

# Spore Production, Discharge, and Dispersal

Nicholas P. Money

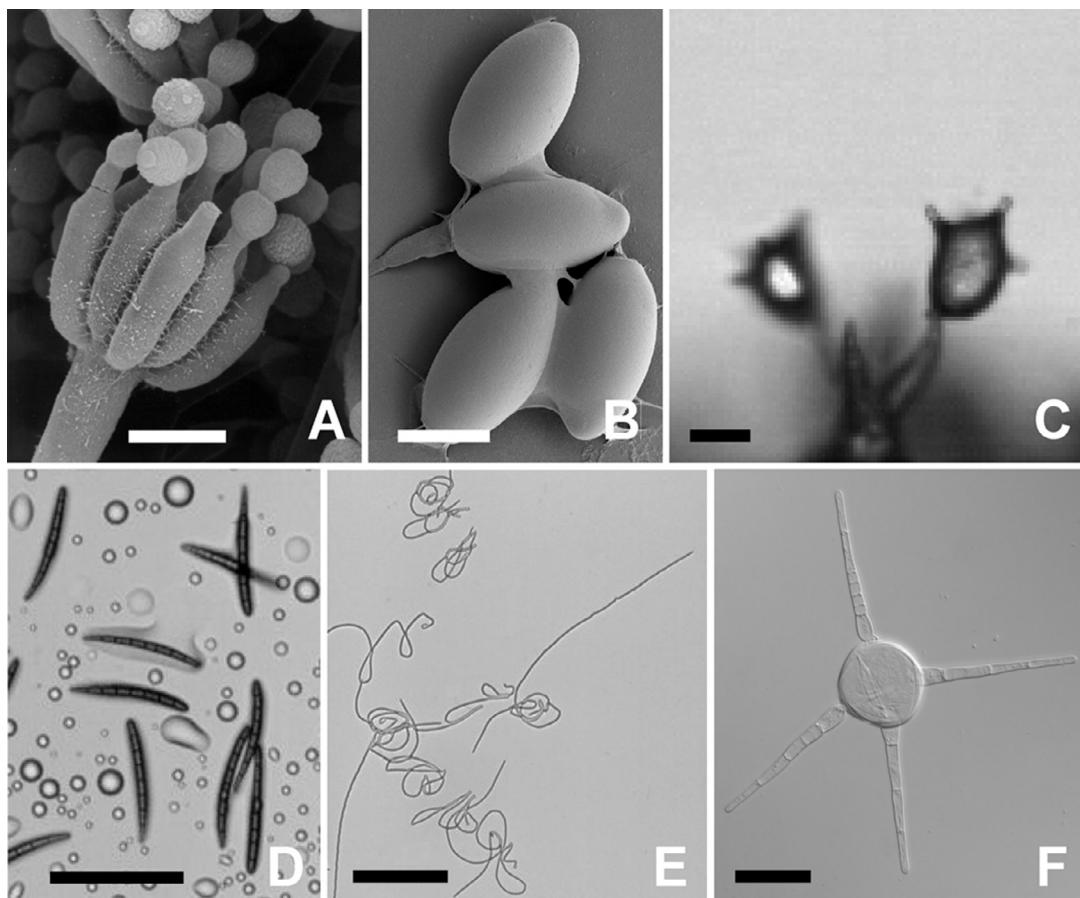
Miami University, Oxford, OH, USA

## DIVERSITY, DEVELOPMENT, AND FUNCTIONS OF SPORES

It is impossible to grasp the diversity of species in Kingdom Fungi by studying their vegetative colonies. Zoosporic fungi have the least complicated feeding structures, with many chytrids, for example, producing nothing more complex than a spherical thallus that absorbs food from the single plant cell in which it develops. The mycelia of non-zoosporic phyla are immeasurably more complicated in terms of their structure and development, but it is rarely possible to identify a fungus beyond its phylum by looking at its hyphae. The presence of dolipore septa is diagnostic of a basidiomycete, but few, if any, fungal biologists could say whether hyphae viewed on a microscope slide belong to a giant puffball or a jelly fungus. When we examine spores, and the structures producing the spores, we have a much better prospect of more detailed identification. Spore morphology is one of the most important features used for visual identification of fungi and the astonishing range of spore types is a powerful reflection of their evolutionary diversity (Figure 3.1). Without assessing differences between spores and fruit bodies, efforts to develop a taxonomy of the fungi would have been futile. The study of spores is a study of evolution.

### Asexual Spores

Nuclei within asexual spores are produced by mitotic division so that the spores are clones of the parent mycelium. The simplest mechanism of spore formation involves the differentiation of pre-formed mycelium. Spores generated in this fashion are called **thallospores**. There are two categories of thallospore: **arthrospores** are produced by the fragmentation of hyphae into compartments separated by septa, and thickening of the cell wall of a hyphal compartment forms a **chlamydospore**. **Sporangiospores** are asexual spores formed inside a walled sporangium. Sporangiospores include the spores of zygomycetes, which are exposed to air by splitting of the mature sporangial wall, and motile zoospores of chytrids expelled into water from their zoosporangia. Asexual spores produced on stalks, or **conidiophores**, are called **conidia** (singular **conidium**).

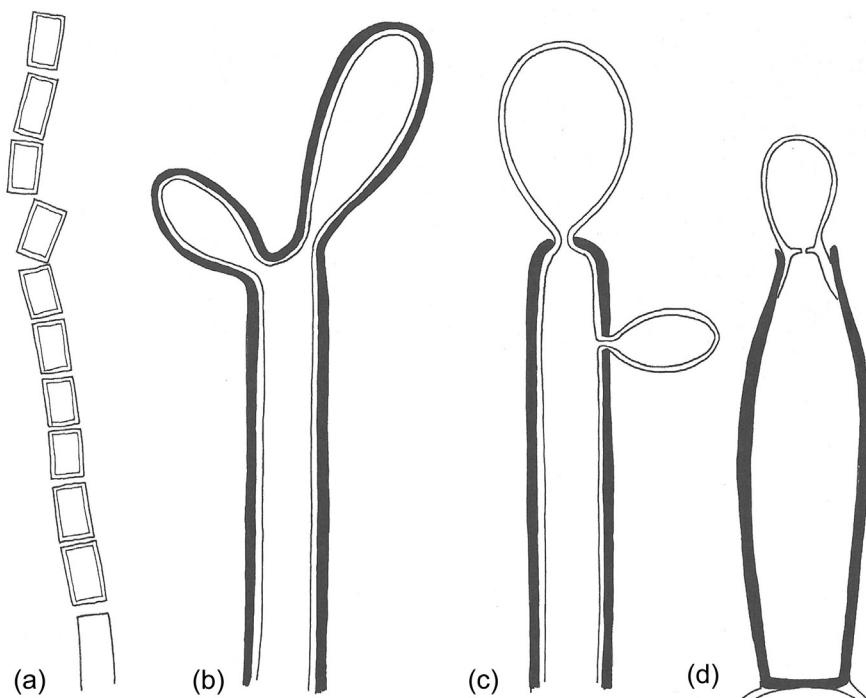


**FIGURE 3.1** Sampling of the morphological diversity of fungal spores. (a) Spherical conidia of *Penicillium* species produced by phialides. (b) Ellipsoidal ascospores of *Podospora anserina*. (c) Polyhedral basidiospores of *Aleurodiscus oakesii*. (d) Fusiform ascospores of *Geoglossum nigritum*. (e) Filamentous ascospores of *Cordyceps militaris*. (f) Star-shaped aquatic conidium of *Brachiosphaera tropicalis*. Scale bars (a–c) 10 µm, (d, e) 100 µm, (f) 20 µm. Source: Fischer et al., 2010.

More than 100 terms have been used to discriminate between different types of conidium. These include annellospore, botryo-aleuriospore, closterospore, polarlocularspore, and stalagmospore. Some of these nouns refer to the shape of the spore, others to a mechanism of development (**conidiogenesis**). To anyone other than an expert, the distinctions between many of these spore types are quite opaque. The ‘Dictionary of Fungi’ definition of an annellospore (or annelloconidium), for example, refers to ‘holoblastic conidiogenesis in which the conidiogenous cell by repeated enteroblastic percurrent proliferation produces a basipetal sequence of conidia.’ Deconstruction of this description would require a separate chapter on taxonomic research on the formation of conidia (asexual spores) and we have opted, instead, to refer the reader to this specialized literature. The variety of conidial shapes and developmental processes has little taxonomic value beyond its use in describing species. Many unrelated genera share the same manner of spore formation and closely related species can show entirely different mechanisms of conidiogenesis.

Given the importance of conidiogenesis in the reproduction of fungi of clinical and agricultural significance, it is surprising that this has received so little attention from researchers in recent decades. Electron microscopic studies on conidial fungi have documented many types of developmental mechanisms (more than 40 have been described), but their cellular and molecular controls have not been analyzed in any detail. Most conidia are formed on stalks called conidiophores. They develop at the tips of the conidiophore, or on branches from the main axis of the conidiophore, as single spores, or in chains. Chains of spores are formed in different ways (Figure 3.2). Segmentation of the conidiophore by the development of multiple closely spaced septa creates spores from the existing structure of the conidiophore. Other fungi form chains from the free ends of conidiophores and conidiophore branches. Spores can inflate before they are separated from other cells by the formation of a septum (described as blastic development), or separate without prior expansion (thallic). Chains can develop with the youngest spores at the base (basipetal arrangement) or youngest at the tip (acropetal). The variation can seem limitless.

Chains of spores produced from cells called phialides are very common among ascomycetes, including *Aspergillus* and *Penicillium* (Figure 3.3). Phialides are vase-shaped cells



**FIGURE 3.2** Modes of conidium formation. (a) Thallic development: the conidial initial does not enlarge before it separates from the conidiophore. (b) Holoblastic development: all cell wall layers expand during conidium formation. (c) Enteroblastic development: only the inner layer of the cell wall expands through an aperture in the outer wall of the conidiophore. (d) Phialidic development: conidium is formed by the synthesis of new cell wall material within the neck of the phialide. Source: Webster, J., Weber, R.W.S., 2007. *Introduction to Fungi*, third edition. Cambridge University Press.

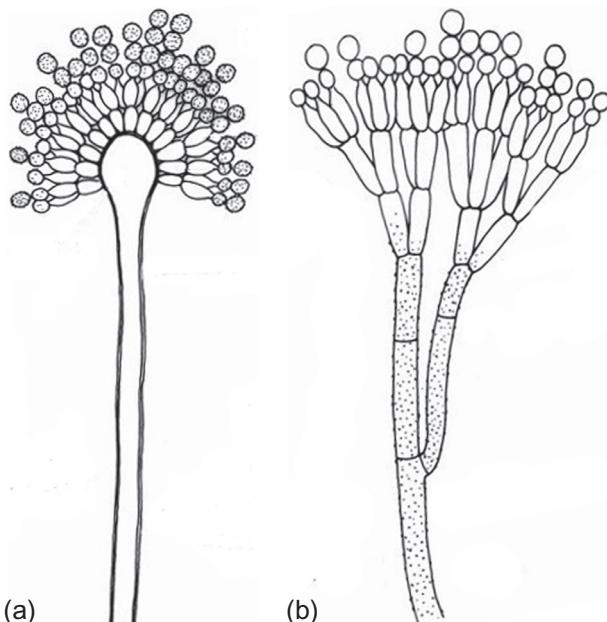


FIGURE 3.3 Conidiophores of (a) *Aspergillus* and (b) *Penicillium* bearing clusters of phialides that generate chains of spores.

(shaped, more explicitly, like a Greek amphora), that develop on conidiophores. They produce chains of conidia by extruding new cell walls through their open necks (think of pulling a turtleneck sweater over your head) and pinching-off uninucleate portions of cytoplasm (Figure 3.2d). The formation of each conidium is coordinated with mitosis in the phialide such that the conidium is separated from the phialide by the formation of a septum through the axis of the mitotic spindle. Phialides produce basipetal chains of spores. In *Aspergillus*, multiple phialides develop over the dome of the swollen tip of the conidiophore called the ampulla. As each phialide generates a chain of conidia, the single conidiophores can support hundreds of spherical spores. The conidiophore of *Penicillium* has a branched apex with one or more phialides produced at the tip of each branch. Like *Aspergillus*, the activity of multiple phialides allows a single conidiophore to support a mass of conidia. These fungi produce millions of dry spores per square centimetre of colony surface.

Conidiogenesis requires hyphae to switch from programs of indeterminate elongation to more determinate growth processes that shape and detach spores. The alterations in cellular form during sporulation have been studied using electron microscopy but very little is known about the underlying molecular genetic controls. The formation of a single phialide must involve a period of limited elongation of a hyphal branch, cessation of extension, and swelling of the cell behind the apex to produce the ampulla shape. Subsequent events include the formation of a septum at the base of the phialide, perforated by a pore to maintain cytoplasmic continuity with the subtending conidiophore, followed by successive programs of tip swelling, differentiation of conidium initials, and separation from the phialide tip by septation. The enzymes involved in conidiogenesis include catalysts for cell wall synthesis

and reorganization of the cytoskeleton. Again, however, this hypothetical laundry list is not supported, nor embellished, by critical studies on the genetics of spore formation. Mutant analysis has identified genes involved in the signalling pathways involved in conidiogenesis in *Aspergillus*. These include genes that exert broad regulatory control over conidiogenesis, genes that encode transmembrane receptors and G-proteins, MAP kinase genes, and multiple transcription factors. However, we remain a long way from solving how each conidial fungus exerts precise control over the shape and size of its spores. This is another example of a fertile area for researchers that would produce significant advances with the application of existing molecular technology coupled with creative experimental design.

## Sexual Spores

Fungi produce three types of sexual spore: basidiospores (Basidiomycota), ascospores (Ascomycota), and zygospor e (zygomycetes). Because these spores are among the defining features of the major phyla, their development is described in Chapter 1 in the context of fungal life cycles.

## Spore Functions

Spores serve as vehicles for transmitting fungi through space and time. This sentence is a bit melodramatic, but captures the fundamental function of spores. Asexual spores carry identical copies of the genes of the parent colony. Sexual spores transmit versions of genes recombined from parental strains. Spores are often formed in response to nutrient limitation. When a colony is in contact with an abundance of nutrients it invests in hyphal growth and branching, which has the effect of capturing energy from an expanding field of activity. When the food is exhausted, it makes more sense to escape from the coming famine and seek a new source of nutrients. This can be done by moving away from the parent mycelium, or by staying put and waiting for a fresh pulse of nutrients to become available in the same spot. To these ends, many spores are adapted for dispersal, for carrying the genome of individual strains through air or water. Following dispersal, deposition of spores some distance from the mycelium may provide access to an environment suitable for the development of a new colony. Other kinds of spores are survival capsules, adapted for allowing the individual to ride out hostile environmental conditions with the potential for future germination. Speaking broadly, the first category of spores may be relatively fragile and poorly provisioned for long-term survival. The second category – resting spores – tend to have thicker cell walls and are packed with nutrient reserves that support longer-term survival. Nutrient limitation is not the only stimulus for spore formation. Mushroom formation follows seasonal patterns for many species and the timing of fruiting may bear little relation to the availability of food.

Airborne spores are subject to rapid dehydration and conidia and basidiospores of plant pathogenic fungi often lose viability within a few hours. Spores of ectomycorrhizal basidiomycetes can survive for at least 4 years under various storage conditions. In the soil, these spores create a ‘spore bank’ that is primed for establishing symbioses with new tree seedlings. Rust teliospores will not germinate until they have endured a 6-month period of dormancy and, beyond the required dormancy, teliospores can survive in the soil for at least 4 years.

Under controlled environmental conditions in the laboratory, some rust teliospores survive for more than 14 years. Experiments on teliospores of *Tilletia indica*, which causes Karnal bunt of wheat, show that only 3–4% of the spores remain viable after 7 years. Regression analysis based on these investigations indicates zero viability of spores within 13–18 years under field conditions and 38 years in the lab.

## SPORE SIZE AND SHAPE

Spores vary greatly in size, from 3 µm-long basidiospores of certain bracket fungi, to the 'giant' spores of lichenized Ascomycota that measure up to 300 × 100 µm. Corresponding estimates of spore mass, based on density measurements between  $1.0 \times 10^3$  and  $1.3 \times 10^3$  kg m<sup>-3</sup>, range from 1 pg to 2 µg. Most variations in spore shape are quite puzzling from an adaptive perspective. Our familiarity with the physical behaviour of large objects like ourselves can lead to fuzzy thinking about the aerodynamics of spores. Spores with appendages may look like they would remain airborne for longer than simple spherical spores, but the mass of the microscopic spore has a far greater influence upon its sedimentation rate. (This is true of spores in aquatic environments too and they are addressed later in this chapter.) We know this because sedimentation rates estimated for non-spherical spores using figures for the size of spheres of equal mass provide a good match to experimental data. Sedimentation rates for spherical spores with a diameter of 5–10 µm range from 1 to 4 mm s<sup>-1</sup>, meaning that they take 4–17 min to fall 1 m through still air. Many mushrooms produce spores within this size range. The slow settling speed prolongs their exposure to air currents beneath the mushroom cap that can sweep them away from the fruit body. Some of the variations in spore shape may be related to developmental constraints and to mechanisms of spore discharge. The different shapes and sizes of ascospores are fitted to the asci in which they develop (and vice versa) and spore and ascus shape affect the launch process by controlling whether spores are discharged one at a time with a pause between shots, in a stream with one spores following the next, or as a single projectile of connected spores. In basidiomycetes, spore shape affects discharge distance by controlling the size of the fluid droplet (Buller's drop) whose motion catapults the spore into the air (pp. 86–89).

## SPORE DISCHARGE

In this book, we use the term **spore discharge** to refer to the separation of fungal spores from their parent colonies and fruit bodies, and **spore dispersal** for their subsequent movement. Discharge often launches spores over a short distance, whereas dispersal can involve travel over vast distances through the atmosphere. The spores of many fungi are displaced from their parent colonies by physical disturbance resulting from airflow, raindrops, vibration of the surface supporting the colony, or by the activities of animals. These are referred to as **passive discharge mechanisms**. **Active discharge mechanisms** are powered by hydrostatic pressure, fast movements induced by cytoplasmic dehydration, and by the utilization of surface tension force.

## Physical Obstacles to Fungal Motion: Air Viscosity and Boundary Layers

The physical challenges encountered by fungi and other microorganisms are very different from those experienced by large animals. Gazelles and exceptional humans achieve horizontal leaps of 9 m. The arc of their flight paths is dominated by inertial forces and neither animal is slowed by the viscosity of the air through which it moves. Things are very different for ascomycetes that shoot their spores from cup-shaped apothecia and for basidiomycete yeasts that propel their ballistospores from the surface of infected flower petals. This is because air represents a viscous obstacle to fungal movement and remarkable launch speeds are necessary to propel spores even for short distances. The ratio of inertial to viscous forces is described by the non-dimensional term Reynolds number ( $R_e$ ): big animals experience high  $R_e$ ; spore movement is a low  $R_e$  process. The same scaling principles make greater intuitive sense in the more viscous medium of water. A dolphin can glide many metres through the ocean after a single stroke of its flukes (high  $R_e$ , inertia trumps viscosity), whereas a chytrid zoospore stops dead the instant it stops lashing its posterior flagellum (low  $R_e$ , viscosity rules).

The horizontal movement of actively discharged fungal spores ceases within a fraction of a second after launch and the spore falls toward the ground. This is the case with coprophilous (dung) fungi whose spores (or spore clusters or sporangia) are shot onto vegetation surrounding their colonies. For the majority of fungi, however, discharge mechanisms get spores airborne and longer-distance dispersal is driven by wind. Wind dispersal cannot occur, obviously, unless the fungus reaches mobile air currents. This poses a problem for a microscopic particle that develops within the boundary layer of slow-moving air close to the colony surface. When air flows around any solid object, drag creates a mantle of slow-moving air close to the object's surface; at the interface itself, the air is stationary. There is an inverse relationship between airspeed and boundary layer thickness, and this is another situation in which the  $R_e$  term is useful. At low airspeed, air movement is dominated by viscosity, the boundary layer is thickest, and  $R_e$  is low. Inertia dominates airflow patterns at higher airspeeds and under these conditions of elevated  $R_e$ , eddies and vortices develop. The boundary layer surrounding leaves of terrestrial plants varies from approximately 0.1 to 9.0 mm in thickness depending upon leaf size and wind speed. The same considerations apply to the motion of water around solids, but boundary layers are much thicker, and  $R_e$ 's lower, in the aquatic environment.

## Discharge by Airflow and Drying, Electrostatics, and Cavitation

### *Airflow and Drying*

The physical disturbance of fungal colonies by airflow is thought to serve as the main stimulus for separating conidia from their conidiophores. Airflow also causes the release of other kinds of spores including the sporangiospores of zygomycetes, and uredospores and teliospores in rusts. Despite the importance of this passive spore release mechanism, it has not been examined in detail in many species. Interesting experiments using miniature wind tunnels have shown that low airspeeds are effective at releasing uredospores from infected cereal leaves. In some instances, clumps of spores are liberated into the airflow rather than single spores. Other experiments have reached a surprising conclusion. Dense clouds of spores are

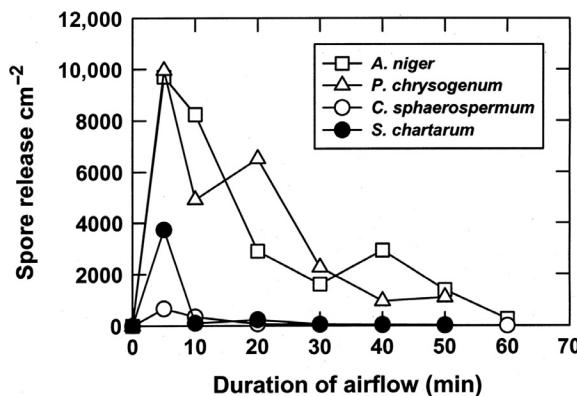
ejected into the airflow when colonies are exposed to wind, but this is followed by a sharp decline in spore release even when airflow is continuous. The number of spores released at any airspeed is increased at low levels of relative humidity, but the pattern of an initial burst of spore release followed by a greatly reduced aerosolisation seems to be a common phenomenon. Studies on conidial ascomycetes show that 98% or more of the spores remain attached to the surface of a colony at low airspeeds (Figure 3.4). Conidia of many fungi seem to be firmly attached to their conidiophores, remaining in chains and clumps despite considerable buffeting. Physical calculations and experiments employing miniature strain gauges show that shear forces produced by wind speeds of 10 m per second, or 36 km per hour, are necessary to separate the spores of some fungi from their colonies. The apparent strong adhesion between spores and their conidiophores is counterintuitive. One possibility is that passive spore release works by allowing colonies to maintain a slow trickle of conidia into the air over a long period of time. A more likely explanation is that the culture conditions and design of experiments fail to replicate the behaviour of fungi in nature, but the source of this discrepancy is not clear. The effectiveness of passive spore release seems evident from the ubiquity of fungi that utilize this dispersal mechanism and the high densities of their spores in air samples.

### *Electrostatics*

The surface of fungal spores becomes electrically charged in dry air and these charges have a significant effect upon spore motion. It has been proposed that electrostatic charges are involved in spore release, but calculations show that forces produced by charge separation are too small to detach conidia from their conidiophores.

### *Cavitation (Explosive Bubble Formation)*

Cavitation occurs in the walled cells of fungi and plants whose contents are subjected to negative pressures sufficient to exceed the tensile strength of water in the cytoplasm. Gas bubble formation inside air-dried ascospores of Sordariomycetes is the most familiar example

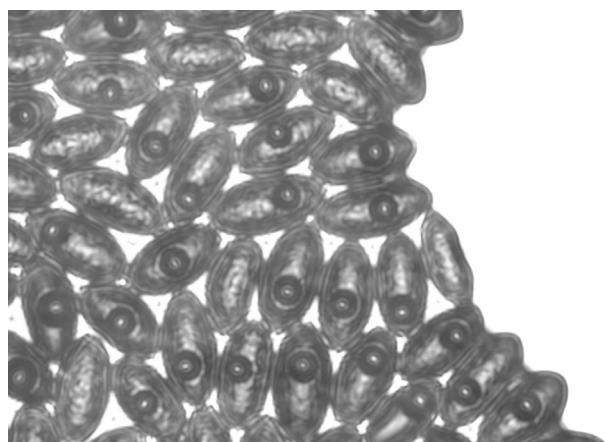


**FIGURE 3.4** Plot showing conidial resistance to release from surfaces exposed to slow airflow (1.6 m per second). Spore release shows initial burst followed by sustained period of low-frequency dispersion. Species in this plot are *Aspergillus niger*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, and *Stachybotrys chartarum*. Source: Tucker, K., Stolze, J.L., Kennedy, A.H., Money, N.P. 2007. Biomechanics of conidial dispersal in the toxic mold *Stachybotrys chartarum*. *Fungal Genet. Biol.* 44, 641–647.

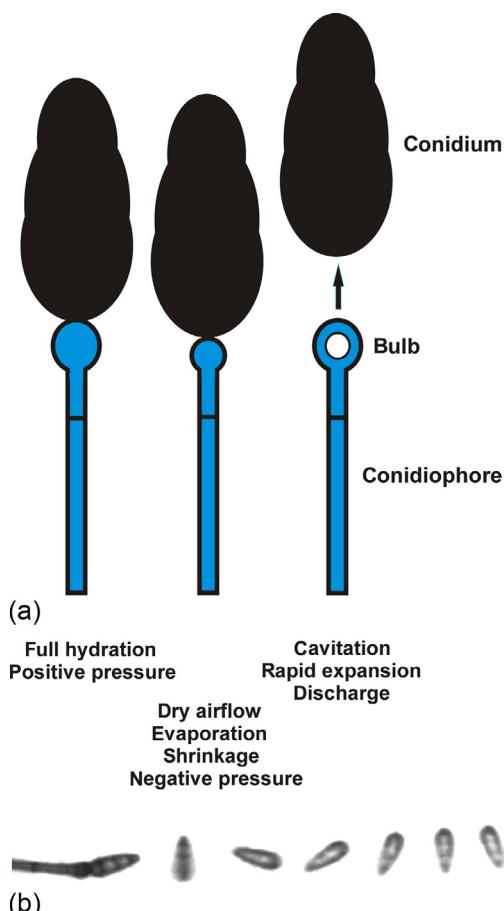
of the cavitation process for mycologists ([Figure 3.5](#)). The analogous cavitation-powered sporangium of ferns is well known among botanists. As water evaporates from the cytoplasm of the spore, or other type of cell, its walls are placed under increasing tension. If the cell wall is pliable it will crumple, allowing the dried cytoplasm to occupy a reduced volume; if the cell wall is rigid it resists compression and, if drying continues, negative pressure causes water in the cytoplasm to fracture creating a vapour-filled bubble. This occurs in the conidia and conidiophores of certain fungi in which it is linked to spore discharge. The mechanism has been studied in the fruit pathogen *Deightoniella torulosa*. The apical cell of the conidiophore of this fungus has a bulbous tip whose sidewalls are thickened ([Figure 3.6](#)). Cavitation causes the thin-walled tip of the bulb to flex outward and this motion propels the spore into the air at speeds of up to 0.6 m per second, propelling the spore over a distance of almost 0.5 mm. Cavitation has also been described in a handful of other conidial fungi, raising the possibility that this discharge mechanism may be widespread.

## Raindrops and Vibration

Dry spores and wet spores are puffed and splashed from the surface of their colonies by raindrops. Spores can be splashed over short distances by this mechanism or can be carried over longer distances by wind either as free spores or associated with water droplets. The impact of raindrops exerts much larger forces on colonies than wind disturbance. A raindrop with a diameter of 0.5 mm is one million times heavier than a spore; scaling up to human dimensions, the 'raindrop' would weigh 100 kilotons and splatter the target with the force of 200 kg of high explosive! Raindrops can discharge spores after falling freely and reaching their terminal velocity or when travelling at slower speeds as secondary droplets shed from vegetation. Raindrops can also discharge spores indirectly by causing vibration. Finally, tiny droplets of water in mist capture and disperse spores as they move through vegetation colonized by fungi.



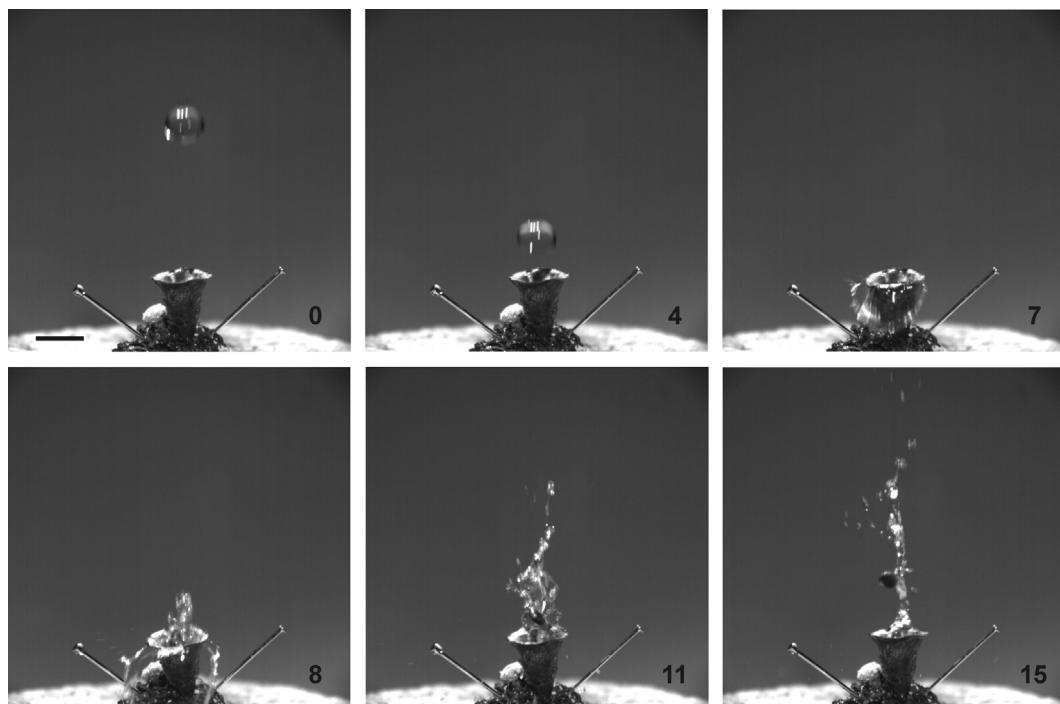
**FIGURE 3.5** Ascospores of *Neurospora tetrasperma* exposed to dry air, containing single cavitation bubbles in their cytoplasm.



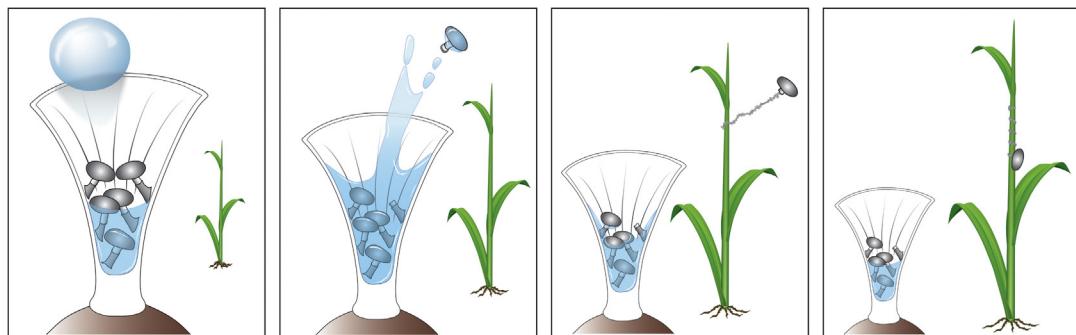
**FIGURE 3.6** Conidial discharge in *Deightoniella torulosa*. (a) Diagram showing mechanism of conidial discharge involving shrinkage of conidiophore bulb, followed by explosive expansion driven by the formation of a cavitation bubble. (b) Composite of individual frames from high-speed video recording of conidial discharge showing launch and rotation of spore as it moves from left to right of the microscope field of view. The mean launch speed of *Deightoniella* conidia is 0.24 m per second. Video captured at 50,000 frames per second.

Raindrops compress the loosely packed spore bags of puffballs (Agaricales) and earth-balls (Boletales) forcing clouds of basidiospores into the air through irregular tears in the fruit body jacket, or peridium, or through a more regular pore or nozzle on its upper surface. The same mechanism discharges spores from earth-stars (Gastrales), which often have a more intricate nozzle formed by a cone of tooth-shaped flaps. The most complex mechanism of splash discharge has evolved in the bird's nest fungi (Agaricales). The basidiospores of these fungi are enclosed within packets called peridioles. The peridioles are exposed in the mature fruit body that creates a structure that looks, superficially, like an egg-filled nest. The fruit bodies vary from irregularly shaped blobs whose walls break open and ooze the peridioles in a mucilaginous matrix (*Mycocalia* and some species of *Nidularia*), to wide-mouthed

cups (*Crucibulum* and *Nidula*), and more angular conical cups (*Cyathus*). Peridiole discharge in *Cyathus* is a spectacular mechanism (Figure 3.7). Each peridiole is tethered to the interior of the fruit body by a cord that is coiled inside a purse. When a raindrop hits the lip of the fruit body it plunges to the bottom of the cone, dislodging the peridiole as water is ejected in a high arc into the air. The peridiole is carried with the ejected water and can fly at 5 m per second (18 km/h) over a horizontal distance of more than one metre. The cord remains coiled during the flight of the peridiole, unravelling if its free end brushes against a leaf or twig (Figure 3.8). The free end of the cord (the hapteron) is coated with a powerful adhesive that sticks to vegetation; when this happens, the momentum of the peridiole carries it beyond the obstacle and its cord unravels, acting as a brake. The cord performs an analogous function to the elastic tether used in bungee jumping. Peridioles of *Cyathus* are often found with their cords coiled around twigs or leaf petioles close to the parent fruit body. This complex mechanism may be an adaptation associated with the coprophilous nature of some bird's nest fungi, situating the discharged peridiole away from the dung in a perfect position for unintentional ingestion by an herbivore (see section on [Dispersal of Spores by Animals](#)).



**FIGURE 3.7** Splash discharge of peridia in the bird's nest fungus, *Cyathus olla*. Selected frames from high-speed video obtained at a frame rate of 3000 frames per second. Time in milliseconds from beginning of sequence is shown in the bottom right of each frame. The peridiole is ejected by the upward displacement of water from the interior of the basidiome when water drop hits the rim of the peridium. Scale bar = 3 mm. Source: Hassett, M.O., et al., 2013. *Splash and grab: biomechanics of peridiole ejection and function of the funicular cord in bird's nest fungi*. *Fungal Biol.* 117, 708–714.

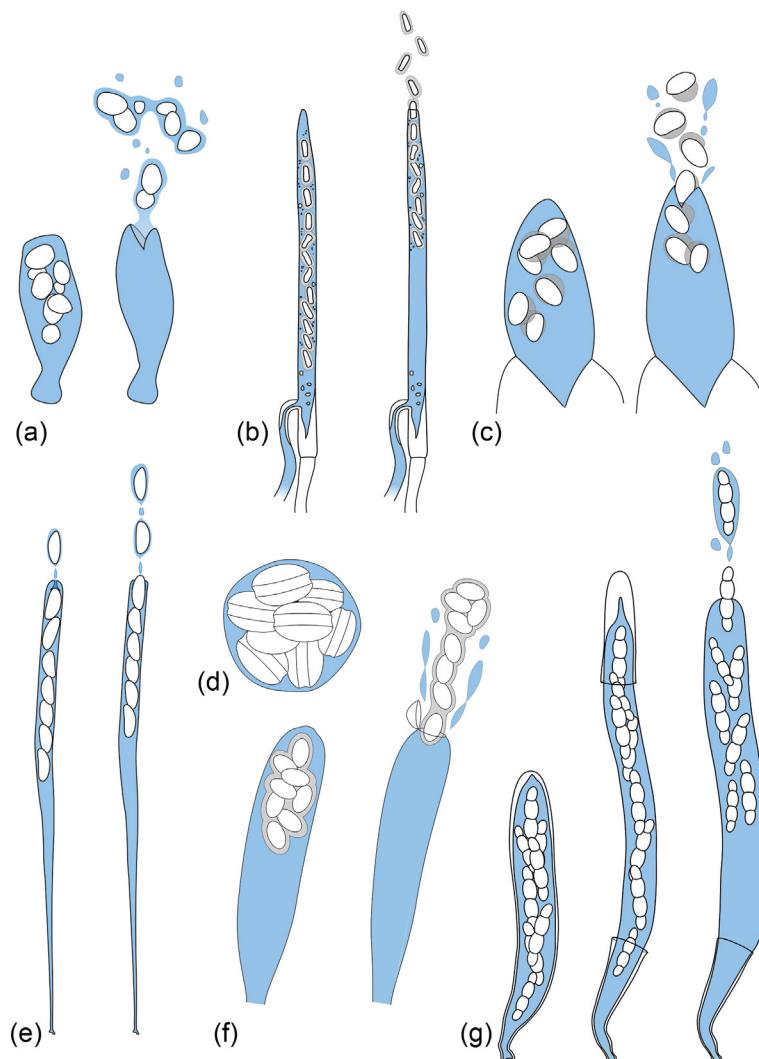


**FIGURE 3.8** Diagram showing splash discharge of peridiolae of the bird's nest fungus and their mechanism of attachment to vegetation. The funicular cord is packed within a purse before discharge. The force of the raindrop fractures the purse leaving the sticky end of the cord exposed during the flight of the peridiole. Deployment of the funicular cord occurs when the hapteron contacts an obstacle. The process is completed in less than 200 ms. Source: Hassett, M.O., et al., 2013. *Splash and grab: biomechanics of peridiole ejection and function of the funicular cord in bird's nest fungi*. *Fungal Biol.* 117, 708–714.

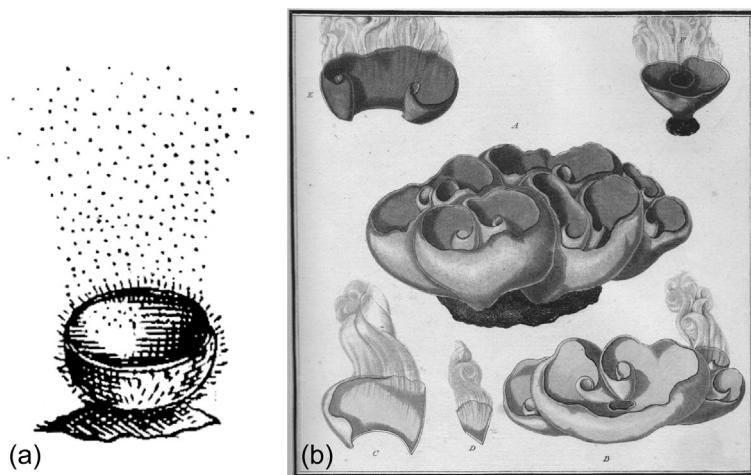
## Turgor Pressure

### Ascospore Discharge

Explosive discharge of ascospores drives some of the fastest movements in nature with launch speeds exceeding 30 m per second, or 100 km per hour clocked using ultra-high-speed video cameras. The ecological and agricultural importance of the mechanism is apparent from the observation that the Ascomycota is the largest fungal phylum and the most numerous group of plant pathogens. The considerable diversity in ascus structure, ascospore development, and spore morphology among the Ascomycota has significant effects on the dynamics of the discharge process (Figure 3.9). The simplest mechanism operates in pathogens like species of *Taphrina* that belong to the basal lineage of the Ascomycota called the Taphrinomycotina. For example, in *Taphrina deformans*, which causes peach leaf curl, the ascus is exposed on the infected leaf surface, splits open at its tip, and expels a cloud of infectious spores. In the Saccharomycotina, most of the yeast species engage in passive distribution of ascospores that relies upon digestion of the ascus wall. Exceptions to this include the discharge of needle-shaped spores from *Eremothecium* and *Metschnikowia*, and the slow extrusion of ascospores in *Dipodascus*. In the Pezizomycotina, ascospores are formed within multicellular fruit bodies or ascomata. The chasmothecia of the Erysiphales (powdery mildews, e.g. *Phyllosticta*) crack open to expose their explosive ascospores; cleistothecia of the Eurotiales (e.g., *Eurotium*) contain non-explosive ascospores that spill from the ascocarps when its wall fragments. Apothecial ascomycetes with operculate ascospores include *Ascobolus immersus*, a coprophilous (dung-inhabiting) fungus that has been used for research on the mechanism of ascospore discharge. The 'enormous' ascospores of this species (up to 1 mm long), project from the ascocarp, open via an operculum, and expel clusters of eight spores embedded in mucilage at a velocity of up to 18 m per second. Other species of *Ascobolus*, and ascomycetes that form larger apothecia, release plumes of ascospores from the discharge of multiple ascospores. This is called 'puffing' and creates an up-draft of air that serves to drag volleys of spores to greater heights than spores discharged when single ascospores fire (Figure 3.10). Puffing is also seen in the stalked apothecia of *Sclerotinia*, which is an inoperculate apothecial fungus.

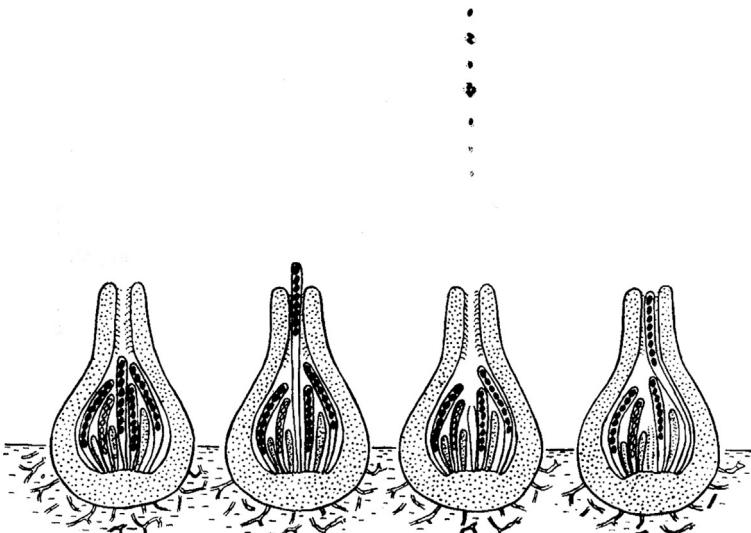


**FIGURE 3.9** Diversity of ascus morphology and ascospore discharge mechanisms. (a) *Taphrina deformans* (Taphrinomycotina). Ascii of this plant pathogen are exposed on the surface of peach leaves. They split open at the tip and discharge multiple spores in a single shot. (b) *Dipodascus macrosporus* (Saccharomycotina) produces ascospores with a mucilage coating. The spores are extruded slowly through the torn apex of the ascus. This fungus forms exposed asci. (c) Single asci of the powdery mildew *Podosphaera pannosa* (Pezizomycotina, Erysiphales) form in chasmothecia (one ascus per ascoma) and open via a slit at the apex. (d) Multiple non-explosive asci of *Emericella nidulans* (Pezizomycotina, Eurotiales) form inside cleistothecia. The ascospores of this fungus are ornamented with a double flange. (e) Asci of *Xylaria hypoxylon* (Pezizomycotina, Xylariales) discharge spores through constricting ring (apical apparatus). Ascomata of this fungus are perithecia. (f) Operculate asci of *Ascobolus immersus* (Pezizomycotina, Pezizales) expel spores when lid or operculum flips open. Ascomata of this species are apothecia. (g) Ascospore discharge from fissitunicate asci of *Pleospora herbarum* (Pezizomycotina, Pleosporales) occurs after outer wall of ascus ruptures to allow expansion of the inner wall. The fruit body of this species is a pseudothecium. Source: Mark Fischer, Mount St. Joseph University, Cincinnati.



**FIGURE 3.10** Classical illustrations of mass discharge or puffing of ascospores from apothecia. (a) First published illustration by Micheli, P.A. 1729. *Nova Plantarum Genera*. Florence, Bernardi Paperinii. (b) Source: Bulliard, P. 1791. *Histoire des champignons de la France, ou, Traité élémentaire renfermant dans un ordre méthodique les descriptions et les figures des champignons qui croissent naturellement en France*. Paris. Chez L'auteur, Barrois, Belin, Croullebois, Bazan.

In perithecial ascomycetes, the ascus apex is thickened in the form of an apical apparatus that operates as a sphincter, maintaining ascus pressure and separating spores as they are discharged. The discharge process in these fungi has been studied in greatest detail in *Podospora* and *Sordaria* (Figure 3.11); pathogens with this type of ascus include species of *Nectria* and *Gibberella*. Explosive ascospore discharge is not a universal feature of the



**FIGURE 3.11** Ascospore discharge in *Sordaria fimicola*. Asci elongate through the neck of the perithecium, one at a time, discharge their spores, and retract, providing space for expansion of the next ascus. Source: Ingold, C.T. 1971. *Fungal Spores: Their Liberation and Dispersal*. Clarendon Press, Oxford.

perithecial ascomycetes. Many species exude masses of spores and sticky sap to form cirri that protrude from their perithecia. Spores of these fungi are dispersed, secondarily, by insects. *Ophiostoma* species work in this fashion, their ascus walls dissolving within the peritheciium to liberate an ooze of spores. The distribution of the slow-exudation process among groups of perithecial ascomycetes that exhibit explosive spore discharge is consistent with the loss of this 'violent' mechanism in multiple lineages.

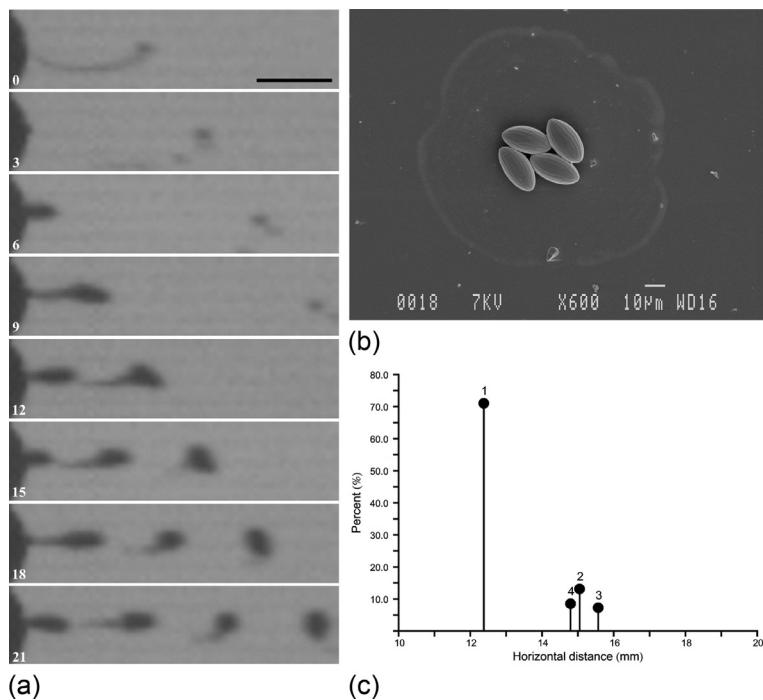
Finally, many ascomycetes with bitunicate asci engage in a two-stage discharge mechanism in which the outer wall (ectotunica) of the ascus ruptures, allowing the inner wall (endotunica) to elongate, and discharge the spores when its apex ruptures. The bitunicate ascus, also referred to as fissitunicate, is formed in fruit bodies called **pseudothecia** that resemble perithecia or other types of fruit body. Important pathogens that form bitunicate asci include species of *Cochliobolus*, *Mycosphaerella*, *Pleospora*, and *Venturia*.

The explosive action of the individual ascus has been studied for more than a century, and in most species we know that the ascus functions as a pressurized spore gun whose dehiscence spouts spores into the air along with a stream of sap. Progress in understanding the biomechanics of ascus function has come from a combination of high-speed video microscopy, spectroscopic analysis of ascus fluid, and mathematical modelling (Figure 3.12). Ascii of *Neurospora tetrasperma* discharge four ascospores that may remain connected by mucilage during flight, or separate from one another. Before discharge, the spores are bathed in fluid containing inorganic ions (potassium and chloride) and sugar alcohols that generate an internal turgor pressure of a few atmospheres. When the tip of the ascus bursts open, the spores and surrounding fluid are expelled at an average speed of 16 m per second. The spores and fluid droplets travel over a horizontal distance of up to 20 mm. A similar mechanism has been demonstrated in *Gibberella zae*, which causes head blight of cereals.

Discharge distance varies from a few millimetres to tens of centimetres among the ascomycetes. Species of *Podospora* are among the record holders, with *Podospora dicipliens* firing its spores for more than 0.5 m. These spores are shot over a distance of 16,000-times the length of the spore. A comparably impressive cannon would propel a human over a distance of 30 km. In reality, gravity brings a human fired from a circus cannon to the ground just a few metres from the muzzle. Gravity certainly affects the range of the ascus, but drag from the air acts as a far greater brake. Spores decelerate during flight at a rate that depends on this drag force and the mass (or inertia) of the spore according to Newton's Second Law, force = mass × acceleration. As the size of the spore increases, both the drag and mass increase. The mass, however, growing as the cube of the spore volume, becomes increasingly significant so that larger spores experience less deceleration than smaller ones and traverse greater distances. For the same ascus turgor pressure and launch speed, larger projectiles travel farther than smaller ones. This principle probably accounts for the evolution of mucilage coats and appendages that connect the spores and increase projectile mass in coprophilous ascomycetes like *Ascobolus* and *Podospora*, whose asci exhibit the greatest range.

### Active Conidial Discharge

A number of turgor-driven mechanisms of conidial discharge have been described, but there has been little recent work on their operation. It seems likely that these are quite widespread and may be important in determining the spread of crop diseases. Conidia of the rice blast fungus, *Magnaporthe grisea* (Pezizomycotina), are propelled over a distance of



**FIGURE 3.12** Ascospore discharge in *Neurospora tetrasperma*. (a) Launch process captured using high-speed video camera running at one million frames per second. Composite shows every third frame from 21 µs of footage. In the first two frames, a plug of cell wall material is discharged from the tip of the ascus. This is followed by the release of the four spores inside the ascus. The maximum launch speed in this fungus is 32 m per second or 115 km per hour. The spores tumble during flight. Scale bar = 20 µm. (b) Scanning electron micrograph of spores shot from single ascus onto a glass slide. The spores are surrounded by a dried deposit of ascus sap and the plug of material discharged from the tip of the ascus rests below the spores in this image. (c) Distribution of spore discharge distances measured from spore deposits: 70% of the spores travelled singly and covered a mean distance of 12 mm; spores that travelled in groups of 2, 3, or 4 spores connected by strings of ascus sap moved farther. This illustrates the relationship between projectile mass and range.

up to 0.5 mm under humid conditions. Details of the mechanism are unclear, but it seems to involve the rupture of pressurized stalks that connect the spores to their conidiophores. A clearer case of turgor-driven conidial discharge is seen in species of *Nigrospora*. This genus includes *Nigrospora oryzae* that causes ear rot of corn. The globose conidium of *Nigrospora* is subtended by a supporting cell and connecting ampulliform cell. Squirting of fluid from the ampulliform cell through a nozzle in the supporting cell propels the 20 µm diameter conidium over a distance of 1–2 cm. Conidial discharge may also be driven by supporting cells whose walls spring outward under pressure but do not burst. This mechanism has been described in *Epicoccum nigrum*, *Arthrinium cuspidatum*, and *Xylospasma furcata*. The large conidia of entomopathogenic species of *Conidiobolus*, *Erynia*, *Entomophthora* (secondary conidia), and *Furia* are discharged by a similarly rapid pressure-driven eversion of the two-ply septum between the spore and conidiophore apex. The range of this mechanism can reach several centimetres. A related process is also involved in discharging rust aeciospores. Rather than



FIGURE 3.13 Sporangium of the chytrid *Obelidium mucronatum* expelling mass of zoospores. The wall of the sporangium is elastic and shrinks as the contents are discharged. Source: Joyce Longcore, University of Maine.

septal eversion, pressure boosted by high humidity results in the sudden rounding-off and mutual repulsion of aeciospores, but the mechanical details have not been examined. The efficacy of the mechanism is obvious, however, from the shower of spores shot to distances of up to 1.0 cm from mature aecia.

#### Zoospore Discharge

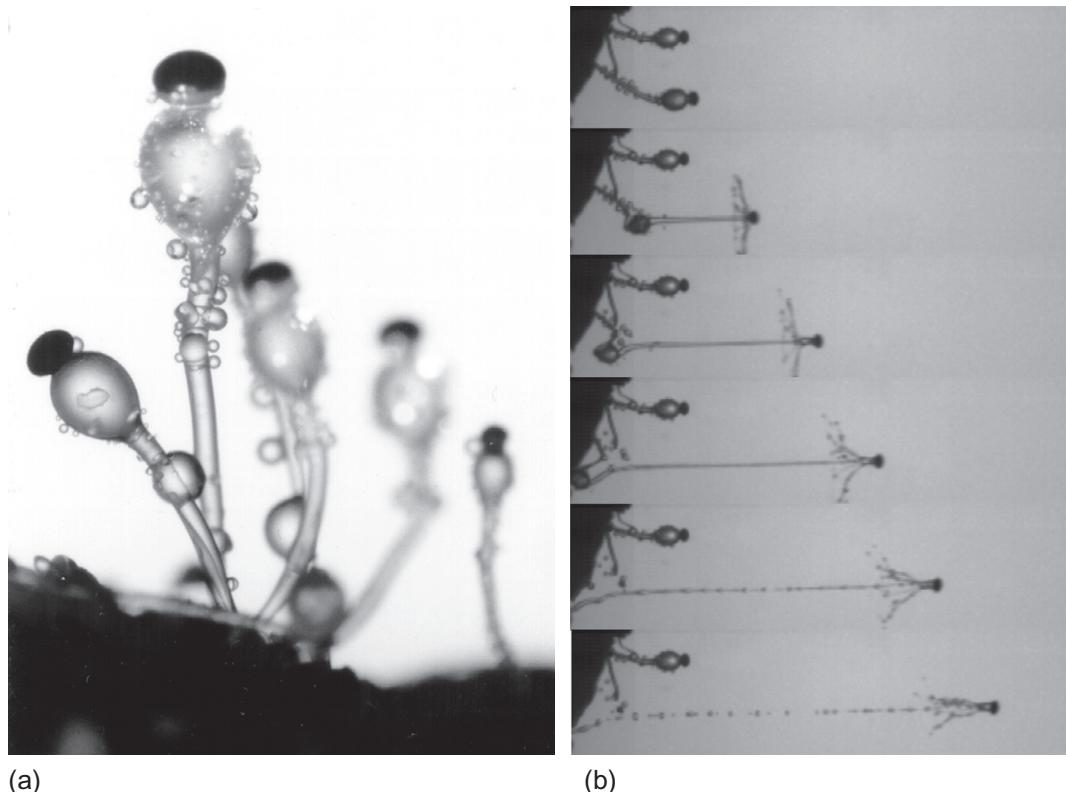
There is considerable variation in the process of sporangial emptying in the Chytridiomycota (Figure 3.13). In the simplest cases, there is no obvious pressure-driven discharge: the papilla opens, sometimes at the tip of an elongated discharge tube, and the spores swim around in the chamber of the sporangium until they find the exit. In some species the papilla is capped by an operculum that is shed to initiate discharge and in others the spores pour into a vesicle. Hydrostatic pressure seems to be more important in these cases. Comparable mechanisms are found in zoosporic water moulds (Oomycota, Straminipila). In these protists, the spores are shot into the water when a papilla at the tip of the sporangium breaks, or its material is stretched into a vesicle, releasing pressure from the sporangium.

#### *Pilobolus* (Zygomycete), *Basidiobolus*, and *Entomophthora*

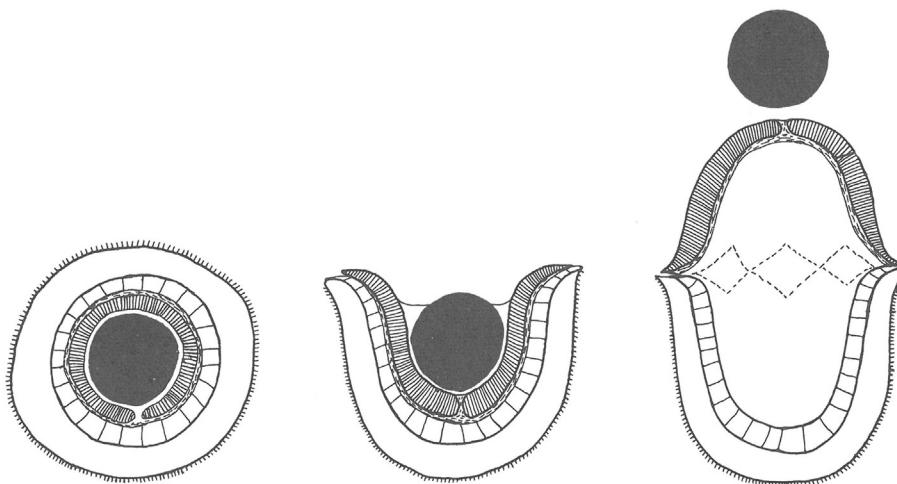
The *Pilobolus* ‘squirt gun’ is probably the best known device for spore discharge. Turgor pressure is generated osmotically within a fluid-filled translucent sporangiophore that develops from the herbivore dung on which *Pilobolus* thrives. In the commonest species, *Pilobolus kleinii*, a black-pigmented sporangium filled with 30,000–90,000 spores forms at the tip of the sporangiophore. The sporangiophore is usually 2–5 mm in height and its sporangium has a diameter of 0.5 mm. The region of the sporangiophore beneath the sporangium swells to

form a bulb that functions as a lens to direct the phototropic bending of the sporangiophore towards sunlight. The fluid within the sporangiophore contains dissolved salts and sugar alcohols and is pressurized to 5 atmospheres (500 kPa). Discharge of the sporangium occurs when a ring of cell wall at the tip of the vesicle fractures, propelling the sporangium and jet of sporangiophore fluid over a horizontal distance of up to 2.5 m (Figure 3.14). The average launch speed is 9 m per second or 32 km per hour.

There are a handful of similar mechanisms among the fungi, including the processes of conidial discharge in *Basidiobolus* and *Entomophthora*. In *Basidiobolus*, the apical part of the conidiophore is discharged along with the spore, so that the projectile resembles a microscopic rocket; the spore and this subtending conidiophore tip may separate after launch by the septal eversion process that launches some conidial fungi (see section on [Active Conidial Discharge](#)). In *Entomophthora*, the primary conidium is discharged along with a stream of sap



**FIGURE 3.14** Sporangia and sporangial discharge in *Pilobolus kleinii*. (a) The translucent stalk is a few millimetres tall and filled with pressurized fluid. The swelling at the top serves as a lens that focuses sunlight on pigments that allow the fungus to point toward the sun. (b) Discharge of the black sporangium occurs when it separates from the stalk and is blasted up to 2.5 m through the air at a speed of 32 km per hour. This sequence of images was edited from a high-speed video recording captured at 50,000 frames per second.

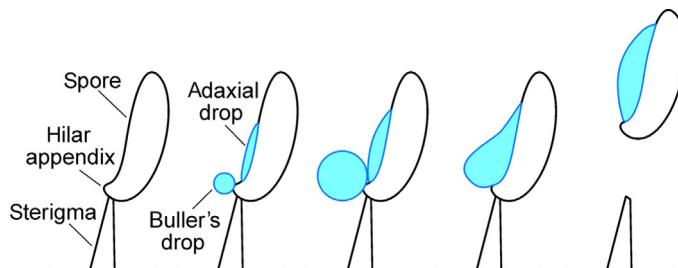


**FIGURE 3.15** The artillery fungus, *Sphaerobolus stellatus* sliced through the centre to show its multiple tissue layers. (Left) unopened fruit body; (middle) open fruit body with black capsule bathed in fluid within cup, and (right) capsule (gleba) jettisoned from triggered cup. Source: Burnett, J.H., 1976. *Fundamentals of Mycology*, second edition. Edward Arnold, London.

from its conidiophore; the septal eversion mechanism is responsible for discharging the secondary conidium of *Entomophthora* species (p. 82), illustrating how different mechanisms of spore liberation can operate in a single fungal species.

#### *Sphaerobolus* (*Basidiomycota*)

*Sphaerobolus* propels a 1 mm-diameter spherical spore-filled projectile called the gleba over distances of up to 6 m (Figure 3.15). The anatomy of the basidiome of *Sphaerobolus* is unusually complex for a fungal organ. It is spherical, no more than 2 mm in diameter, and is composed of six layers of interwoven hyphae surrounding the central gleba. At maturity, the surface of the organ fractures and opens outward to form a star-shape, with the exposed gleba sitting in its centre. In this form, the mature basidiome is best described as a cup within a cup. A slightly flaccid tennis ball, pushed inward on one side to form an unstable dimple, serves as an excellent model for the ripe fruit body. The gleba is bathed in fluid derived from the innermost tissue layer and is supported by the underlying pair of tissue layers that form an elastic 'membrane' called the peridium. The two layers of the peridium consist of a palisade of elongated, radially oriented cells whose long axes point inward, and a backing layer of tangentially oriented thin hyphae. The palisade cells solubilise sugars from glycogen reserves and become pressurized by osmosis. When the peridium is untriggered in its concave form, the exposed ends of the palisade cells are more compressed than their bases. The resulting strain within the walls of these cells is relieved when the peridium flips outward, propelling the gleba into the air at a velocity of 9 m per second (the same as *Pilobolus*). This is associated with an audible 'pop'. Buller (1933) estimated that the power required for glebal discharge is about  $1 \times 10^{-4}$  horsepower or 0.1 W.



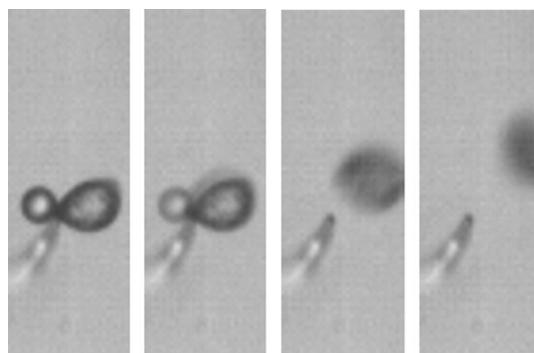
**FIGURE 3.16** Mechanism of ballistospore discharge. Source: Mark Fischer, Mount St. Joseph University, Cincinnati.

## Surface Tension

Most Basidiomycota utilize the process of ballistospore discharge (ballistospory), which is powered by the rapid motion of a fluid droplet over the spore surface (Figure 3.16). This mechanism is responsible for launching basidiospores from the gills, spines, and tube surfaces of mushroom-forming fungi, and also features in the life cycles of basidiomycete yeasts and the phytopathogenic rusts and smuts. The wide distribution of ballistospory and the similarity of the mechanism throughout the phylum suggest that it had a single origin in an ancestral group. Ballistospores have an asymmetric shape, with a prominent bulge at their base, called the hilar appendix, adjacent to the region of contact with the pointed stalk or sterigma. A few seconds before discharge, fluid begins to condense on the spore surface in two locations: (i) as a prominent droplet on the hilar appendix (called Buller's drop), and (ii) on the adjacent spore surface (called the adaxial drop). Once initiated, Buller's drop expands for a few seconds, and then, the spore and drop are discharged from the sterigma. Discharge occurs when Buller's drop reaches a critical diameter and contacts and coalesces with the adaxial drop on the adjacent spore surface. When Buller's drop moves, mass is redistributed from the hilar appendix in the direction of the free end of the spore. This imparts momentum to the spore, and the spore springs from its sterigma. Fluid movement is driven by the reduction in free energy (surface tension) when the two drops fuse, so we refer to the mechanism as a surface tension catapult.

The fluid that accumulates on the hilar appendix (Buller's drop), and on the adjacent spore surface (adaxial drop), occurs by condensation of water from the surrounding air. Condensation follows the release of mannitol and other osmotically active compounds onto the spore surface that cause a localised reduction in the chemical activity of water (or water potential) and vapour pressure. It is not clear how the osmolytes are delivered to the spore surface in sufficient concentration to act as nuclei for condensation of water, but electron microscopic studies suggest that these compounds may be prepackaged in the form of a discrete organelle in the cytoplasm of the maturing spore.

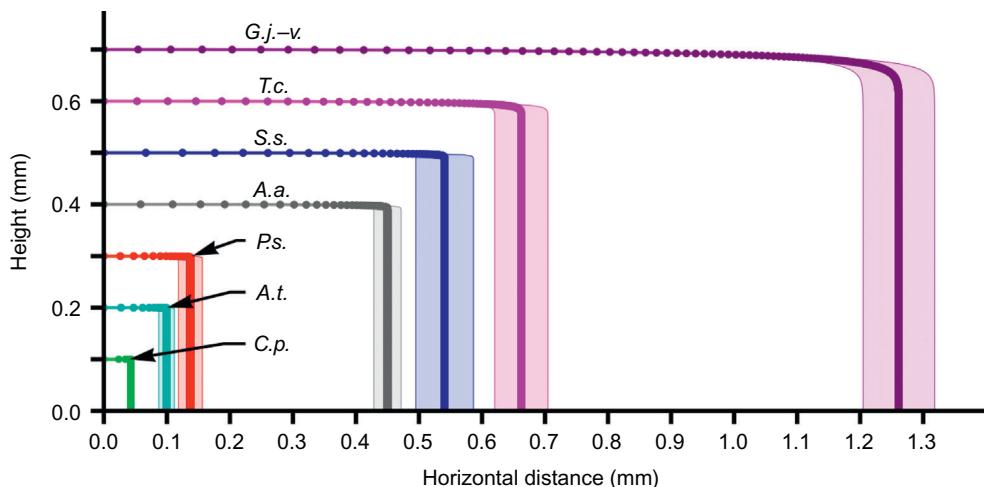
The basidiomycete yeast, *Itersonilia perplexans*, causes petal blight of *Chrysanthemum* and also causes a destructive disease called black streak on edible burdock (*Arctium lappa*). It forms large ballistospores in pure culture and has been used for biomechanical research on the discharge mechanism for many years. The launch of the spore is too fast for conventional video analysis, but has been captured using ultra-high-speed video cameras. The average initial velocity of the spore is  $0.7 \text{ m s}^{-1}$ , which is slow relative to explosive discharge of a cospores. In terms of acceleration, however, this is an impressive launch: motion of Buller's



**FIGURE 3.17** Basidiospore discharge shown in successive images from high-speed video recording captured at 100,000 frames per second. The fluid droplet at the base of the spore in the first frame coalesces with fluid on the adjacent spore surface in the second frame, which makes the spore jump into the air at an acceleration of 10,000 g. Source: Pringle, A., et al., 2005. *The captured launch of a ballistospore*. Mycologia 97, 866–871.

drop and separation of the spore from the sterigma is completed in less than  $10\text{ }\mu\text{s}$ , implying an acceleration of 10,000 g. (Figure 3.17). A variety of other basidiomycetes have been studied using high speed video and maximum launch speeds approach 2 m per second. This is interesting in light of C. T. Ingold's maxim, that 'the basidium is a spore-gun of precise range'. The ballistospore discharge mechanism is capable of propelling spores over distances of no more than 1–2 mm. The maximum range for *Itersonilia* is estimated at 1.2 mm, but many spores are propelled over distances of only a few tenths of one millimetre. This must be sufficient to clear the boundary layer on the surface of the host plant. The boundary layer varies from 0.1 to 9.0 mm in thickness depending upon wind speed. If the fungus is positioned so that its spores will fall free from the plant surface after discharge, the boundary layer does not present a problem. Spore wastage may be significant for the colony on the upper surface of a petal, however, because they will fall back on the plant after a brief flight. Similar considerations apply to other ballistosporic yeasts, jelly fungi, rusts, and smuts. For mushroom-forming species, the limited range of the discharge mechanism must be tightly controlled to ensure release into the surrounding air. Spores formed on gills, for example, must be propelled over a limited distance so that they do not hit the opposite gill. The control of discharge distance is a complex issue that involves modifications in spore morphology that affect the size of Buller's drop (Figure 3.18). These variations have been studied in many species of Basidiomycota using high-speed video.

Models of the effect of viscous drag upon spores of different sizes show good agreement between the launch speeds measured from video data and the range of the mechanism. For example, *Itersonilia* spores will travel almost 0.4 mm with an initial velocity of 0.7 m per second. The rapid acceleration of a stationary spore into air is an impressive feat of microengineering, but it is important to recognize that spore motion is also an example of impressive deceleration: as soon as the spore is discharged, the viscosity of the air begins to drag it to a halt. At any lower initial velocities, the microscopic particle would make little headway at all through air. The direction of the launch has very little effect upon the distance that the spore is propelled. In other words, the same initial velocity will shoot a spore upwards or downwards over the same distance. The influence of gravity is imperceptible until the spore is braked by air viscosity. For spores that are shot horizontally, the typical flight path may be



**FIGURE 3.18** Model spore trajectories for seven basidiomycete species based on measurements of spore size and launch speed and using Stokes model of viscous drag. To aid visualization, spores were launched horizontally from arbitrary heights. Positions of spores at 50  $\mu$ s intervals indicated by dots. The variation in horizontal range predicted from the measured variation in launch speeds ( $\pm$  standard error; Table 1) for each species is represented by the shaded region around each trajectory. Species initials: *G.j.-v.*, *Gymnosporangium juniperi-virginianae*; *T.c.*, *Tilletia caries*; *S.s.*, *Sporobolomyces salmonicolor*; *A.a.*, *Auricularia auricula*; *P.s.*, *Polyporus squamosus*; *A.t.*, *Armillaria tabescens*, and *C.p.*, *Claviciporina pyxidata*. Source: Stolze-Rybaczynski, J. L., et al., 2009. Adaptation of the spore discharge mechanism in the Basidiomycota. PLoS ONE 4(1): e4163.

aptly described as a ‘Wile E. Coyote Trajectory’, recalling the tragic canine featured in Warner Brothers cartoons. This trajectory is critical to the effectiveness of the mechanism in mushrooms whose spores are propelled from the surface of gills, spines, and tubes.

Calculations show that the hypothetical link between drop movement and spore discharge makes sense energetically: minuscule droplets of fluid certainly possess sufficient energy (in the form of surface free energy or surface tension) to discharge spores the appropriate distance. Second, it is clear from mathematical models that any change in spore and drop size will have dramatic effects on the launch speed and discharge distance. For constant spore size, an increase in the radius of Buller’s drop will tend to catapult the spore over a greater distance. Similarly, any decrease in drop size will reduce the launch speed and range. Calculations show that the relative size of the spore and drop are the crucial variables. The hydrophobic nature of the spore surface surrounding the hilar appendix causes the water condensing in this spot to remain as a discrete drop until the moment of discharge. Because Buller’s drop forms on the end of the hilar appendix, longer appendices will tend to support larger drops because the drop must expand further before it will contact and run over the adjacent spore surface. This is one example of the kind of microscopic morphological detail that may control the dynamics of the launch process. The remarkable variation in spore morphology among the Basidiomycota may reflect the control of discharge speed and distance necessitated by the evolution of diverse fruit body forms.

Experiments in wind tunnels show that mushroom shapes may enhance spore release by interrupting airflow, reducing airspeed directly beneath the cap in a way that protects falling

spores from being blown back onto the gills. Evaporation of water from mushrooms has additional effects on dispersal. Evaporative cooling of the spore-producing tissues has been measured with thermocouples and this temperature drop is thought to promote condensation of water on the spore surface. Evaporation of water from mushrooms has the added effect of creating local airflow patterns that may sweep spores away from the fruit body.

The reliance of the ballistospore discharge mechanism upon condensation limits spore discharge to wet environments. Discharge of the ballistosporic stages of pathogenic yeasts, rusts, and smuts can occur only under conditions of high ambient humidity. Mushroom-forming fungi have some degree of control over the humidity of the air between their gills, but even these organisms are restricted to moist habitats and fruit after rainfall. One mushroom, discovered in Oregon, produces submerged fruit bodies in rivers and maintains air pockets between its gills in the form of trapped bubbles. Ballistospore discharge proceeds in the usual fashion and the spores accumulate in rafts at the bottom of the gills and drift downstream in water currents. The surface tension catapult mechanism is not found in other fungi, but an analogous form of fluid movement discharges the spores of the protostelid slime mould *Schizoplasmodium cavostelioides* and related species.

## DISPERSAL OF SPORES AND AEROMYCOLOGY

### **Plant Disease Epidemiology**

The concentration of airborne fungal spores decreases in proportion to the distance from their point of origin. For spores dispersed from an infected tree, for example, the number of spores per cubic metre of air is highest close to the plant surface and decreases in the direction of the airflow. Dispersal patterns are of obvious significance to plant pathologists trying to predict the spread of an epidemic disease and the modelling of spore clouds is part of the specialized research field called plant disease epidemiology. Models of dispersal and disease transmission tend to be very complicated because a large number of variables affect spore movement. The concentration of spores at a particular distance from the source is determined by multiple factors including: (i) the number of spores released per minute, (ii) windspeed, (iii) air turbulence, (iv) convection currents, and (v) the sedimentation rate of the spores. When this physical puzzle is scaled up from a single source to an infected stand of trees, or to fields of infected crops, the complexities of disease forecasting become evident. Epidemiologists must also consider genetic variations in plant disease resistance, weather conditions, and other factors in forecasting the severity of the disease once the spores are deposited on the plant surface.

### **Mushroom Spores and Atmospheric Chemistry**

Experiments on mushrooms have shown that most spores released from the cap are deposited quite close to the basidiome ([Figure 3.19](#)). In a study of ectomycorrhizal mushrooms, 95% of the spores fell within 1m of the basidiome. This seems exceedingly wasteful, given the likely premium upon longer distance dispersal from the parent colony, and may speak to the reasons that fungi produce such large numbers of spores. It has been suggested that

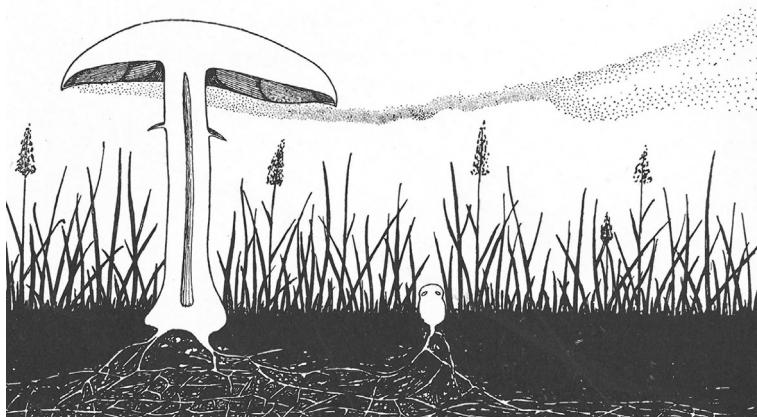


FIGURE 3.19 Cloud of spores dispersed from gills of *Agaricus arvensis*, the horse mushroom. Source: Buller, A.H.R., 1909. *Researches on Fungi*, vol. 1. Longmans, Green, and Co., London.

sampling limitations may have led investigators to conclude that such a high proportion of the total spore output settles close to the fruit body. Nevertheless, dense spore deposits do accumulate beneath mushrooms producing a visible carpet on the surrounding vegetation. Spore deposits on the surface of the caps of bracket fungi reveal the additional wastage of spores deposited from air currents swirling around the fruit body.

Irrespective of the proportion of spores that land close to their source, the fecundity of many species ensures that large numbers of spores are carried longer distances from the parent colony. If only 5% of the spores released from a mushroom travel more than 1 m, at least 135 million spores per day will escape the immediate neighbourhood of a single basidiome of *Agaricus campestris* (that discharges an estimated 2.7 billion spores), and more than one billion spores per day will disperse from *Ganoderma applanatum* (that discharges 5 trillion spores during 6 months of annual activity). Fungi invest huge resources in sporulation and natural selection has 'calculated' the likely losses and small probability of long-distance transmission and survival. The fate of the spores that do land close to the parent mycelium is unknown.

Despite the impediments to becoming airborne, an estimated 50 million tons of fungal spores are dispersed in the atmosphere every year, corresponding to more than  $10^{23}$  spores. This approximation comes from studies by atmospheric chemists who have measured the concentration of mannitol in the air above rainforests. Sugar alcohols are a good proxy for spore numbers because they are carried on basidiospores and ascospores: mannitol, which is concentrated in Buller's drops and in ascus sap, clings to the spore surface after discharge. Spore clouds above rainforests are particularly rich in basidiospores from saprotrophic and mycorrhizal species. It has been suggested that these mushroom spores may act as nuclei for cloud formation, supporting the heavy rainfall that sustains these ecosystems.

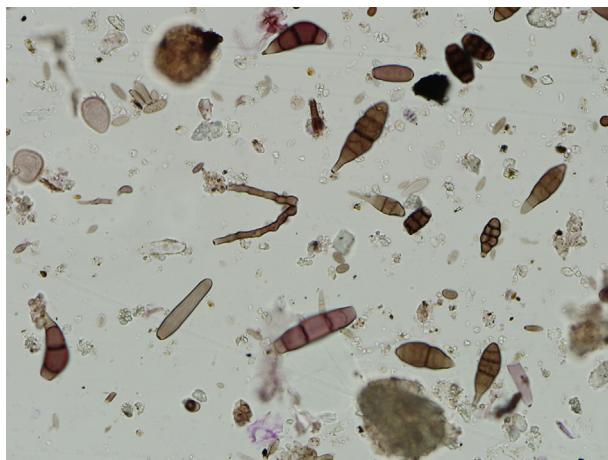
## Sampling Methods

Until the introduction of molecular methods for identifying fungi, there was no substitute for the painstaking microscopic examination of air samples to determine the numbers of airborne spores and their identity. A very simple form of air sampling is carried out by exposing microscope slides coated with adhesive. This was used in the nineteenth century to collect spores of plant pathogens moving through crops and, early in the twentieth century, for analyzing air samples using aircraft and balloons. The spores on these slides can be identified, or, at least, assigned to a genus or larger taxonomic grouping. They can be counted too, but it is impossible to relate these numbers to the concentration of spores in the air unless the volume of air passing over the slides is known. This limitation was addressed with the development of a series of ingenious instruments for air sampling.

The earliest volumetric sampling devices were tested in the nineteenth century and relied on trapping spores in cotton plugs. Impactor traps that deposited spores on sticky slides were introduced in the 1940s and refined as the Burkard spore trap (based on the original Hirst trap) that remains in use today. This device employs a vacuum pump to suck air through a slit at a defined rate and particles are collected on sticky slides or adhesive tape. By moving the slide or tape beneath the slit at a controlled speed using a clockwork mechanism, the Burkard trap is used to record spore identity and concentration for up to 1 week. Rotorod traps offer a competing design in which spores are collected on the surface of adhesive tape attached to U-shaped arms that are rotated through the air by a motor. Both types of trap are also used for pollen counting. Other kinds of impactor traps expose Petri dishes containing culture medium to the air. These allow the investigator to estimate the concentration of viable spores from the number of colonies that develop in the Petri dish after a short incubation.

Filtration devices that employ disposable cassettes have become very popular instruments for studying airborne spores. After filtering a measured volume of air using a vacuum pump, the filters are removed from the cassettes and transferred to a microscope slide for identifying and counting of spores (Figure 3.20). The standard unit for spore concentration is number per cubic metre. Spore concentrations in outdoor air vary greatly, of course, according to geographical location, seasonal patterns of plant growth and decomposition, and weather conditions. Average spore concentrations in cities range from a few hundred to a few thousand spores per cubic metre. A study in San Diego showed averages of 200 spores  $\text{m}^{-3}$  during calm wind conditions rising to 80,000 spores  $\text{m}^{-3}$  during high wind. Similar numbers have been recorded in many cities in North America and Europe, with exceptional readings in excess of 100,000 spores per cubic metre.

Most samples of outdoor air are populated by spores of ascomycetes, with conidia of *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* being the most frequent types. Other fungi are more common where particular crops are cultivated. The frequency of mushroom spores rises in heavily forested locations. Record spore concentrations have been associated with farming practices. A British study of farm buildings found a maximum concentration of 68 million spores  $\text{m}^{-3}$  when mouldy hay was shaken. Spores of *Aspergillus* and *Mucor* dominated these extraordinary samples. Higher spore numbers quoted in various sources may have conflated spores produced by actinobacteria (called actinomycetes in older literature) with the somewhat lower frequencies of fungal spores.



**FIGURE 3.20** Air sample with spores and spore fragments captured by filtration. *Source: Estelle Levetin, The University of Tulsa.*

Besides their significance in forecasting allergy symptoms, information on the prevalence of fungal spores is also of interest to patients suffering from a variety of illnesses including chronic obstructive pulmonary disease and cystic fibrosis. Both conditions are exacerbated by lung infections caused by fungi, including *Pneumocystis jiroveci*, *Scedosporium prolificans*, and species of *Aspergillus*. Inhalation is the route for many fungal infections, including histoplasmosis, coccidioidomycosis, and deep-seated mycoses of the central nervous system caused by *Cryptococcus* species. Information on the concentrations of spores of these opportunistic pathogens is useful for understanding geographic hotspots of infection by different fungi.

The study of aeromycology is benefitting from the introduction of molecular methods and metagenomic research in identifying broad patterns in the diversity of fungi. Molecular analysis of air samples taken from locations in the middle of continents, coastal ecosystems, and above the ocean showed that mushroom spores dominated the air in continental locations. The proportion of ascomycete spores increased over coastal habitats and exceeded the numbers of mushroom spores in the air samples collected at sea. Related studies have tracked seasonal changes and shown, as one would predict, increases in mushroom spores during peak periods of fruiting. Molecular studies have also revealed a surprising diversity of fungi in buildings and found a greater diversity of fungi in temperate compared with tropical locations. Surprisingly, the latitude of the sampled building is a better indicator of fungal diversity than its construction materials. This is interesting in light of the observed correlation between the incidence of childhood asthma and distance from the equator.

## DISPERSAL OF SPORES BY ANIMALS

Animals are important vectors for the dispersal of fungal spores. Given the great sweep of fungal diversity and abundance of invertebrates in the habitats in which fungi grow, we are probably aware of only a small fraction of these dispersal mechanisms. The discharge mech-

anisms that have evolved in many coprophilous fungi, including bird's nest fungi, diverse ascomycetes, and the zygomycete *Pilobolus*, deposit spores on vegetation that is eaten by herbivores. After passage through the digestive system of the animal, the spores are released at varying distances from the parent mycelium when the animal defecates. The vector does not gain any benefit from these interactions. The fungus is simply exploiting an available source of mobility in its reproductive strategy. In at least one case the vector is harmed by its interaction with a coprophilous fungus. Parasitic nematodes can hitch a ride on the *Pilobolus* sporangium when it is squirted into the air and cause lung disease in mammals.

Besides the carriage of spores inside animals (endozoochory), fungi are dispersed on the surface of their vectors (ectozoochory). Ascospores that ooze from their ascii to form sticky cirrhi that are smeared on the bodies of insects. In some cases, the ascomycete may secrete a specific chemical attractant; in others, contact between fungus and vector is non-specific. Stinkhorns and other members of the Phallales attract invertebrate vectors to their fruit bodies using volatile chemicals in a way that resembles the relationships between flowers and their animal pollinators (Chapter 1). A comparable strategy is used by ascomycete truffles and basidiomycete false truffles that form subterranean or **hypogeous** fruit bodies. Ascomycete truffles are members of the Pezizales and evolved from cup fungi that discharge their ascospores into the air. The black or Périgord truffle, *Tuber melanosporum*, and the white truffle, *Tuber magnatum*, are the best-known species, but there are hundreds of other truffles. Dispersal of *Tuber* species by mammalian vectors is stimulated by fungal synthesis of a potent mammalian pheromone called alpha-androstenol. Boars are among the animals that produce this steroid in their saliva and this accounts for the attraction of sows to truffles and their traditional use in truffle hunting.

False truffles have evolved from gilled mushrooms in the Russulales and poroid mushrooms in the Boletales. Evolution of the hypogeous morphology is thought to involve an intermediate stage in which the spore-producing tissues remain covered as the fruit body develops. Fruit bodies of this kind are called **secotioid**. The secotioid morphology may reduce water loss by evaporation from the fertile tissues, but limits, or completely eliminates, wind dispersal of spores. The evolution of the secotioid and hypogeous fruit bodies is accompanied by the loss of the spore discharge mechanism using Buller's drop and adaptations that favour consumption by animals. The loss of ballistospory in false truffles is matched by a loss of active ascospore discharge in ascomycete truffles. This process is thought to have occurred during the evolution of *Rhizopogon*, a genus of ectomycorrhizal false truffles related to species of *Suillus* (Boletales) that form poroid mushrooms. *Rhizopogon* spores are spread by numerous species of small mammal in coniferous forests.

Provocative experiments on a bioluminescent mushroom species, *Neonothopanus gardneri*, suggest that the emission of green light according to a circadian rhythm acts as a lure for insects during darkness. Investigators found that acrylic models of fruit bodies illuminated with green light-emitting diodes were more effective at attracting insects than non-luminescent control models. The role of insects as vectors for spore dispersal in *Neonothopanus gardneri* is unproven, but this species grows in Brazilian forests at the base of palm trees where the fruit bodies are shielded from wind. In sheltered habitats of this kind, insect dispersal may be an important adjunct to wind dispersal for luminescent and nonluminescent mushrooms.

## AQUATIC SPORES

### Non-Motile Aquatic Spores

Spores of aquatic fungi engaged in leaf decomposition are plentiful in calcareous streams. These are conidia of ascomycetes, and a smaller number of basidiomycetes, called Ingoldian fungi after C. T. Ingold (1905–2010), who discovered them in the 1930s. It has been suggested that some of the Ingoldian fungi are endophytes and that their colonies are dispersed by leaf abscission. Ingoldian spores are striking for their elaborate shapes, including stars with four limbs connected to a central hub (tetraradiate conidia), crescents, sigmoids, commas, and miniature cloves (Figure 3.21). They form at the tips of conidiophores that develop at the surface of leaves and become concentrated in bubbles of white foam that accumulate around rocks and fallen logs obstructing water flow. Collection of foam samples provides a convenient way to study these fungi and pure cultures can be obtained by harvesting spores from leaves after a short incubation in the lab.

Spore morphology is no more important in determining sedimentation rate in water than it is for spores dispersed in air. The high viscosity of water relative to air slows the sedimentation rate of spores from millimetres *per second* to millimetres *per minute*, but most experiments show that conidia with appendages fall through the water column at the same speed as more compact spores. Indeed, one experiment showed that intact spores of marine fungi settled faster than spores whose appendages had been disrupted by sonication. The unusual shapes of aquatic spores require an alternative explanation.

The most compelling answer is that the broader span of spores with unusual shapes increases the probability that they will collide with submerged plant materials. The largest

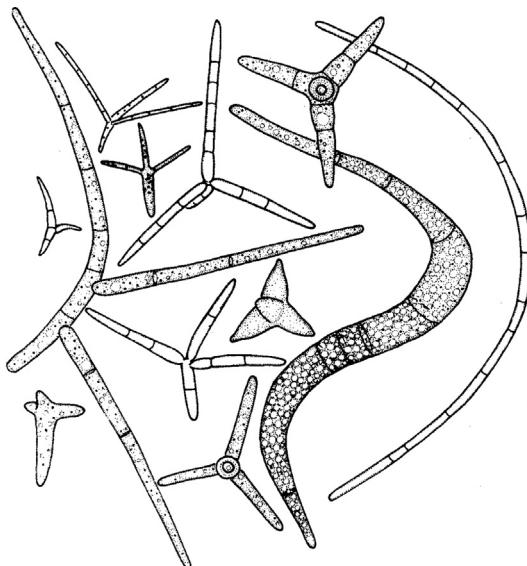


FIGURE 3.21 Variety of conidia produced by Ingoldian fungi in freshwater habitats. Source: Webster, J., Weber, R.W.S., 2007. *Introduction to Fungi*, third edition. Cambridge University Press.

conidia produced by the Ingoldian *Brachiosphaera tropicalis* have an effective diameter of 0.4 mm ([Figure 3.1f](#)). A spherical spore of this diameter would weigh approximately 40 µg; the tetraradiate spore with a central hub and slender arms is 400 times lighter, producing a similar probability of hitting a leaf fragment yet saving a considerable investment in cytoplasm. This calculation is a little simplistic because the spherical spore might reduce its volume of active cytoplasm by expanding a large fluid-filled vacuole. Also, the surface area of the tetraradiate spore is only 30 times less than the surface of the sphere, which means that the economy in cell wall production is more modest than the potential reduction in cytoplasm. Nevertheless, the concept of the spore as a search vehicle probably explains the significance of the beautiful spore shapes in these fungi. The utility of the spore morphology with multiple appendages is evident from its convergent development in basidiospores of the marine wood-rotting basidiomycete *Nia vibrissa*.

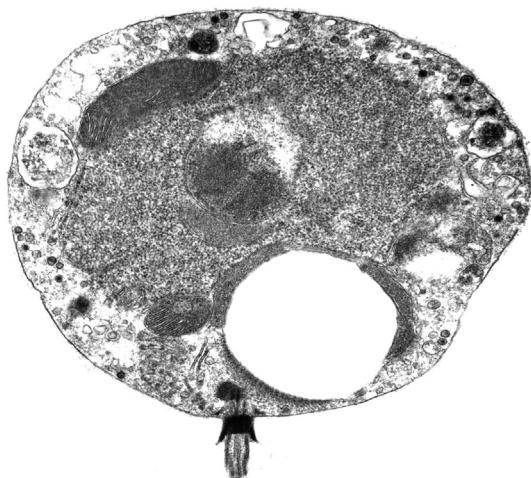
The extended shapes of these aquatic spores may confer other advantages. Experiments on tetraradiate conidia show that when one arm of a spore strikes a target, the spore pivots around this point of attachment allowing the fungus to make a stable three-point landing. Leaf colonization begins when the tips of the arms cement themselves to the surface and slender hyphae grow from the triangle of contacts. Enhanced dispersal in surface films is another possible benefit of this spore morphology and helps explain the concentration of spores in foam. It has been suggested that spores trapped in foam may become airborne as the bubbles collapse. This would explain how some of these aquatic fungi establish themselves as endophytes in plants growing above the water.

A different adaptation is observed in aeroaquatic conidia that form at the air–water interface in stagnant ponds. These spores develop by helical growth of hyphae to form barrels with an air bubble trapped in the centre. Dispersal occurs by floating on the surface of the water and these fungi colonize leaves and decaying wood. Many other fungi that grow on plant debris in aquatic environments do not show any obvious morphological adaptations to their habitats.

## Motile Aquatic Spores

Motile fungal spores called zoospores have a single posterior flagellum that pushes them head first through the water ([Figure 3.22](#)). This is the structural signature of the Opisthokonta supergrouping that encompasses the fungi and animals. Zoospores are produced by Blastocladiomycota, Chytridiomycota, Neocallimastigomycota, and diverse zoosporic fungi of uncertain taxonomic assignment included in the Cryptomycota (Chapter 1). Exceptions to the uniflagellate structure of fungal zoospores are found in some of the anaerobic gut fungi in the Neocallimastigomycota that produce spores with multiple flagella. The majority of zoospores function in dispersal and allow the fungus to locate new sources of nutrients. Motile uniflagellate cells also serve as gametes in the sexual cycles of Blastocladiomycota.

The fungal zoospore is a spherical or ovoid cell that lacks a cell wall. The absence of a wall means that the cell must regulate water influx without developing turgor pressure: unregulated osmosis would burst the naked zoospore. Contractile vacuoles have been observed in some chytrid zoospores and additional control of water influx is achieved via active ion exchange through the spore membrane. Zoospore propulsion is driven by high-frequency undulation of the flagellum from base to the tail and a velocity of 100 µm per second (20-times



**FIGURE 3.22** Transmission electron micrograph of a single zoospore of the chytrid *Chytridium lagenaria*. The base of the single flagellum is visible at the bottom of the spore. The large circular structure is a contractile vacuole. *Source: Peter Letcher, University of Alabama.*

cell length per second) is typical for a chytrid. Zoospores can swim for many hours in a culture dish or glass microscope chamber. They spend most of the time swimming in straight lines or following circular paths. Unlike the flagellate cells of many other eukaryotic micro-organisms, chytrid spores swim without rotation of the cell. This smooth gliding motion is interrupted by momentary flicks, jerks, and changes in direction. Changes in direction are controlled by bending of the flagellum toward its base so that it acts as a rudder. Zoospores stop swimming periodically too, even though the flagellum keeps lashing from side-to-side and curling around the stationary cell. Chytrid zoospores show amoeboid motion over surfaces for relatively short distances and can switch repeatedly between this behaviour and swimming freely in the water.

Unlike airborne spores, which have no need to draw upon nutrient reserves until germination, zoospores are powered by the continuous oxidation of lipids and other stored fuels. This limits their period of activity. A zoospore swimming at an average speed of  $25\text{ }\mu\text{m}$  per second (allowing for frequent stops) for 5 h would travel 0.5 m. Observations on the erratic swimming patterns of zoospores in the lab suggest that a journey over this distance in a straight line is unlikely. Flagellar movement and amoeboid locomotion are probably effective over quite short distances and allow the spores to explore limited zone in which they can detect chemical gradients that provide cues to nutrient availability. Experiments have shown that dissolved amino acids and sugars attract chytrid zoospores. It is likely that more distinctive compounds released from host cells are also used for chemotaxis by species that infect plants and animals. Adhesion to host surfaces is accompanied by retraction of the flagellum into the cell and the formation of a cell wall to create a cyst. Penetration of the host cell occurs via the growth of a penetration hypha from the cyst. Motile zoospores and cysts that are unattached to surfaces may be dispersed passively over long distances in water trickling through soils and carried by water movement in aquatic habitats.

Much more is known about mechanisms of zoospore dispersal in plant pathogenic oomycetes (*Stramenopila*) including species of *Phytophthora* and *Pythium*. Zoospores of these microorganisms have paired flagella that emerge from the side of the kidney-shaped cell. One flagellum points ahead of the swimming zoospore and is covered with fine filaments called mastigonemes, and the other lashes behind the cell. Both flagella undulate from base to tip. The presence of the mastigonemes on the anterior flagellum redirects its thrust so that it pulls the spore through the water. The posterior flagellum acts as a rudder and does not generate much propulsion. Oomycete zoospores rotate around the long axis of the cell and follow a wider helical path as they swim. Like the zoospores of fungi, swimming zoospores of oomycetes show frequent changes in direction and are adapted for nutrient detection over distances of a few centimetres.

## Further Reading

- Fischer, M.W.F., Stolze-Rybaczynski, J.L., Davis, D.J., Cui, Y., Money, N.P., 2010. Solving the aerodynamics of fungal flight: How air viscosity slows spore motion. *Fungal Biol.* 114, 943–948.
- Ingold, C.T., 1971. *Fungal Spores: Their Liberation and Dispersal*. Clarendon Press, Oxford.
- Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. (Eds.), 2008. *Dictionary of the Fungi*. 10th ed. CAB International, Wallingford, United Kingdom.
- Lacy, M.E., West, J.S., 2007. *The Air Spora: A Manual for Catching and Identifying Airborne Biological Particles*. Springer-Verlag, Berlin, Heidelberg, New York.
- Money, N.P., Fischer, M.W.F., 2009. Biomechanics of spore discharge in phytopathogens. In: Deising, H. (Ed.), *The Mycota*, second ed. In: Plant Relationships, Vol. 5. Springer Verlag, Berlin, Heidelberg, New York, pp. 115–133.
- Webster, J., 1987. Convergent evolution and the functional significance of spore shape in aquatic and semi-aquatic fungi. In: Rayner, A.D.M., Brasier, C., Moore, D. (Eds.), *Evolutionary Biology of the Fungi*. Cambridge University Press, Cambridge, pp. 191–201.