CHAPTER 20

Reaching the Wind

Boundary Layer Escape as a Constraint on Ascomycete Spore Dispersal

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CONTENTS

20.1 INTRODUCTION

Dispersal shapes the biogeography of organisms, and because dispersal is a control on gene flow, it also structures genetic diversity across populations and influences rates of speciation. Fungal spores may be dispersed long distances by air or water. Spores carried by storms or atmospheric circulation can travel between continents (Watson and De Sousa 1982; Pringle et al. 2005a; Kellogg and Griffin 2006) and research has focused on predicting and identifying rare long distance dispersal events (Brown and Hovmøller 2002; Aylor 2003; Muñoz et al. 2004). In contrast to animals, fungi are thought to have little control over the processes of dispersal, and the common view is: "…the migration of birds and mammals in search of new sites and sources of food occurs in an orderly, coordinated way with minimum wastage of progenies. Plant pathogens, on the other hand, produce enormous numbers of spores that are passively transported, scattered in all directions, and finally land on non-target sites in uncongenial environments as well as on congenial hosts" (Nagarajan and Singh 1990).

While dispersal in air or water appears passive (but see Dressaire et al. 2016), fungi actively manipulate discharge. Ascomycete and basidiomycete spores are explosively launched (Buller 1909, 1922, 1924; Ingold 1971; Money 1998; Trail 2007), and the structures involved appear exquisitely engineered to propel spores into local habitats (Roper et al. 2010). Discharge is rarely considered by ecologists, and since the start of the twentieth century it has remained the purview of a handful of mycologists (Buller 1909, 1922, 1924; Ingold 1971; Money 1998; Trail 2007) and plant pathologists (Meredith 1973; Aylor 1990).

While the structures used to launch spores are fascinating adaptations in and of themselves, spores also play a role in human health and the earth's climate. Asthma and allergy symptoms are associated with exposure to spores (Salo et al. 2006; Sahakian et al. 2008; Wolf et al. 2010; Pringle 2013), and elevated CO_2 concentrations appear to stimulate sporulation (Klironomos et al. 1997; Corden and Millington 2001; Wolf et al. 2010). A current hypothesis suggests a connection between the increasing prevalence of asthma and allergies, and global change (Wolf et al. 2010). Fungal spores are ubiquitous in the earth's atmosphere (Fröhlich-Nowoisky et al. 2009), and a growing body of work explores the role of spores as cloud condensation and ice nuclei (Iannone et al. 2011; Després et al. 2012; Fröhlich-Nowoisky et al. 2012).

Dispersal is a needed focus of current research for other reasons as well. Discerning patterns of dispersal will be essential in resolving questions about the biogeography of microbes (Fitter 2005), and understanding mechanisms will be critical in predicting the spread of recently emerged and

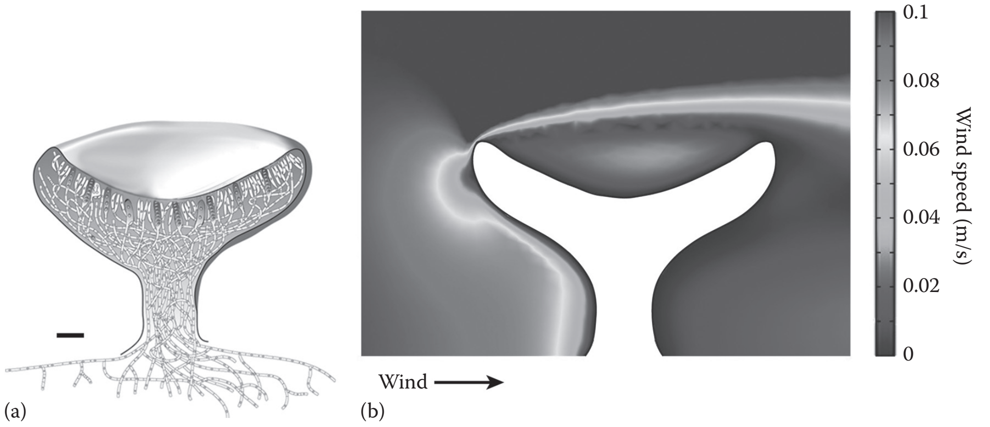

Figure 20.1 **(See color insert.)** The boundary layer is the sheath of nearly still air surrounding any object. (a) Drawing of a *Sclerotinia sclerotiorum sporocarp* (scale bar = 1 mm). Note spores within asci at the fertile surface. (b) Vertical cut showing wind speeds. Different colors mark different speeds. Dark gray delineates the boundary layer: compare spore size to boundary layer height. Speeds are the result of COMSOL finite element simulations of the three-dimensional Navier-Stokes equations with wind blowing at 10 cm/s from the left, adaptive triangular mesh, open boundaries at the borders of the computational domain, and no slip on the sporocarp.

devastating diseases of amphibians (Berger et al. 1998), bats (Foley et al. 2011), humans (Kidd et al. 2004), and plants, including crops (Aylor 1999; Meredith 1973; Rizzo et al. 2002; Isard et al. 2011). Dispersal is also likely to control the range shifts of nonpathogenic species, whether caused by accidental introductions to new habitats (Vellinga et al. 2009), or global change (van Herk et al. 2002).

In this review, we parse the startling morphological diversity of spore-shooting apparatuses, and use physical principles to provide a synthesis of their forms and function. We focus on sexual spores of terrestrial ascomycete fungi. Ascomycota is the largest phylum of fungi, and includes devastating pathogens, as well as decomposers, lichens, and mycorrhizal fungi. Ascomycetes are defined by a conserved form of ballistospory: sexual spores are formed within fluid-filled sacs, or asci. At maturity, osmolytes (Trail 2007) cause the ascus to swell and become turgid. Once a critical pressure is reached, controlled rupture ejects the spores and sap. Species with very large spores, for example, *Ascobolus immersus* (60 × 40 μm), may launch spores a meter or more (Fischer et al. 2004), but the smaller spores of most other species travel much smaller distances (Schmale et al. 2005). The active launch of fungal spores is critical, because as compared to seeds, spores are very small (Pringle 2013). Spores decelerate rapidly after release, and it is difficult for a spore to cross the boundary layer of nearly stagnant air surrounding a sporocarp.

20.2 THE PROBLEM AND HOW TO SOLVE IT: CROSSING THE BOUNDARY LAYER

The problems of very small projectiles seem unintuitive when compared to the physics of familiar larger projectiles; a bullet, baseball, or the leap of a gazelle. Spores are very small objects, often less than 15 μm long (Pentecost 1981). Their small size enables spores to be carried by weak winds. But to reach a wind, a spore must first cross the boundary layer of nearly still air surrounding the sporocarp and its substrate. Boundary layers emerge whenever air moves over an object; friction between the object and the wind causes air next to the object to slow down and creates a stagnant cover. Boundary layers around sporocarps will vary dramatically in thickness, depending on the geometry of the sporocarp and wind speed, but are typically on order of a few mm in thickness; equivalent to hundreds or even thousands of spore lengths (Figure 20.1).

20.2.1 Calculating the Thickness of a Boundary Layer

Sporocarps may form on the ground or on elevated substrates, for example, plant stems or leaves. Away from the sporocarp, wind will travel at normal speed U_{wind}. The U_{wind} around a sporocarp growing in a sheltered environment, for example, a sclerotium of a *Sclerotinia* species under a crop canopy, may be tens of centimeters per second.

At the surface of the sporocarp, strong friction causes wind speed to drop to zero. The fact of "no slip," or zero flow velocity, at an object boundary is a central tenet of fluid dynamics, discovered in the early part of the twentieth century by Ludwig Prandtl (Schlichting and Gersten 2000). The region over which the flow velocity decreases from its unobstructed speed U_{wind} to zero at the sporocarp surface is called the *boundary layer*. The thickness of this boundary layer, delta is given by

$$\delta = \sqrt{\frac{\nu L}{U_{\text{wind}}}}, \tag{20.1}$$

where ν is the kinematic viscosity of the air (equal to the ratio η/ρ_{air} of viscosity η, to density of air ρ_{air}; for air at room temperature, $\nu = 0.1$ cm^2/s) and L is the length scale over which air is moving (e.g., the span of the sporocarp and the leaf on which it grows).

Although detailed theories of boundary layer characteristics can be complex (Schlichting and Gersten 2000), the basic physics underlying Equation 20.1 is simple: imagine a fluid particle moving in the wind which comes very close to the surface of the sporocarp. The particle slows down dramatically, due to the flow's interaction with the solid surface. This particle can only return to the free stream wind velocity by being jiggled and buffeted by neighboring fluid particles, which also have been slowed down by the sporocarp. The jiggling and buffeting causes the motion of the particles to be diffusive, so that the particle will move according to the law

$$y(t) = \sqrt{\nu t}, \tag{20.2}$$

where $y(t)$ is the distance that the particle moves away from the sporocarp in a time t.

Now the total amount of time that it takes for the wind to blow over the sporocarp and its substrate of size L is

$$t = L/U_{\text{wind}}. \tag{20.3}$$

This is the maximum time the particle has to return to the free stream. Hence, combining Equation 20.3 with Equation 20.2 yields the (maximum) thickness of the boundary layer, Equation 20.1.

Our discussion assumes the boundary layer is laminar, in other words, stable, steady, and not turbulent. The small sizes of sporocarps and sheltered habitats in which they often grow make this a realistic assumption, but more complex boundary layers are possible.

20.2.2 Mechanisms Enabling Spores to Escape Boundary Layers

Mechanisms enabling spores to escape boundary layers are critical to the fitness of individuals. If the range of a spore is less than the thickness of the boundary layer, the spore will fall back on its parent and be unlikely to establish as an independent fungus.

Spores are ejected at enormous speeds (typically 1–20 m/s, Yafetto et al. 2008), but decelerate much more rapidly than macroscopic projectiles. The range of any forcibly ejected body is determined by a balance of forces: the resistance caused by air-drag, causing deceleration, the inertia of the body itself, working to maintain the body at its original speed, and gravity. Small objects have great difficulty moving through still air because of the different ways resistance and inertia scale with the body size. For a spore, resistance stems almost entirely from the viscosity (or "stickiness") of the air. The viscous force on a spore is directly proportional to its size (Purcell 1977; Vogel 2005; Fischer et al. 2010). So, leaving all other parameters the same, halving the size of a spore halves the total viscous force. On the other hand, the inertial force scales with the spore volume, that is, is proportional to the cube of its size. Halving the size of a spore reduces its inertia eightfold. Vogel (2005) draws an analogy between forcibly ejecting a spore and throwing a balloon. In both cases, resistance greatly exceeds inertia, causing rapid decelerations.

If a spore is ejected from the ascus with velocity V_{spore}, it decelerates according to

$$m\frac{\mathrm{d}v}{\mathrm{d}t} = -\zeta v, \tag{20.4}$$

which balances the spore's inertia with air drag. In Equation 20.4, ζ is the viscous drag coefficient, linearly proportional to the spore size. Equation 20.4 can be solved to find the maximum distance the spore can travel:

$$x = V_{\text{spore}}\frac{m}{\zeta}. \tag{20.5}$$

For the spore to escape the boundary layer, this distance must exceed the boundary layer thickness

$$V_{\text{spore}}\frac{m}{\zeta} \geq \delta = \sqrt{\frac{\nu L}{U_{\text{wind}}}}. \tag{20.6}$$

Equation 20.6 is the fundamental constraint of boundary layer escape. It includes three different parameters which can be manipulated by fungi to maximize the probability of spore dispersal (Table 20.1, Figure 20.2); V_{spore}, m/ζ, and L/U_{wind}:

20.2.2.1 Manipulating V_{spore}

The spore ejection velocity V_{spore} is determined by the chemistries causing osmotic imbalance and pressure within the ascus, and the various morphologies maintaining the swollen ascus and propelling the spore out of the ascus. By equating the spore's kinetic energy to the work done to accelerate the spore, we can relate V_{spore} to the overpressure Δp and spore density ρ_{spore}:

$$V_{\text{spore}} = \sqrt{\frac{2\Delta p}{\rho_{\text{spore}}}}. \tag{20.7}$$

In the absence of dissipation, the predicted speed is independent of the spore shape and size, defined as the radius of a sphere with the same volume of the spore (Roper et al. 2008). Although different species use different osmolytes to create turgor pressure (Fischer et al. 2004), overpressure appears to be broadly conserved at about 0.3 MPa (but see Trail et al. 2005), suggesting most species function at a limit caused by physiological or biomechanical constraints, for example, the strength of the ascus wall (Fritz et al. 2013; Trail and Seminara 2014).

Table 20.1 Escaping the Boundary Layer, and Traveling in Wind

Adaptation	Mechanism	Examples	Key Citation
(1) Increase Velocity			
Match spore size to ascus opening	Optimization is key: if a spore is too large, friction will slow it down during launch. If a spore is too small, fluid surrounding the spore will be lost, decreasing the propulsive force pushing it.	Poricidal Ascomycetes	Fritz et al. (2013)
(2) Increase the Ratio m/ζ: Minimize Drag or Make Ejected Mass Heavier			
a. Streamlined/ drag minimizing spore shapes	Shapes of many species match theoretically generated, idealized drag-minimizing forms.	Species of *Neurospora*	Roper et al. (2008)
b. Puffing	Coordinated ejection of thousands of spores generates a wind.	*Sclerotinia sclerotium*	Roper et al. (2010)
c. Spores are shot as a group, but are not bound by mucous	Clumped spores would have greater mass and more easily cross the boundary layer.	Species of *Podospora*	Ingold (1928)
d. Mucilaginous appendages or sheaths	Mucous sticks spores together. By promoting cohesion during launch, projectile mass is increased, but mucous would also allow spores to disassociate and disperse independently after ejection.	Species of *Podospora*	Ingold (1939)
(3) Manipulate the Boundary Layer			
Long, pointy fruit bodies/sporocarp optimization	Boundary layers are minimized around the tips of elongate objects.	Species of *Cordyceps*	
(4) Decrease the Ratio m/ζ: Increase Drag or Make Traveling Parts Lighter			
a. Other appendages, and sheaths	Appendages and sheaths add mass to facilitate crossing the boundary layer, but also increase drag.	Various species of Xylariaceae, including species of *Astrocystis* and *Rosellinia*	Gareth Jones (2006)
b. Evaporation	Spores bound by a quick-drying fluid will cross the boundary layer as a single mass and then travel independently.	*Ascobolus immersus*	Buller (1909)
c. Polyspory/disarticulating spores	Polyspores cross the boundary layer as a single mass, but disarticulate into more easily carried part spores after ejection.	*Capronia populicola* (polyspores are formed by septation of ascospores)	Barr (1991), Ramaley (1997)

Disparate ascomycete species have independently evolved different solutions to reach and travel in dispersive air flows.[1] Single species may use multiple mechanisms, for example, the spores of *Podospora curvula* have mucoid appendages and are also shot as a group (Ingold 1928, 1939). Other mechanisms facilitate staying aloft in air currents. A single mechanism may play a role in negating both constraints: appendages add mass, easing passage through the boundary layer, but then increase drag, enabling longer drifts in wind. Numbers and letters match those found in Figure 20.2. Neither this table nor Figure 20.2 provide an exhaustive list of potential adaptations. We consider terrestrial species with explosively ejected spores, and exclude, for example, marine species and the Eurotiomycetes (Geiser et al. 2006), whose spores are dispersed after the deliquescence of asci.

However, Equation 20.7 neglects a critical aspect of spore ejection from the ascus: there is friction within the ascus which causes the real speed of spore ejection to be less than predicted by the equation. In many species, spores are forced through an apical ring, an elastic seal at the tip of the ascus. This ring functions like an O-ring in engineering applications. As the spore leaves the ascus, it deforms the ring in such a way that there is a thin layer of epiplasmic fluid separating the ring and the ejecting spore. This thin fluid layer lubricates the system and its thickness is optimized to minimize the total loss of energy from two distinct mechanisms (Fritz et al. 2013). First, if the lubricating fluid layer is too thin, the velocity gradient between the fast moving spore and the stationary ring is extremely large, and this gradient causes the spore to decelerate dramatically as a result of viscous friction. On the other hand, if the fluid layer is too thick, this effect is reduced, but then a large amount of fluid leaves the ascus through the fluid gap. As turgor pressure inside the ascus is maintained by the fluid, this sudden leak of fluid causes the driving force to plummet. Hence, large gap thickness is also detrimental, and an intermediate gap thickness exists

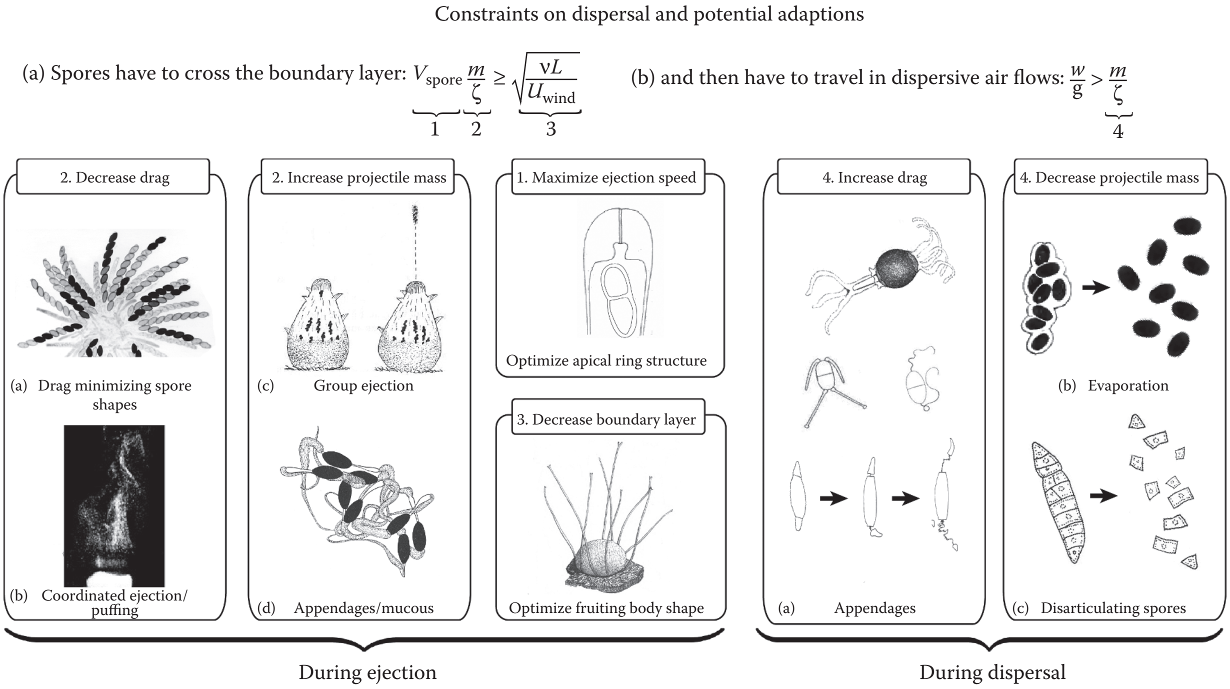

Figure 20.2 The diversity of mechanisms used to facilitate spore dispersal. Species can 1) maximize launch speed by matching the spore size to the geometry of the apical ring; minimize drag or make an ejected mass heavier by 2a) evolving specific shapes, 2b) "puffing," 2c) launching spores as a group, or 2d) using mucous to bind spores; and minimize the width of the boundary layer by 3) growing long, pointy sporocarps. After spores cross the boundary layer species may increase the drag of individual spores by 4a) using appendages or sheaths; and may decrease projectile mass by 4b) evaporating liquid from bound spores, so that each will travel independently, or 4c) launching "polyspores" which subsequently divide into smaller "part-spores." Note equation on the right-hand side is a more general form of Equation 20.11 from the text.

where dissipation is minimal. The sum of these two effects is minimized if a precise relation between the spore size and ring geometry is satisfied. Over 90% of the morphologies examined by Fritz et al. (2013) appear to be within 2% of the optimum (Figure 20.3 left), whereas species with nonfunctional apparatuses lie far away from the optimal line (Figure 20.3 right).

Equation 20.7 is a physical constraint on the ejection of single spores. By ejecting many spores at once, species can avoid the constraint. The synchronized ejection of hundreds or even hundreds of thousands of spores by "puffing" fungi is a cooperative mechanism by which a sporocarp creates a jet of air, enhancing spore range (Equation 20.5) by a factor of 10 or more (Roper et al. 2010).

High-speed movies of synchronized ejection by the crop pathogen *Sclerotinia sclerotiorum* (Figure 20.4a) show that spores decelerate dramatically in a thin layer of air directly above the sporocarp, of the order of the inertial range x (Equation 20.5). However, as spores decelerate, they accelerate the surrounding air. Beyond this thin layer, air and spores move together as a heavy plume whose range is mainly limited by gravity (Figure 20.4b):

$$x_{\max} = \left(\frac{\rho_{\text{air}} + m\, n_{\text{spore}}}{2m\, n_{\text{spore}}\, g}\right)U^2, \tag{20.8}$$

where:

n_{spore} is the number of spores per unit volume
m is the mass of a spore
g is the gravity acceleration
U is the initial velocity of the plume that depends on how densely packed and well synchronized the spores are on the sporocarp

For typical spore densities of 1000 asci/mm^2, and a launch velocity of 4 m/s, the gravity limited range of a 3 mm sporocarp is $x_{\max} = 80$ cm, which is about 20 times the range of an isolated spore x, from Equation 20.5 (Figure 20.4b). Plumes created by smaller apothecia are stopped short of this maximum height by viscous resistance, but asymptotics and numerics show that even small apothecia reach a considerable fraction of $x_{\max}$. Remarkably, although the ranges of individual spores are severely limited by drag, cooperating spores behave like almost frictionless projectiles.

Equation 20.8 shows that synchronization is a formidable adaptation that enables apothecial fungi to cross the boundary layer. But to ensure effective dispersal, "puffing" fungi need to coordinate the ejection of thousands of spores. As the benefits of synchronization are shared unequally among spores, which may be genetically different, cooperation needs to be stabilized against the invasion of cheaters.

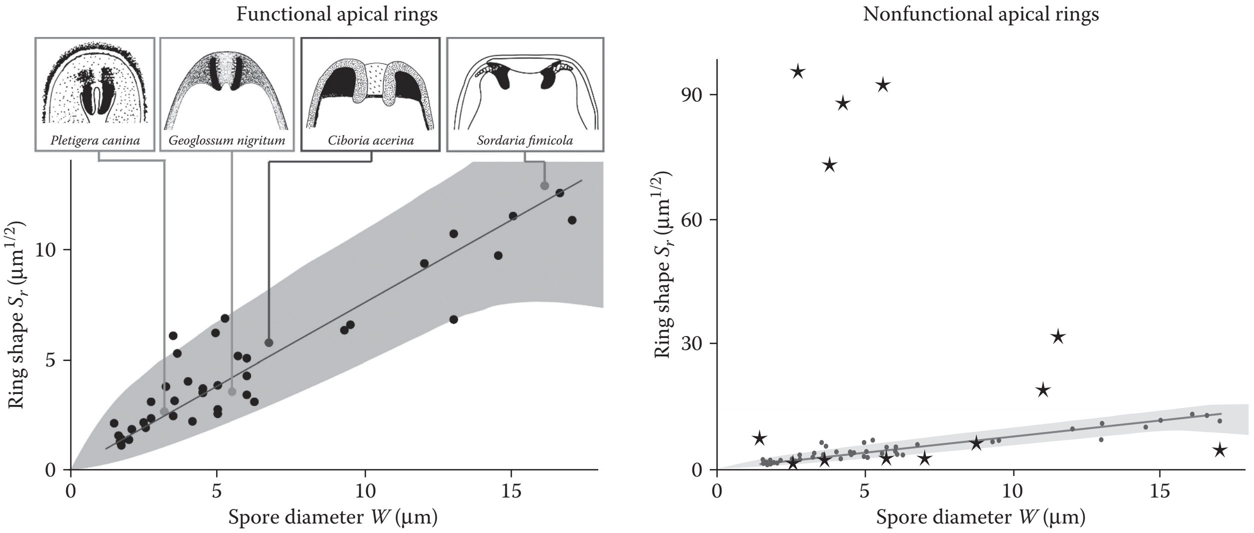

Figure 20.3 Geometries and spore diameters of species with functional (left) and nonfunctional (right) apical rings (Fritz et al. 2013). Gray shading marks the theoretically predicted optimum enabling minimal energy loss; shading takes the same form on left and right panels although the *y*-axis changes. Dots mark individual species with functional apical rings, while stars mark species with nonfunctional apical rings. Data on geometries and spore diameters taken from the literature (Fritz et al. 2013).

High-speed movies of puffing in *A. furfurasceous* show that ejection is self-organized (Figure 20.4c): it starts when a small number of asci fire their spores and proceeds as a wave that crosses the entire apothecium. Spatial coordination may serve as a signal for synchronization and at the same time as a physical mechanism for policing against cheaters.

20.2.2.2 Manipulating m/ζ

The mass-to-drag ratio m/ζ is the timescale over which an ejected spore will decelerate. If this timescale is large, a spore will decelerate more slowly and travel farther. The ratio is sensitive to the spore size: the mass of a spherical spore is $m = 4/3\rho_{\text{spore}}\,\pi a^3$, where a is the radius of a sphere with the same volume of the spore and ρ_{spore} is the density of the spore that we will consider roughly constant, while $\zeta = 6\pi\eta a$. Thus,

$$m/\zeta = \frac{2}{9} a^2 \big/ v\left(\rho_{\text{spore}}/\rho_{\text{air}}\right). \tag{20.9}$$

A twofold increase in spore radius produces a fourfold increase in range. If we combine Equation 20.6 with Equation 20.9, we obtain a minimum size enabling escape from the boundary layer:

$$a^2 \geq \frac{9}{2}\frac{v}{V_{\text{spore}}}\left(\frac{\rho_{\text{air}}}{\rho_{\text{spore}}}\right)\sqrt{\frac{vL}{U_{\text{wind}}}}. \tag{20.10}$$

However, after ejection, spores are dispersed by wind, and smaller spores will be carried more easily. For the upward thrust of a wind w, to carry the spore, it must be greater than the spore's weight. This requires $mg \leq \zeta w$ or, for spherical spores:

$$a^2 \leq \frac{9}{2} v \frac{\rho_{\text{air}}}{\rho_{\text{spore}}} \frac{w}{g}. \tag{20.11}$$

Equations 20.10 and 20.11 are lower and upper bounds on spore size, and identify a tradeoff: larger spores are more likely to cross boundary layers, but smaller spores will travel more easily in dispersive winds (Figure 20.5).

To manipulate m/ζ, a species may use a number of different tools (Figure 20.2). To increase mass, fungi may evolve very large spores, eject spores as a group, or create mucoid appendages to stick smaller spores together. Temporarily tethering spores with mucous increases their likelihood of entering dispersive air flows, and when the spores break apart, individual spores will still be carried by weak air flows. A "polyspore" grows as a single larger spore with septa or divisions, and serves the same purpose; polyspory would allow a single large mass to cross the boundary layer and subsequently disassociate into smaller parts (Barr 1991; Ramaley 1997). Each of these adapations has evolved independently in multiple ascomycete orders.

The size of an individual spore may be constrained by aerodynamics or the limits of maternal investment, but spore shape is another variable enabling a species to alter m/ζ. The shape that minimizes drag for a prescribed spore volume was computed numerically in Roper et al. (2008) and the result for three representative volumes is reproduced in Figure 20.6 (left). Over the range of real spore sizes, spores with drag-minimizing shapes travel only 5%–10% farther than

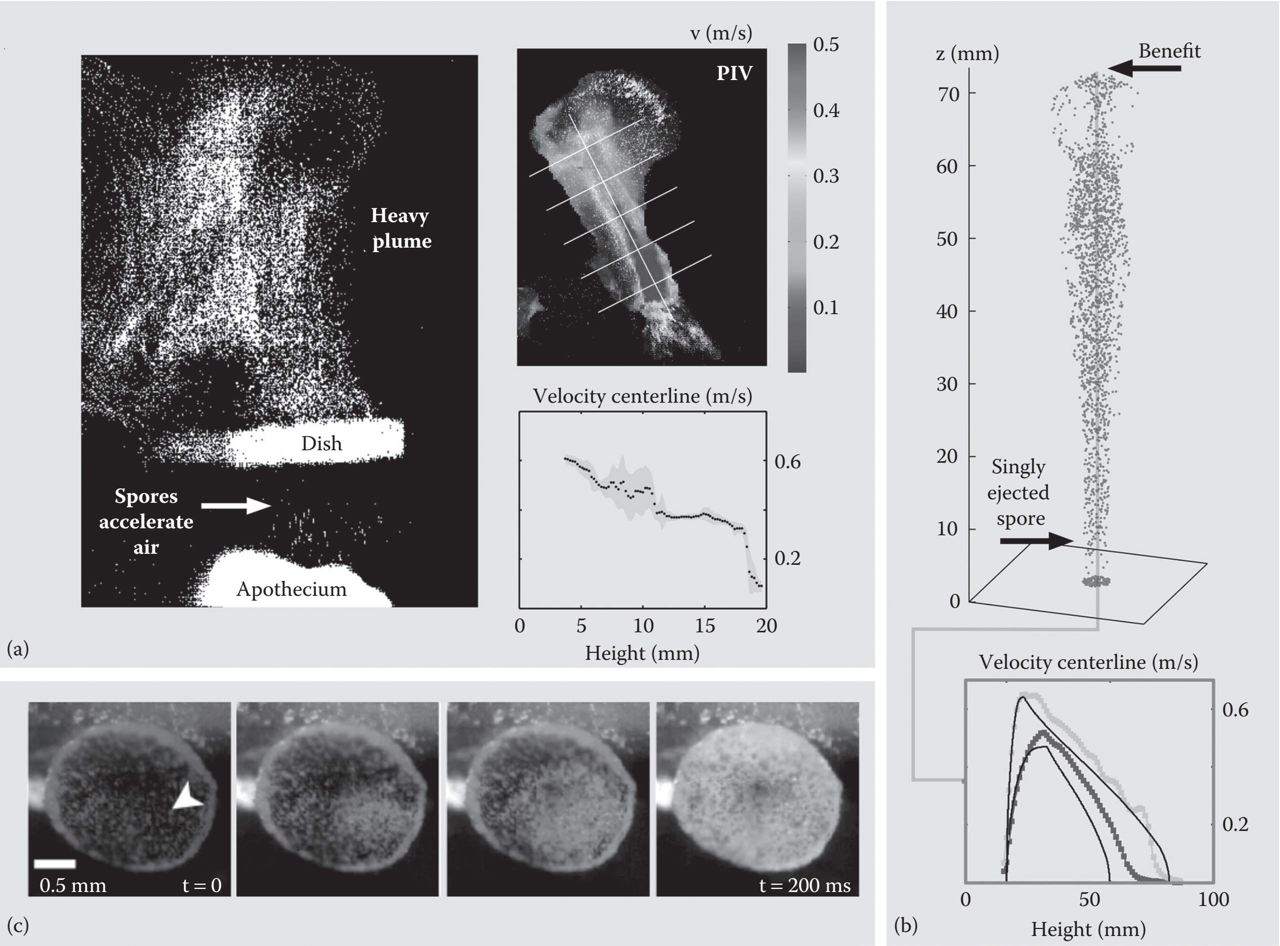

Figure 20.4 **(See color insert.)** "Puffing" (Roper et al. 2010). (a) A laser split into a plane and shot through a puffing event reveals trajectories of individual spores, which can be tracked with particle image velocimetry (PIV) and used to calculate a velocity profile. Images of *S. sclerotinia*. Compare with (b), a velocity profile generated from theory (Roper et al. 2010). Theory confirms the benefit to a spore in a puffing event, compared to a singly ejected spore. (c) Puffing from an apothecium of an *Ascobolus* sp. grown from rabbit dung reveals synchronous ejection; spores are shot from ever widening, concentric circles of tissue. These kinds of coordinated behaviors may serve as a control on cheating by spores, which might otherwise evolve to shoot last and into an already created wind, as opposed to first and into still air. Discussions of cheating assume some level of genetic heterogeneity among the spores of an apothecium.

spherical spores with matched volumes. However, the comparatively modest effects of spore shape on range seem to be associated with strong selective forces. Real spore shapes were analyzed across the Ascomycota from a phylogeny of more than 100 species that eject their spores individually and do not possess any appendages or septa that would alter the ejection dynamics. Three representative examples are reproduced in Figure 20.6 (right), and compared with idealized drag-minimizing shapes (in white). Seventy-three of the 102 species were found to be within 1% of the drag-minimizing optimum, assuming an ejection speed of between 1 and 3.5 m/s (Roper et al. 2008). In fact some of the outliers may be optimized for speeds larger than the speeds assumed in the study. For nonforcibly ejected species, the fraction of drag-minimizing shapes plummets: only 29 out of 65 species within an insect dispersed group and 9 out of 57 species within a group that produces fruiting bodies underground possess drag-minimizing shapes, suggesting that the spore shape is under considerable selective pressure.

20.2.2.3 Manipulating L/U_{wind}

The thickness of a boundary layer is also controlled by environmental parameters, including the distance air travels over an object L, and wind speed U_{wind} (Equation 20.1). These will vary according to the location and size of the sporocarp and its substrate. A species may fruit preferentially on smaller substrates or at sites with greater winds: a plant pathogen might preferentially grow sporocarps on plant stems instead of leaves, and other species might grow asci at the tips of protruding or elevated structures. We are unaware of any data which would specifically test this novel

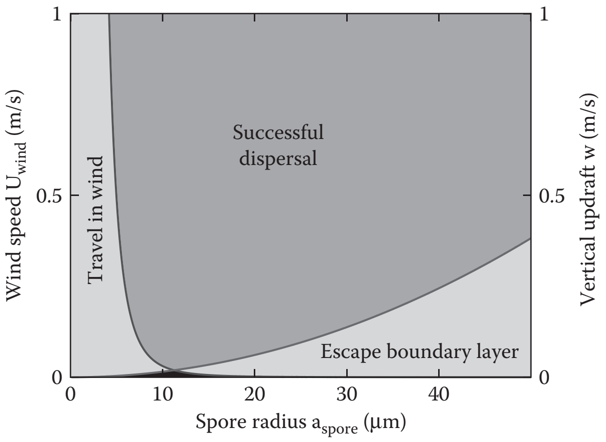

Figure 20.5 Different constraints on spore size. To disperse, a spore must be large enough to escape the boundary layer, but also small enough to travel in wind; the size needed for successful dispersal will change according to wind speeds and vertical updrafts but fundamentally the radius of a successful spore will lie between the curves given by Equations 20.10 and 20.11. Although these curves were computed for spherical spores, aspherical spores will follow qualitatively similar bounds.

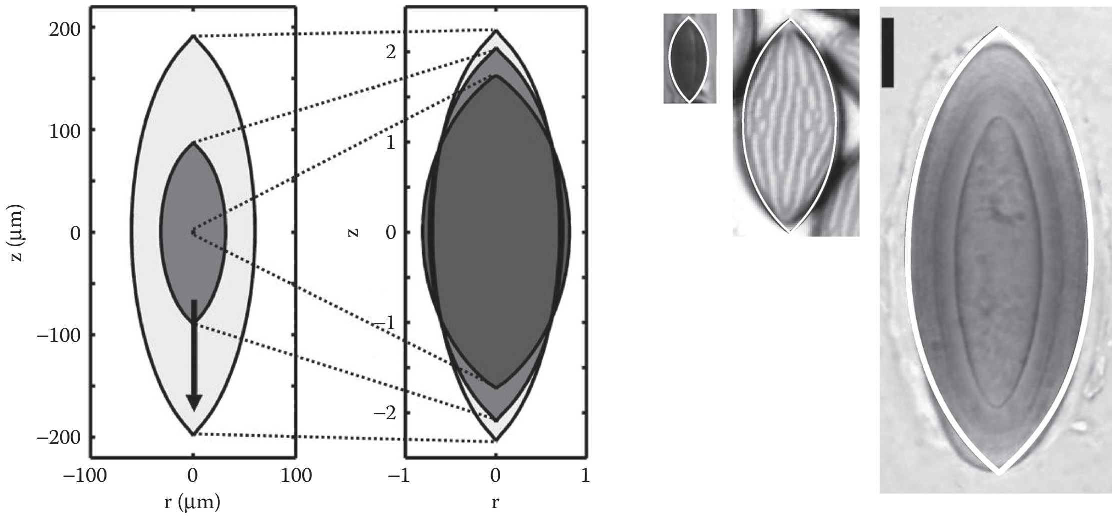

Figure 20.6 Ascomycete species with singly ejected spores appear to evolve drag-minimizing spore shapes (Roper et al. 2008). Left panel displays theoretical drag-minimizing shapes for three different spore volumes; right panel displays images of three different spores with the idealized, drag-minimizing shape drawn in white over the real spore shape. Species are (from left to right) *Astrocystis cepiformis*, *Neurospora crassa*, and *Pertusaria islandica*. Scale bar = 10 μm. Despite the symmetry of three images at left and three images at right, no correspondence between spore volumes at left and spore sizes at right is implied.

hypothesis. And for other species, the sporocarp's location will be dictated by the size and exact placement of the parent: epiphytic fungi colonize just a few square millimeters of a particular leaf, while many competing fungi occupy a single pellet of dung. But remarkable adaptations may allow some species to influence sporocarp placement. Entomogenous fungi prey on insects, and modify the behaviors of infected ants (Evans 1982). Responding to unknown cues from the fungus, the final act of an infected ant is to leave its nest and climb a nearby plant; ants are "grasping the stems with their mandibles before dying in this exposed position" (Evans 1982). Since wind speed increases with height from the ground, the climb exposes the emerging sporocarp to higher wind speeds, and a thinner boundary layer.

Fungi have evolved many different tools to enable the movement of spores through boundary layers (Table 20.1, Figure 20.2). Of course, spores do a lot else besides penetrating boundary layers: spores must carry the resources to enable germination, and the emerging germling may need to quickly find a mate or symbiont. Larger spores will more effectively cross boundary layers, but will also carry more resources, and for this reason may be more fit than smaller spores of the same species. Aspects of morphology may also serve multiple purposes; for example, spore appendages may increase mass and also facilitate the attachment of spores to substrates (Gareth Jones 2006). Nonetheless, dispersal is clearly the paramount work of spores. Despite a long recognition of this fact (Buller 1909), it is remarkable how very little data are available on the mechanics of spore discharge.

20.3 CONCLUSIONS

Thinking of dispersal as an active, dynamic process makes clear why global dispersal is difficult: to reach the atmosphere is nontrivial, and most spores do not arrive to distant habitats. Biologists have assumed fungi will easily disperse across the globe, but correlations between geographic and genetic distances prove that genuinely cosmopolitan species are rare (Koufopanou et al. 1997; Pringle et al. 2005a; Taylor et al. 2006).

The fluid mechanics of dispersal creates a tradeoff: larger spores will more effectively escape boundary layers, but smaller spores will more easily travel in weak air currents. Selection for effective penetration of boundary layers may constrain selection for long distance dispersal. The specific mechanisms of global dispersal in species with broad ranges are more or less unknown (but see Aylor 2003), but once in the atmosphere, smaller sizes would appear to facilitate travel. Recently published data (Fröhlich-Nowoisky et al. 2012) provide support for the hypothesis; fungal spores found above oceans are smaller than the spores above continental land masses.

Understanding the mechanics of discharge is a key to understanding the vast array of sizes and shapes of spores and sporocarps (Figure 20.2). Physical constraints associated with dispersal can explain morphological diversity, and although molecular data have emerged as critical tools for describing new species and phyla (Porter et al. 2011), the morphologies of reproductive structures remain key components of modern taxonomy (Korf 2005; Peterson and Pfister 2010). However, many features of spores, spore appendages, and asci have unknown or imputed functions. For example, operculate species initiate spore ejection by popping open a cap at the ascus apex; these fungi may eject all of their spores at once (Ingold 1928). By contrast, the apical ring of inoperculate species appears narrow, and it may force spores to be ejected one-at-a-time (Pringsheim 1858; Buller 1909; Ingold 1939; Roper et al. 2008). The rarity of intermediate forms (Samuelson 1975) suggests strong selection for one or the other mode of ejection, but the adaptive differences of the two modes are not understood.

Deposition is a rarely considered aspect of dispersal, but to reach a new habitat, a spore must also settle out of the atmosphere. Although spores may act as nucleating agents for water (Després et al. 2012), and reach the ground in snow or rain (Aylor and Sutton 1992), many spores are coated with water-repellent proteins, named hydrophobins (Whiteford and Spanu 2002). Spores with hydrophobins may use as yet unknown mechanisms to settle out of the atmosphere, and we speculate morphology (Figure 20.2) will also play a role in facilitating deposition.

We have focused on Ascomycota, but similar analyses may explain spore and sporocarp diversity within the Basidiomycota, a phylum of mushroom-forming fungi characterized by the use of surface-tension catapults to eject spores (Money 1998; Pringle et al. 2005b). But there are two caveats. First, in most basidiomycete species, spores are formed on the underside of the mushroom's cap. Spores are launched almost horizontally, and then fall vertically through the boundary layer; gravity, rather than inertia, carries the spores to dispersive airflows (but see Dressaire et al. 2016). Second, because of the close packing of either pores or gills, a spore must be ejected away from the surface from which it is launched, but not so far as to collide with the opposite surface. Spore morphologies may be more strongly constrained by the geometries of mushrooms than by the problems of crossing the boundary layer and staying aloft in dispersive airflows (Stolze-Rybczynski et al. 2009; Galente et al. 2011). Differences in ascomycete and basidiomycete discharge mechanisms may influence gross differences in spore size, and the relative reach of the two kinds of spores (Ingold 1971; Fröhlich-Nowoisky et al. 2012).

In fungi, sex appears strategically coupled with the active discharge of spores. Mating and meiosis are often triggered by hostile environments (Lee et al. 2010), and for the genetic models, *Saccharomyces cerevisiae* and *Neurospora crassa*, the stress is nitrogen limitation. The active discharge of sexual spores may be a strategy to use genetically variable offspring to search for new, favorable habitats. In contrast, many asexual spores are passively liberated (although some are not, Meredith 1965, 1973). Asexual spores are blown or washed away from the parent fungus (Gregory 1961), and do not actively cross a boundary layer. On average, asexual spores may travel shorter distances than sexual spores, and remain nearer the parent. However, remarkably little is known about the relative reach of either kind of spore.

A near exclusive focus on long distance dispersal and what may be a poorly understood hypothesis about the global reach of spores has left ecologists with many untested assumptions about the ability of fungi to move across landscapes. Meanwhile, over the last century, humans have moved scores of pathogens, mutualists, and decomposers to novel ranges (Desprez-Loustau et al. 2010; Vellinga et al. 2009), where species may or may not establish and spread. Dispersal will be critical to success in a new habitat; for example, drag-minimizing spore shapes (Roper et al. 2008) predict which pathogens are likely to become invasive (Aurore et al. 2011). Species manipulate dispersal with an array of extraordinary tools, and parsing the mechanics of discharge may be key to understanding the biodiversity of fungi in a changing world.

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