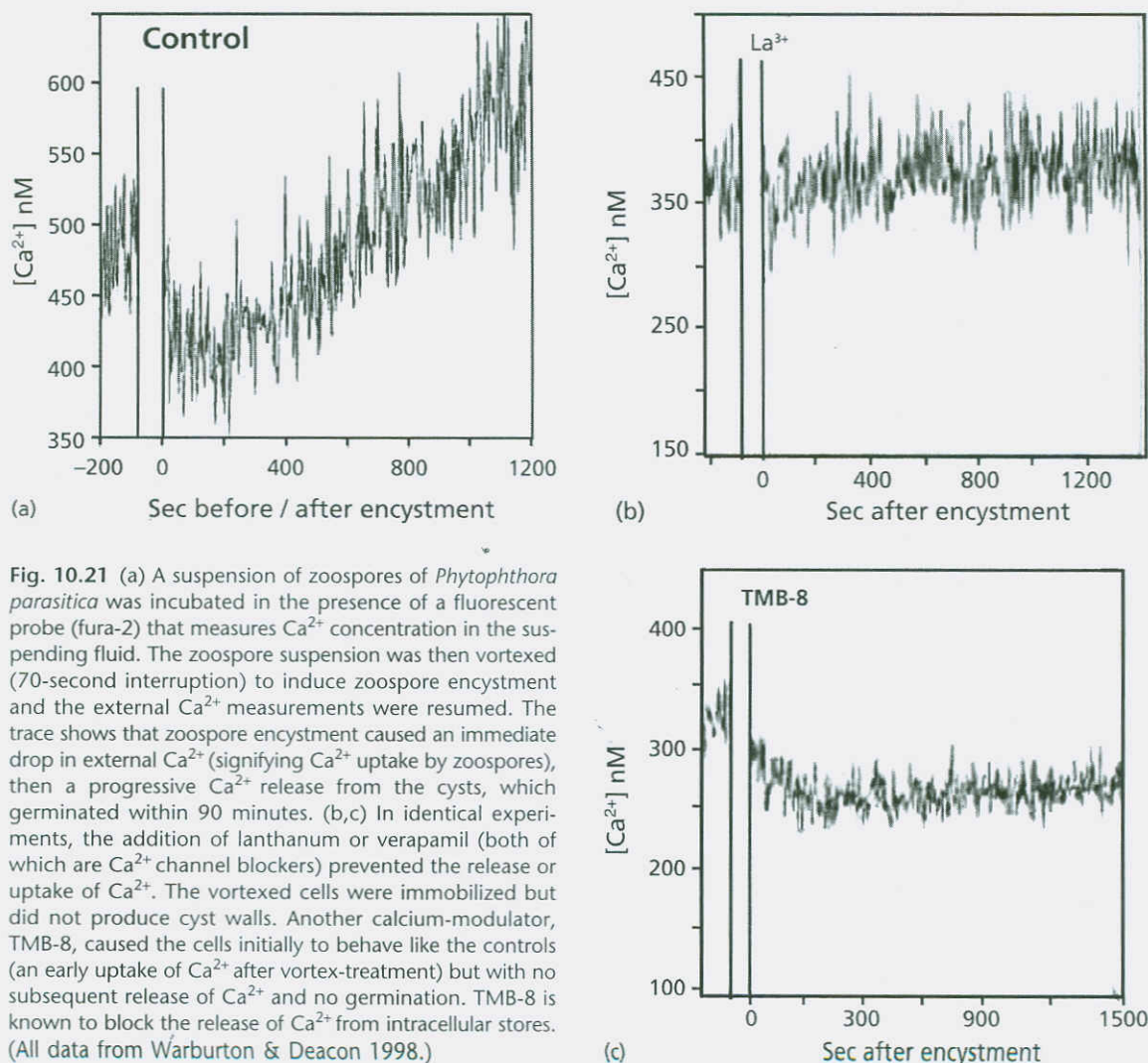


Fig. 10.21 (a) A suspension of zoospores of *Phytophthora parasitica* was incubated in the presence of a fluorescent probe (fura-2) that measures Ca²⁺ concentration in the suspending fluid. The zoospore suspension was then vortexed (70-second interruption) to induce zoospore encystment and the external Ca²⁺ measurements were resumed. The trace shows that zoospore encystment caused an immediate drop in external Ca²⁺ (signifying Ca²⁺ uptake by zoospores), then a progressive Ca²⁺ release from the cysts, which germinated within 90 minutes. (b,c) In identical experiments, the addition of lanthanum or verapamil (both of which are Ca²⁺ channel blockers) prevented the release or uptake of Ca²⁺. The vortexed cells were immobilized but did not produce cyst walls. Another calcium-modulator, TMB-8, caused the cells initially to behave like the controls (an early uptake of Ca²⁺ after vortex-treatment) but with no subsequent release of Ca²⁺ and no germination. TMB-8 is known to block the release of Ca²⁺ from intracellular stores. (All data from Warburton & Deacon 1998.)

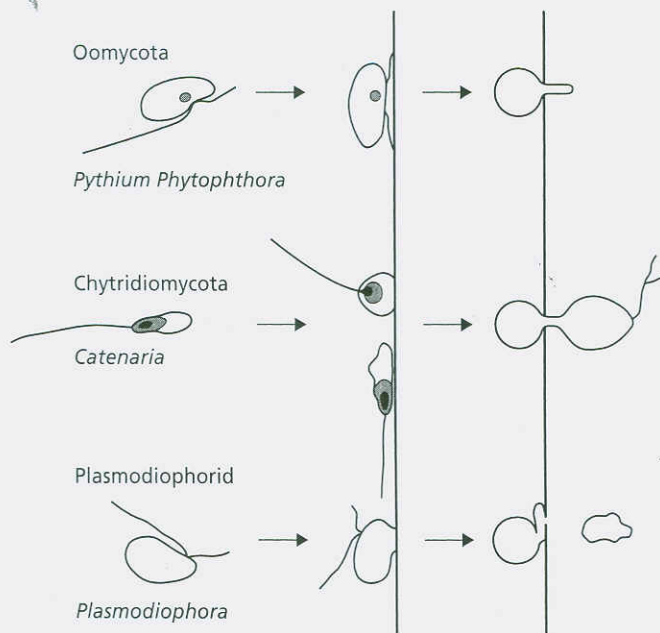


Fig. 10.22 Comparison of the docking and penetration of a host by three types of zoospore of fungus (see text for details). In all three types of organism the zoospore shows precise orientation during encystment on a host surface, related to the fact that these zoospores have a predetermined site from which they penetrate the host.

Collectively, the results in Fig. 10.21 demonstrate a central role of Ca^{2+} uptake and subsequent release in the transition from a zoospore to a germinating cyst.

In normal conditions, when zoospores encyst on a host surface they germinate within 20 or 30 minutes by the emergence of a germ-tube, which usually penetrates the host directly. However, the zoospores of *Pythium* and *Phytophthora* also have a default option. If a zoospore does not locate a suitable host after several hours of swimming, it encysts before its nutrient reserves are depleted and the cyst then releases a further zoospore (Fig. 10.19). This process of repeated emergence can occur two or three times before the zoospore exhausts its nutrient reserves.

Parallel development among zoospore species

Many of the features described above for Oomycota are also found in other zoospore organisms, but the details vary (Fig. 10.22). For example, zoospores of Oomycota always dock onto a host or other surface with the flagella and ventral groove located next to the surface. The Chytridiomycota always seem to dock "head-on," with the flagellum orientated away from the host. Plasmodiophorids show yet another variation: their zoospores always seem to dock with the two flagella orientated away from the host. But these obligately parasitic organisms also display a unique mode of behavior. When the zoospore settles and encysts on a

host surface, the cyst vacuole enlarges and a small **adhesorium** is produced at the site of contact with the host. Then a pre-formed bullet-like **stylet** is shot through the host wall and the contents of the cyst enter the host cell as a wall-less **plasmodium**. Again, this provides evidence of precisely orientated encystment, because the stylet must be positioned correctly to ensure that the protoplast will be delivered through the host cell wall.

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 (Table 10.2). 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.

There are different degrees of specialization in these virus-vector relationships. *Olpidium* spp. usually transmit isometric viruses such as cucumber necrosis virus and tobacco necrosis virus, but these viruses have additional (and perhaps more important) modes of transmission. The swimming zoospores acquire these viruses from soil when the virus particles adhere to a

Table 10.2 Some important viruses that are vectored by zoosporic fungi.

<i>Virus type and features</i>	<i>Examples</i>	<i>Host</i>	<i>Vector</i>
Furoviruses: always vectored by fungi			
Straight tubular particles, 250–300 + 100–150 × 20 nm	Soil-borne wheat mosaic	Wheat, barley	<i>Polymyxa graminis</i>
	Beet necrotic yellow vein	Sugar beet, spinach	<i>Polymyxa betae</i>
Single-stranded RNA; genome divided between more than one particle	Potato mop top	<i>Solanum</i> spp.	<i>Spongospora subterranea</i>
	Peanut clump	Peanut	<i>Polymyxa graminis</i>
	Oat golden stripe	<i>Avena</i> spp.	<i>Polymyxa graminis</i>
	Broad bean necrosis	<i>Vicia faba</i>	<i>Polymyxa graminis</i>
Barley yellow mosaic type: always vectored by fungi			
Filamentous particles, 350–700 × 13 nm	Barley yellow mosaic	<i>Hordeum</i>	<i>Polymyxa graminis</i>
	Wheat yellow mosaic	<i>Triticum</i>	<i>Polymyxa graminis</i>
	Wheat spindle streak	<i>Triticum</i>	<i>Polymyxa graminis</i>
	Oat mosaic	<i>Avena</i>	<i>Polymyxa graminis</i>
	Rice necrosis mosaic	<i>Oryza</i>	<i>Polymyxa graminis</i>
Tobacco stunt type: characteristically vectored by fungi			
Straight tubular particles, 200–375 × 22 nm	Tobacco stunt	<i>Nicotiana</i> spp.	<i>Olpidium brassicae</i>
	Lettuce big vein	Lettuce	<i>Olpidium brassicae</i>
Double-stranded RNA			
Tobacco necrosis type: various means of transmission, including fungi			
Isometric particles, 26–30 nm	Tobacco necrosis	Tulip, <i>Solanum</i> , <i>Vicia</i> , many other plants	<i>Olpidium brassicae</i>
Single-stranded RNA	Cucumber necrosis	Cucumber	<i>Olpidium</i> spp.
	Melon necrotic spot	Melon, cucumber	<i>Olpidium radicale</i>

zoospore surface component. The virus presumably remains on the plasma membrane when the zoospore encysts, and will later be carried on the membrane of the protoplast that enters the host. By contrast, the zoospores of *Polymyxa* and *Spongospora* cannot acquire viruses by swimming in virus suspensions; instead, they acquire the virus inside an infected plant. Many of these viruses are of a distinct type, termed **furoviruses** (fungally-transmitted rod-shaped viruses), and they have no other natural means of transmission. They include some of the most economically important soil-borne viruses, such as beet necrotic yellow vein virus, soil-borne wheat mosaic virus, and potato mop-top virus. Other fungus-transmitted viruses, such as the filamentous types (Table 10.2), show some affinities to furoviruses but have a different particle shape.

There is no evidence that any of these viruses multiply within the vectors. But they are carried internally in the resting spores, which can persist in soil for 20

years or more. So, once established in a field site, these viruses are almost impossible to eradicate. Further details of the zoospore-vectored viruses can be found in Adams (1991), Hiruki & Teakle (1987), and Brunt & Richards (1989).

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. The subject is covered in detail by Ingold (1971) and Gregory (1973).

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

shown in Fig. 10.23. Often they 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*). Fungi that grow on

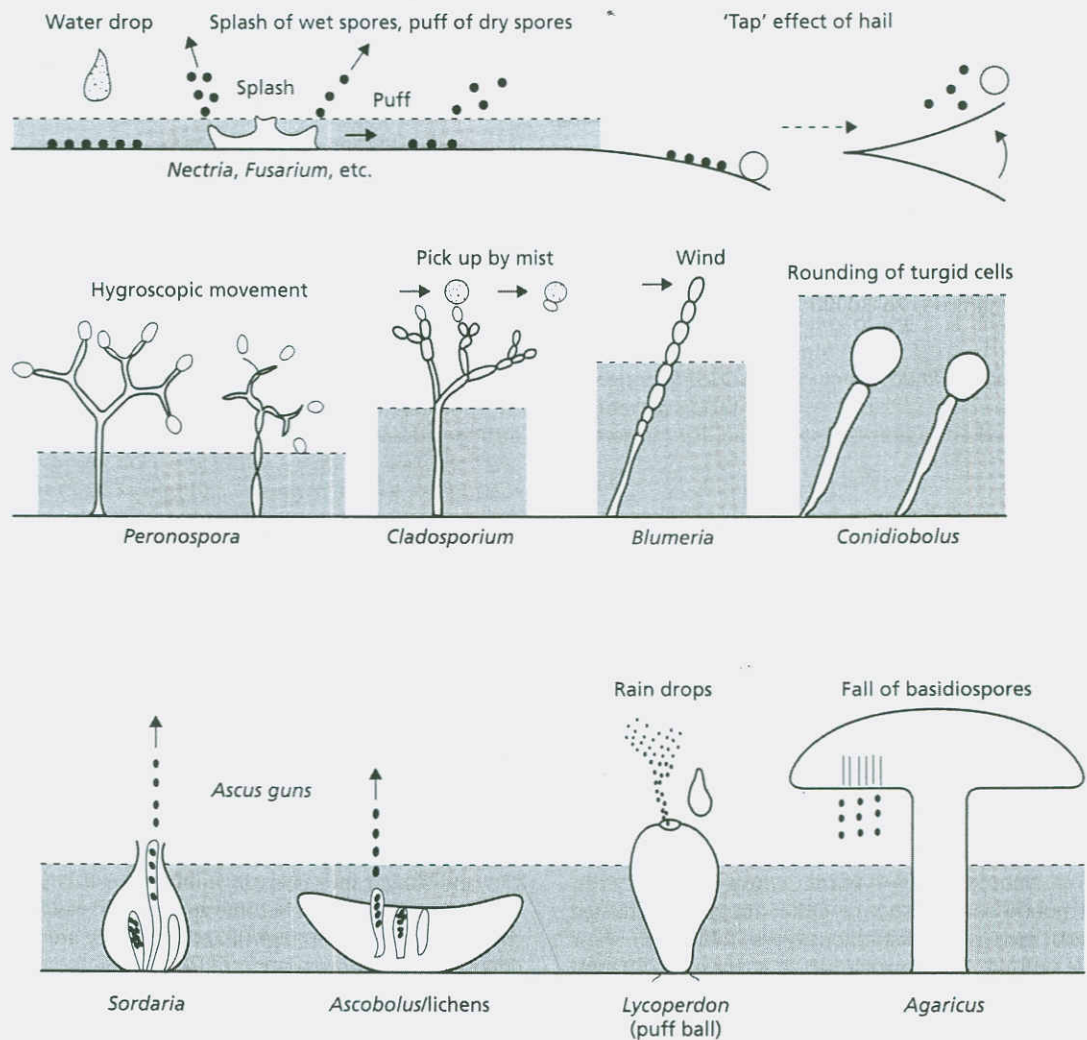


Fig. 10.23 The diversity of mechanisms of spore liberation through a boundary layer of still air (shown by shading).

more rigidly supported surfaces can release the spores by active processes. For example, the asci of many Ascomycota function as small guns, shooting ascospores to 1 or 2 cm distance, to break free of the boundary layer. Spores can also "pop" from a supporting structure when an enclosing wall layer suddenly ruptures, like two balloons that pop apart if compressed and suddenly released (e.g. *Conidiobolus*).

Some other fungi are dispersed by rain-splash. In these cases the spores often are linear or curved (e.g. *Fusarium*, Fig. 10.24) and are produced in mucilage on a pad of tissue (an acervulus) or in a splash cup so that raindrops are caused to fragment on impact and rebound as many tiny droplets which can carry the spores into the air. Splash cups are also found in a group of Basidiomycota that grow on decomposing wood chips, on the surface of organic soils or on dung. A good example is the genus *Cyathus* (see Fig. 2.21).

Raindrops or hail can also release dry spores that are lying on a surface, by "puff" and "tap" mechanisms.

When a raindrop falls on a rigidly supported surface, the drop splodges sideways, and the resulting puff of air disturbs the boundary layer, causing the dry spores to become airborne. Hailstones act differently – they are most effective in redistributing spores from lightly supported surfaces such as leaves. In puffballs (Basidiomycota) such as *Lycoperdon* (see Fig. 2.23) the mature basidiospores are enclosed in a papery fruitbody with an apical pore, so that raindrops "puff" the spores into the air, like bellows.

Toadstools display a different strategy from all those above, made necessary by the deep layer of still air that often exists on a woodland floor. When the stalk (stipe) of a toadstool elongates, the cap (pileus) projects into turbulent air. The basidiospores develop on short stalks (sterigmata) and the spores are popped from the sterigmata when the continuous outer wall surrounding the sterigma and the spore breaks down, so that the spores can drop from the gills or pores and be carried away by wind. Much of the variation in shape and



Fig. 10.24 Curved spores (macroconidia) with several compartments, typical of many species of *Fusarium* and other splash-dispersed fungi.

size of toadstools is related to this strategy – the toadstools with thick, rigid stipes (e.g. *Boletus*, *Amanita*) and the large brackets produced by tree-rotting fungi (e.g. *Ganoderma*) have very closely spaced and deep gills or pores, just wide enough for spores to be popped from the basidia and then to fall vertically into turbulent air. Toadstools with thin, bendable stipes (e.g. *Marasmius oreades*, the common fairy-ring fungus of grass turf) have widely spaced, shallow gills to ensure that the spores fall free. Many of these strategies are illustrated in Chapter 2 (see Figs 2.24–2.32).

This is more than just a catalogue of examples, because it demonstrates how fungi have an **integrated lifestyle**. The only reason for producing a fruitbody is to disperse the microscopic spores, and the only reason for producing a large or massive fruitbody is to overcome the constraints to spore dispersal imposed by a boundary layer.

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.

The use of spore-trapping devices mounted on the outsides of aircraft has provided clear evidence of long-distance dispersal of fungi. Figure 10.25 shows

an example where spores were carried on the westerly winds across the North Sea from the English coast to Denmark. The spore clouds were found to be clustered at different altitudes and distances from the English coast. From knowledge of the wind speeds it was possible to distinguish between spores released on different days in England and also to distinguish between spores released in daytime (e.g. *Cladosporium*) and those released at night (the pink yeast *Sporobolomyces*, and various ascospores). Such long-distance dispersal can be highly significant for plant disease epidemiology, especially when new pathogenic races or fungicide-resistant strains develop and are spread across or between continents.

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. The sedimentation rates can be measured in closed cylinders and, except for unusually shaped spores for which correction factors are needed, the rates are found to agree closely with Stokes's Law for perfect spheres of unit density (1.0). The relevant equation is:

$$V_t = 0.0121r^2$$

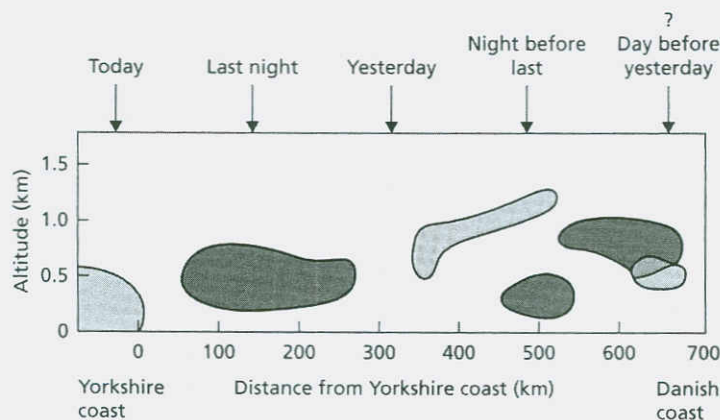


Fig. 10.25 Positions of peak spore concentrations of *Cladosporium* spp. (light shading) and damp-air spore types (dark shading) at different altitudes over the North Sea and at different distances from the English coast. (From Lacey 1988; based on the work of P.H. Gregory and J.L. Monteith.)

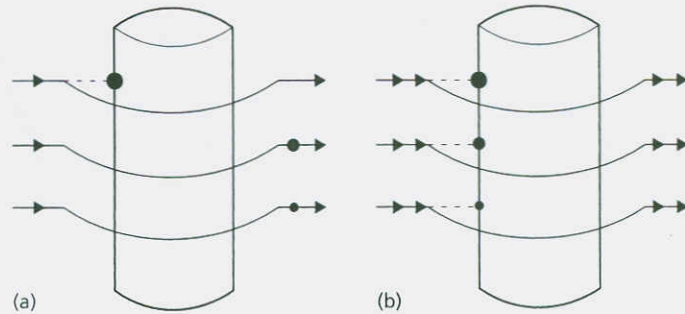


Fig. 10.26 Illustration of the relationship between spore size, wind speed, and impaction of spores onto a cylinder. (a) At low wind speeds only the largest (heaviest) spores impact. (b) At higher wind speeds progressively smaller (lighter) spores impact.

where V_t is the terminal velocity (cm s^{-1}) and r is the spore radius (μm). For example, spores of the cereal smut pathogen *Tilletia caries* ($17 \mu\text{m}$ diam) had a measured V_t of 1.4 cm s^{-1} , whereas puffball spores (*Bovista plumbea*, $5.6 \mu\text{m}$ diam) had a V_t of 0.24 cm s^{-1} . Differences of this order probably have little effect in outdoor environments, and so the spores of all types would remain airborne or settle according to the prevailing conditions. However, small differences can be significant in buildings and in the respiratory tract, discussed later.

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. As shown in Fig. 10.26, 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 \times velocity) of a spore will tend to carry it along its existing path for at least some distance.

Three points arise from this:

- 1 at any given air speed the larger spores (greater mass) have more chance of impacting than do smaller spores;
- 2 as the air speed (velocity) increases, so progressively smaller spores can impact;
- 3 as the size of the receiving object increases, so the deflection of air is greater and this reduces the chances of impaction.

These points are directly relevant to spores in nature. All the fungi that infect leaves or that characteristically grow on leaf surfaces (phyllosphere fungi) have large spores, with sufficient mass and therefore sufficient momentum to impact at normal wind speeds (up to 5 m s^{-1}). Examples include *Cladosporium herbarum* (spores $8\text{--}15 \mu\text{m}$ diameter), *Alternaria* spp. (about $30 \mu\text{m}$), and the leaf-infecting pathogens *Blumeria graminis* (about $30 \mu\text{m}$) and *Puccinia graminis* (about $40 \mu\text{m}$). By contrast, the typical soil fungi such as *Penicillium*, *Aspergillus* and

Trichoderma spp. have spores about $4\text{--}5 \mu\text{m}$ diameter, too small to impact at normal wind speeds but they can sediment out of the air in calm conditions.

The receiving object also determines the efficiency of spore impaction. A classic demonstration of this involved placing young branches of apricot trees in a wind tunnel, and exposing the branches to air containing spores of *Eutypa armeniaca* (Ascomycota), a pathogen of apricot trees. This fungus naturally releases its spores as clusters of eight ascospores held together in mucilage – a relatively large propagule with sufficient momentum to impact at relatively low wind speeds. As shown in Fig. 10.27, at all wind speeds the spore clusters impacted best on the narrow leaf stalks (petioles, about $1\text{--}2 \text{ mm}$ diameter), less well on the thicker young apricot stems, and even less well on the broader leaf blades. And, as the wind speed was increased, so the efficiency of impaction increased. It might be considered that the impaction efficiencies were quite low in all cases – never more than 3%. But in an apricot orchard the spores that do not impact on one shoot system would impact on another. On this basis, it was estimated that most spore clusters would be removed from the air as it travelled through an orchard at 2 m s^{-1} – a typical wind speed that was measured in field conditions. *Eutypa* is a wound pathogen, which commonly infects grape vines and apricots through natural wounds or pruning wounds. After impaction, the fungus relies on secondary spread by rain or irrigation water, which disperses the mucilage and carries the separate spores down to any wound sites.

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. By contrast, nonwettable spores that are covered with rodlets of hydrophobins remain on the

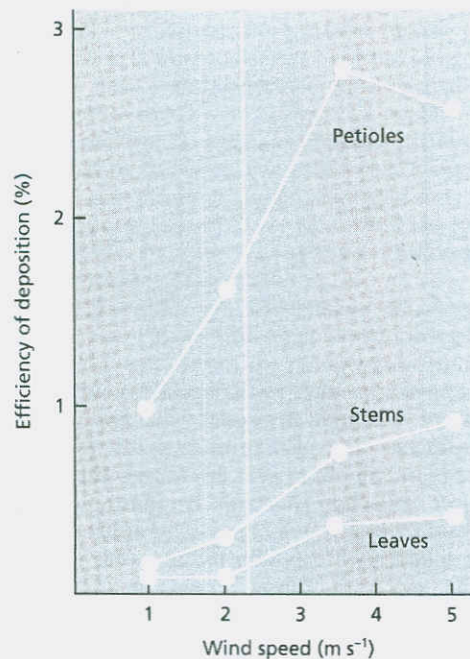


Fig. 10.27 Efficiency of impaction of ascospore tetrads of *Eutypa armeniaca* on leaf blades, petioles, and branch stems of apricot shoots, at different wind speeds. Most impaction occurs on the narrower objects (petioles) and in all cases the efficiency of impaction is increased as the wind speed increases. (From Carter 1965.)

surface of the raindrop, and if this rolls across a non-wettable surface such as a leaf cuticle it will leave the spores behind as a trail. This is easily demonstrated in a laboratory by rolling a water drop over a *Penicillium* colony, when the drop becomes coated with the nonwettable spores. If the spore-laden drop is then rolled across a plastic Petri dish, the spores are left as a trail across the plastic. The nonwettable spores can become airborne again by the "puff and tap" mechanisms mentioned earlier.

Air sampling devices and human health

Spores in the respiratory tract

The respiratory tract of humans and other animals is a natural spore-trapping device, as people with allergies know only too well. The respiratory tract is also the route of entry for spores of several fungal pathogens of humans, discussed in detail in Chapter 16. Three mechanisms of spore deposition in the respiratory tract are important: **impaction**, **sedimentation**, and **boundary layer exchange**.

During each intake of breath the air speed is fastest in the nose, trachea and bronchi (about 100 cm s^{-1}) and it diminishes with successive branching of the bronchioles. Therefore impaction will only occur in the upper respiratory tract, where the air speed is fastest, and only the heaviest (largest) spores will have sufficient momentum to impact. The hairs in the nostrils are narrow and covered with mucilage, making them highly efficient for intercepting the larger fungal spores and pollen grains. Some of these airborne particles cause rhinitis and other typical symptoms of hayfever.

All other particles that are too small to impact will be carried deep into the lungs and reach the terminal bronchioles and alveoli. This includes most particles of $4\text{--}5 \mu\text{m}$ diameter or less, including the spores of many common airborne fungi. Most of these spores are expelled again, but a few will settle onto the mucosal membranes by sedimentation during the brief period (usually less than 1 second) when the air in the alveoli is static between inhalation and exhalation. Particles even smaller than this, including the airborne spores of actinomycetes ($1\text{--}2 \mu\text{m}$ diameter), can be trapped by boundary layer exchange. This is a process in which small particles that are positioned very close to the boundary layer (the lining epithelium) can "flip" into the boundary layer by electrostatic or other forces. The airborne spores of potentially pathogenic fungi such as *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Coccidioides* spp. can settle in the alveoli by sedimentation, as do the spores of several other *Aspergillus* and *Penicillium* species. Some of these fungi, such as *Aspergillus clavatus*, cause acute allergic alveolitis in people who have been repeatedly exposed to spore dusts and have become sensitized. Several occupational diseases are of this type – farmer's lung, malt-worker's lung, etc. Once a spore has been deposited in the alveoli it persists until it is engulfed by a macrophage. By contrast, the upper regions of the respiratory tract are lined with ciliated epithelium which continuously sweeps mucus upwards and removes any particles deposited there.

The importance of airborne spores in relation to crop pathology, human ailments, and air quality in general has led to the design of air-sampling devices for the monitoring of spore loads. We end this chapter by considering the main types of device and the principles on which they operate.

The rotorod sampler

The rotorod sampler is a very simple air-sampling device, used mainly as an experimental tool (Fig. 10.28). It consists of a U-shaped metal rod with two narrow upright arms, attached to a spindle. The upright arms revolve rapidly (about $2000 \text{ rev min}^{-1}$) by a battery or

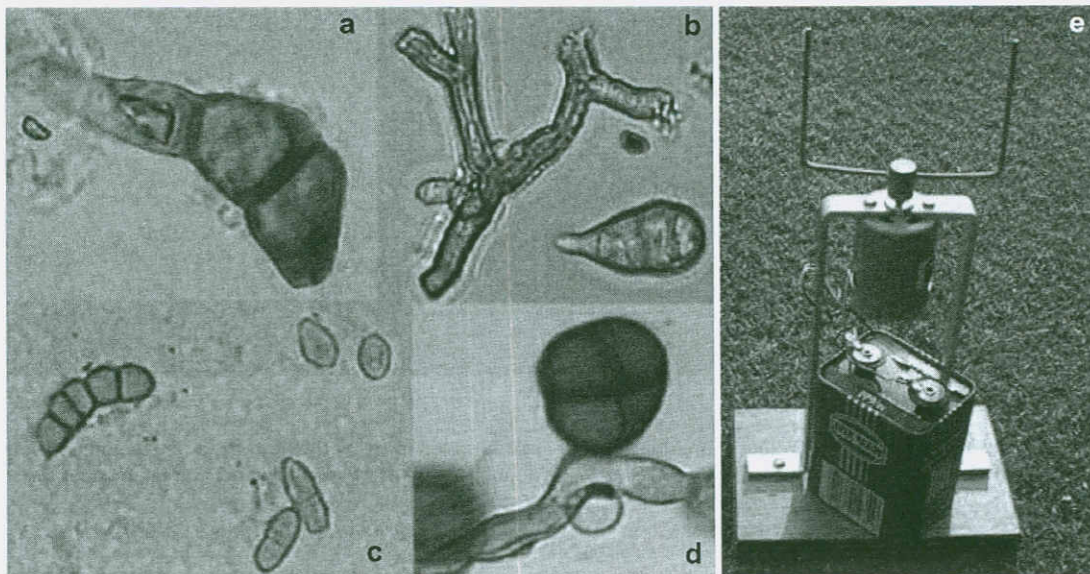


Fig. 10.28 The rotorod air sampler (e), and some representative spores and hyphal fragments commonly seen when rotorod tapes are examined. Images are at various magnifications. (a) Uredospore of *Puccinia graminis*. (b) Darkly pigmented hyphae of *Cladosporium*, with a spore of *Alternaria*. (c) Various pigmented spores, including *Cladosporium* (in the lower part of the frame). (d) An immature spore of *Epicoccum purpurascens* attached to a hyphal fragment.

electric motor. In order to sample spores and other airborne particles, the arms are covered with narrow strips of double-sided, transparent adhesive tape so that spores impact on the tape and can be examined with a microscope. This apparatus is cheap and portable, and is highly efficient at trapping relatively large particles, in the range 10–30 μm diameter, including pollen grains and the spores of most leaf fungi. It is much less efficient at trapping small particles. It can be used to home-in on the source of a particular type of spore, by making successive samplings in a small area. For example, it has been used in a field site in southern Britain to find the source of spores of *Pithomyces chartarum*, a toxigenic fungus that causes the facial eczema condition of sheep (see Fig. 7.20).

The Burkard spore sampler

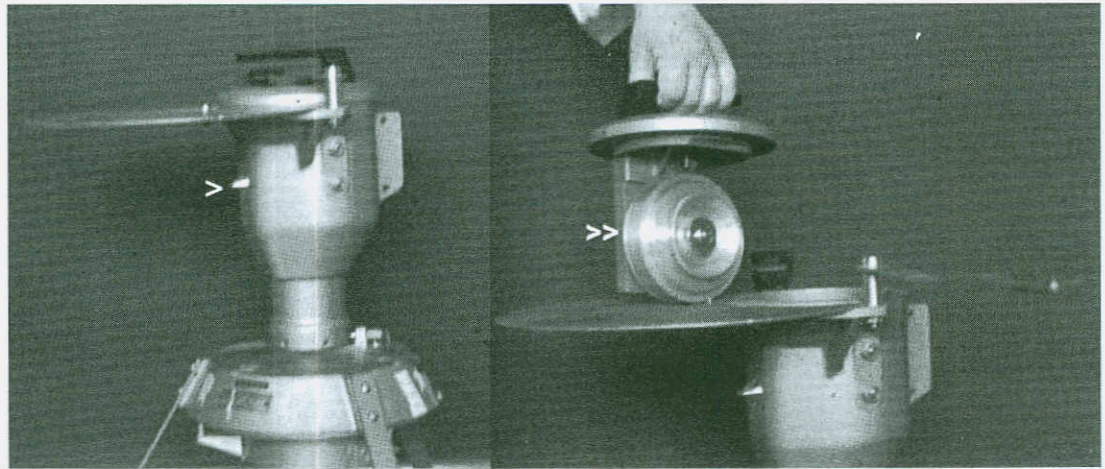
The Burkard spore sampler (Fig. 10.29) is a continuous monitoring device that works on the same principle as the rotorod sampler. It is used commonly in crop epidemiology and for monitoring allergen levels, including the pollen and spore counts announced on radio and television.

This sampler consists of a sealed drum with a narrow slit orifice (arrowhead in Fig. 10.29) beneath a weather-shield. Spores and other particles entering

the orifice impact as a narrow band on a reel (double arrowhead in Fig. 10.29) covered with transparent sticky tape. The reel rotates slowly past the orifice on a daily or weekly cycle, providing a continuous record of the particles present in the air throughout the sampling period. Finally, the tape is removed, cut into sections representing different time periods, and examined microscopically. Like the rotorod sampler, the Burkard sampler is based on the principle of impaction. The air entering the orifice is travelling at relatively low speed, so only the larger (heavier) particles are deposited on the tape. These include many pollen grains, the larger spores of plant-pathogenic fungi, and the spores of many common allergens.

The Anderson spore sampler

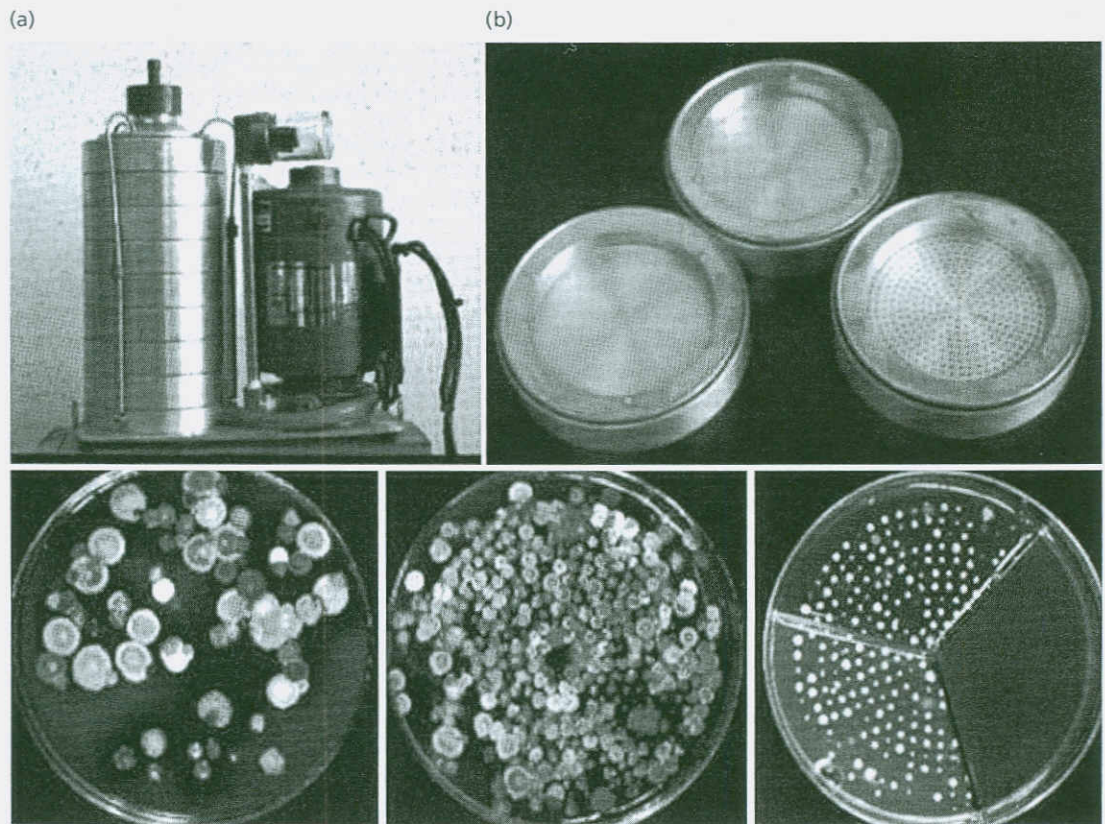
The Anderson spore sampler (Fig. 10.30) is perhaps the most ingenious and is claimed, with some justification, to simulate the deposition of airborne particles in the human respiratory tract (Fig. 10.31). It consists of a stack of perforated metal plates which fit together to form an airtight cylinder, with space for open agar-filled Petri dishes to be inserted between them. Each metal plate has the same number of holes in its base, but these holes become progressively smaller down the stack. Air is drawn in at the top, and down through



(a)

(b)

Fig. 10.29 (a,b) The Burkard continuous monitoring sampler in assembled form (a) showing the air intake orifice, arrow-head and (b) showing the reel covered with adhesive tape (double arrowhead) on which the spores are trapped.



(c)

(d)

(e)

Fig. 10.30 (a) The Anderson spore sampler in assembled form, and (b) three of the metal disks with perforations in their bases. (c-e) Examples of colonies from agar plates at different levels in an Anderson sampler.

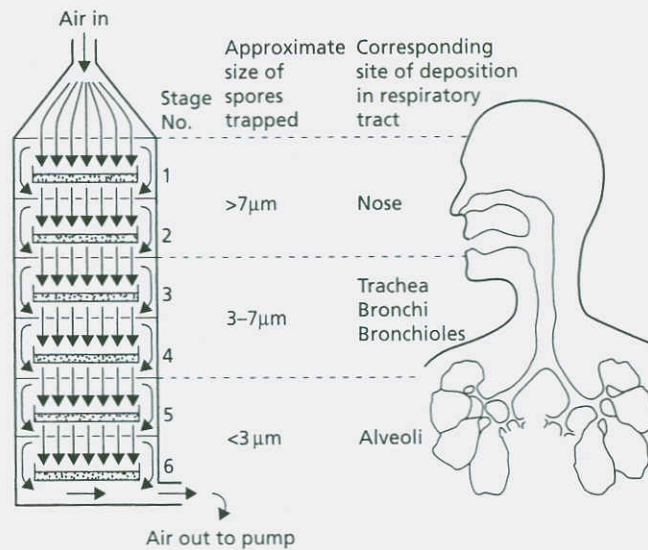


Fig. 10.31 Representation of the Anderson sampler, which simulates the deposition of different sized particles in the human respiratory tract.

the apparatus by a motor-driven suction pump at the base.

The air striking the first agar plate is travelling at low speed, so only large particles will impact on this plate. Any particles that do not impact are carried round the agar plate in the airstream and pass through the perforations at the next stage, and this is repeated down the whole stack of plates. Since the same volume of air has to pass through successively smaller perforations (while the number of perforations remains the same), its speed is increased progressively as it exits the perforations, and this causes successively smaller particles to impact on the agar plates. In fact even the smallest spores such as those of the actinomycetes *Thermoactinomyces vulgaris* and *Faenia rectivirgula* (1–2 µm) will impact at the high air speeds on the lower agar plates. These spores cause debilitating respiratory conditions such as “farmer’s lung” (acute allergic alveolitis).

It is important to note that all the metal plates in an Anderson sampler have perforations that are large enough for spores of all sizes to pass through them. So the Anderson sampler is not a sieving device. Instead, it sorts the airborne particles according to their momentum (mass × velocity). In this respect it acts on the same principle as the rotorod and Burkard spore traps.

After this sampler has run for an appropriate time, depending on the spore load, it is dismantled and the agar plates are incubated to identify the colonies that grow on them. In the example shown (the bottom row of Fig. 10.30) the agar plates were, from left to right, colonies from one of the uppermost agar plates,

colonies from the center of the stack of agar plates, and colonies from the lowest agar plate, consisting entirely of small actinomycete colonies. The agar plate at the extreme right is an example of a split plate with three different types of agar, designed to detect different types of organism simultaneously.

Cited references

- Adams, M.J. (1991) Transmission of plant viruses by fungi. *Annals of Applied Biology* 118, 479–492.
- Brasier, C.M. (1995) Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* 73, S1213–S1221.
- Brunt, A.A. & Richards, K.E. (1989) Biology and molecular biology of furoviruses. *Advances in Virus Research* 36, 1–32.
- Carter, M.V. (1965) Ascospore deposition of *Eutypa armeniacae*. *Australian Journal of Agricultural Research* 16, 825–836.
- Cho, C.W. & Fuller, M.F. (1989) Ultrastructural organization of freeze-substituted zoospores of *Phytophthora palmivora*. *Canadian Journal of Botany* 67, 1493–1499.
- Coley-Smith, J. (1987) Alternative methods of controlling white rot disease of *Allium*. In: *Innovative Approaches to Plant Disease Control* (Chet, I., ed.), pp. 161–177. Wiley, New York.
- Deacon, J.W. & Donaldson, S.P. (1993) Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Pythium* and *Phytophthora*. *Mycological Research* 97, 1153–1171.
- Deacon, J.W. & Mitchell, R.T. (1985) Toxicity of oat roots, oat root extracts, and saponins to zoospores of *Pythium*

- spp. and other fungi. *Transactions of the British Mycological Society* 84, 479-487.
- Deacon, J.W., Donaldson, S.J. & Last, F.T. (1983) Sequences and interactions of mycorrhizal fungi on birch. *Plant and Soil* 71, 257-262.
- Fox, F.M. (1986) Groupings of ectomycorrhizal fungi on birch and pine, based on establishment of mycorrhizas on seedlings from spores in unsterile soils. *Transactions of the British Mycological Society* 87, 371-380.
- Gregory, P.H. (1973) *Microbiology of the Atmosphere*, 2nd edn. Leonard Hill, Aylesbury.
- Gubler, F. & Hardham, A.R. (1988) Secretion of adhesive material during encystment of *Phytophthora cinnamomi* zoospores, characterized by immunogold labeling with monoclonal antibodies to components of peripheral vesicles. *Journal of Cell Science* 90, 225-235.
- Hardham, A.R. (1995) Polarity of vesicle distribution in oomycete zoospores: development of polarity and importance for infection. *Canadian Journal of Botany* 73(suppl.) S400-407.
- Hardham, A.R. (2001) Cell biology of fungal infection of plants. In: *The Mycota VIII. Biology of the Fungal Cell* (Howard, R.J. & Gow, N.A.R., eds), pp. 91-123. Springer-Verlag, Berlin.
- Hardham, A.R., Cahill, D.M., Cope, M., Gabor, B.K., Gubler, F. & Hyde, G.J. (1994) Cell surface antigens of *Phytophthora* spores: biological and taxonomic characterization. *Protoplasma* 181, 213-232.
- Hiruki, C. & Teakle, D.S. (1987) Soil-borne viruses of plants. In: *Current Topics in Vector Research*, vol. 3 (Harris, K.F., ed.), pp. 177-215. Springer-Verlag, New York.
- Hsu, S.C. & Lockwood, J.L. (1973) Soil fungistasis: behavior of nutrient-independent spores and sclerotia in a model system. *Phytopathology* 63, 334-337.
- Ingold, C.T. (1971) *Fungal Spores and their Dispersal*. Clarendon Press, Oxford.
- Lacey, J. (1988) Aerial dispersal and the development of microbial communities. In: *Micro-organisms in Action: concepts and applications in microbial ecology* (Lynch, J.M. & Hobbie, J.E., eds), pp. 207-237. Blackwell Scientific, Oxford.
- Lockwood, J.L. (1977) Fungistasis in soils. *Biological Reviews* 52, 1-43.
- Mitchell, A.G. & Brasier, C.M. (1994) Contrasting structure of European and North American populations of *Ophiostoma ulmi*. *Mycological Research* 98, 576-582.
- Moss, S. (1986) *The Biology of Marine Fungi*. Cambridge University Press, Cambridge.
- Reichle, R.E. & Fuller, M.F. (1967) The fine structure of *Blastocladiella emersonii* zoospores. *American Journal of Botany* 54, 81-92.
- Ron, E.Z. & Rosenberg, E. (2001) Natural roles of biosurfactants. *Environmental Microbiology* 3, 229-236.
- Warburton, A.J. & Deacon, J.W. (1998) Transmembrane Ca^{2+} fluxes associated with zoospore encystment and cyst germination by the phytopathogen *Phytophthora parasitica*. *Fungal Genetics and Biology* 25, 54-62.