

Dispersal of fungal spores on a cooperatively generated wind

Marcus Roper^{a,b,1,2}, Agnese Seminara^{c1,2}, M. M. Bandi^c, Ann Cobb^d, Helene R. Dillard^d, and Anne Pringle^e

^aDepartment of Mathematics and Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720; ^bDepartment of Mathematics, University of Warwick, Coventry, CV4 7AL, United Kingdom; ^cSchool of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138; ^dDepartment of Plant Pathology and Plant-Microbe Biology, Cornell University and New York State Agricultural Experiment Station, Geneva, NY 14456; and ^eDepartment of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138

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Because of their microscopic size, the forcibly ejected spores of ascomycete fungi are quickly brought to rest by drag. Nonetheless some apothecial species, including the pathogen *Sclerotinia sclerotiorum*, disperse with astonishing rapidity between ephemeral habitats. Here we show that by synchronizing the ejection of thousands of spores, these fungi create a flow of air that carries spores through the nearly still air surrounding the apothecium, around intervening obstacles, and to atmospheric currents and new infection sites. High-speed imaging shows that synchronization is self-organized and likely triggered by mechanical stresses. Although many spores are sacrificed to produce the favorable airflow, creating the potential for conflict among spores, the geometry of the spore jet physically targets benefits of the airflow to spores that cooperate maximally in its production. The ability to manipulate a local fluid environment to enhance spore dispersal is a previously overlooked feature of the biology of fungal pathogens, and almost certainly shapes the virulence of species including *S. sclerotiorum*. Synchronous spore ejection may also provide a model for the evolution of stable, self-organized behaviors.

hydrodynamics | cooperation | fungal spores

The forcible launch of sexual spores into dispersive air flows enables ascomycete fungi to propagate between physically distant patches of habitat; for example, the pathogen *Sclerotinia sclerotiorum* disperses from apothecia in the ground to infect the flowers of crop plants (1), and dung fungi in the genus *Ascobolus* must escape from their dung piles to be ingested by animals (2, 3). Although their microscopic size enables spores to be transported by even slow flows of air, it also severely limits the distance that they may travel ballistically. Launched at a speed of $8.4 \text{ m}\cdot\text{s}^{-1}$, the $12 \text{ }\mu\text{m}$ long spores of *S. sclerotiorum* would be decelerated to rest after traveling less than 3 mm (4, 5). In response to this constraint, fungi have evolved multiple adaptations to maximize spore range. For example, spores that cohere during launch benefit from increased inertia (6), while individually ejected spores may be shaped in order to minimize drag (5).

Here we demonstrate the remarkable ability of apothecial fungi to manipulate their own fluid environment and negate the range constraints imposed by fluid drag. It has long been known (7, 8) that in many species spore discharge is almost synchronous between the asci of an individual apothecium, so that hundreds, thousands, or tens of thousands of spores can be discharged in a single puff, lasting a fraction of a second (Fig. 1*A, B*). Discharge may be initiated spontaneously, or by changes in air pressure, or when an apothecium is touched. Buller (9) first connected spore coejection with the creation of a flow of air. In this work we adapt algorithms originally developed to simulate hundreds of thousands of droplets in clouds to prove that the hydrodynamic cooperation of spores creates a flow of air. Our simulations, analytic models, and experiments: (i) quantify the dispersal advantage provided by simultaneous ejection, (ii) elucidate the biomechanical parameters under the control of the fungus, and (iii) demonstrate a previously unreported benefit of synchronized

launch; the dispersal of spores around obstacles. We also use high-speed imaging to probe how the ejection of spores from different asci is synchronized.

Results and Discussion

Simultaneously ejected spores cooperate to create a macroscopic flow of air. To demonstrate this, we simulate the trajectory of each ejected spore, including the acceleration of the surrounding air, by direct numerical simulation (DNS) of the full Navier-Stokes equations (Fig. 2*A* and *SI Appendix*). In these simulations, spores are assumed to be randomly ejected from points uniformly covering the entire apothecium. Our simulations show that within a short ($\sim\text{cm}$ thick) basal region of the jet, rapidly moving spores mobilize the surrounding air. In crossing the basal region spores decelerate while air accelerates until they reach the same speed U uniformly across the width of the jet (Fig. 2*A*). Beyond the basal region spores are transported by the air flow that they have initiated. In addition to increasing spore range, the transition from ballistic to passive dispersal allows spores to avoid impact with obstacles. We saw experimentally that the pressure gradients created within the jet displace spores sideways and around obstacles (Fig. 1*C–F*), enabling spores to reach flowers that are blocked e.g., by leaves*.

The range of cooperating spores can be 20 times greater than the ranges of individually ejected spores. In experiments we observed spore jets more than 10 cm in length (Fig. 1*B, 3B*) compared to the 3 mm range of singly ejected spores. Similar range enhancements were seen in simulations (Fig. 2*A, 3A*). We can quantify how range enhancement depends upon parameters that may vary between individual apothecia; namely the flux (rate of spore ejection), q_s , per unit area of apothecium, the jet diameter D , the mass of each spore, m_s , and the spore launch speed v_s . Since spores follow streamlines except in the basal region, and are therefore constrained by the incompressibility of the surrounding air flow, the density of spores is constant through the jet, and from conservation of mass in the basal region, is equal to $\rho_s \equiv q_s/U$. The speed, U , of the jet at the end of the basal region can then be calculated by equating the momentum flux

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*There are no pressure gradients within an unobstructed spore jet, since the pressure within the jet must be equal to the pressure of the still air surrounding the jet. However, pressure gradients are set up when the jet impacts upon an obstacle. Correspondingly, although spores in a free jet have no direct hydrodynamic coupling to spores ejected earlier or later in the puff, spores that are dispersed around obstacles must be pushed by the spores that follow them.

¹M.R. and A.S. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: mroper@math.berkeley.edu or seminara@seas.harvard.edu.

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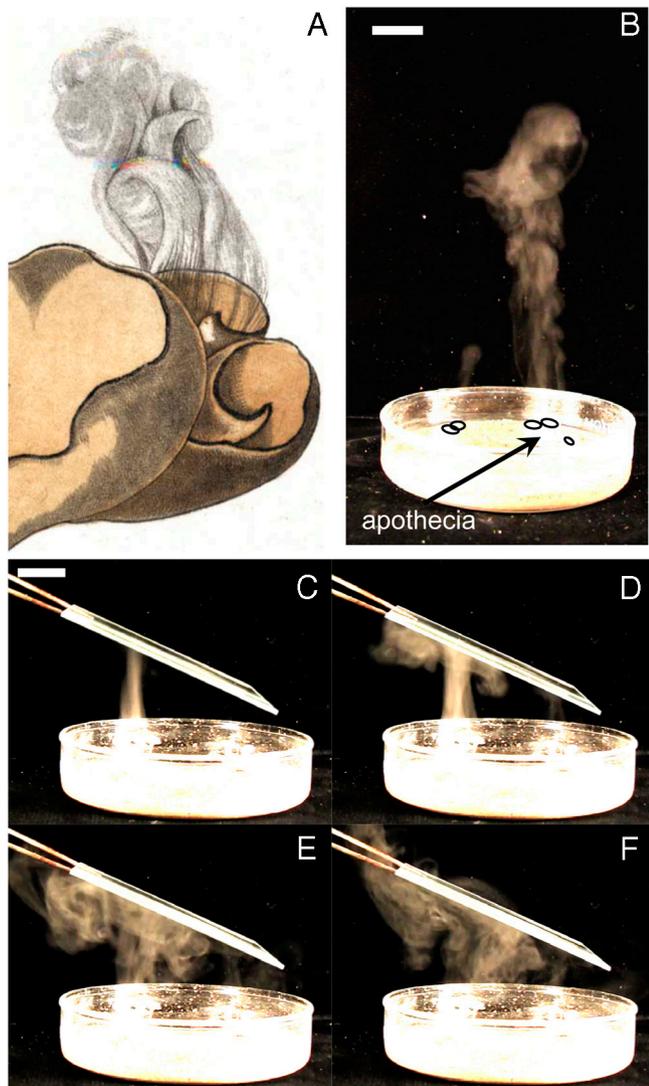


Fig. 1. Synchronized ejection creates a coherent jet of air, enhancing dispersal in open and crowded environments. (A) Early documentation of synchronous spore release in a 1791 drawing of *Otidea cochleata* (8). [Reproduced with permission from *Histoire des champignons de la France* by J.B. Bulliard, Plate number 154, courtesy of the Farlow Library of Cryptogamic Botany]. (B) Spore jets originating from *S. sclerotiorum* apothecia travel much farther than individually ejected spores. (C–F) If the jet impacts upon an obstacle, such as a glass slide, then pressure gradients within the jet displace spores out and around the obstacle. Images are taken at $t = 0, 0.11, 0.32,$ and 0.55 s after initial impact. (Scale bar: 2 cm).

of the spores and of the mobilized air at the end of the basal region with the momentum of the spores at ejection, i.e., $\rho U^2 + m_s q_s U = m_s q_s v_s$, where ρ is the density of air (see *SI Appendix*). Viscous and gravitational stresses then limit the maximum length of the spore jet: we separately estimate the size of these forces. The weight of unit length of jet $\sim m_s q_s g D^2 / U$, where g is the gravitational deceleration. Meanwhile the viscous stress resultant on a unit length of the jet is $\sim \eta u_j$, where η is the viscosity of the surrounding air and u_j the speed of the jet. The relative magnitude of the two resistive forces is given by the dimensionless ratio $Gy \equiv m_s q_s g D^2 / \eta U u_j$. Taking parameters from real fungi (see *SI Appendix*) we estimate that $Gy \gtrsim 5$ at the foot of the jet and increases with height as the jet decelerates and broadens, implying that the range of the jet is limited mainly by gravity: a slug of spore laden air created in the basal region decelerates like a frictionless projectile. To predict the steady range of the jet, we

balance the inertia of a horizontal slice of the steady jet against gravity and viscous forces:

$$(\rho + \rho_s) u_j \frac{du_j}{dz} = -\rho_s g + F_{\text{visc}}[u_j]. \quad [1]$$

Neglecting the viscous force $F_{\text{visc}}[u_j]$ we integrate this equation over the length of the jet $0 < z < z_{\text{max}}$, obtaining an expression for the steady jet range: $z_{\text{max}} = \frac{(\rho + \rho_s) U^2}{2\rho_s g}$. For *S. sclerotiorum*, spore weight limits the range of the jet to 90 mm (Fig. 3B). However jets created by smaller apothecia have smaller values of Gy so are stopped short of this maximum, weight-limited, height by viscous resistance. To quantify the additional resistance, we developed an asymptotic model that includes the viscous drag from shear layers around the circumference of the jet (see *SI Appendix*). Both this analytic model and our DNS show that, even when viscous effects are properly accounted for, spores reach more than 67% of the maximum possible height (Fig. 2A, 3A and *SI Appendix*). Remarkably, although the ranges of individual spores are severely limited by drag, cooperating spores behave like almost frictionless projectiles.

Direct Particle Imaging Velocimetry (PIV) measurements of the spore velocities within real *S. sclerotiorum* spore jets (see *Materials and Methods*) quantitatively confirm our numerical and analytical models for jet initiation and propagation (see Fig. 2 and *Movie S1*). We directly measured the spore launch speed to be $8.4 \text{ m}\cdot\text{s}^{-1}$ (see *SI Appendix* and *Movie S2*), but over the first few millimeters of the jet, spores decelerated to speeds between 0.4 and $0.8 \text{ m}\cdot\text{s}^{-1}$ (Fig. 2B). These speeds are consistent with theoretical values for U , for spore fluxes $q_s = 1.3 \times 10^4 - 5.3 \times 10^4 \text{ spores}/\text{mm}^2\cdot\text{s}^{-1}$, very close to the directly measured value of $3.5 \times 10^4 \text{ spores}/\text{mm}^2$. Above this basal region, spore speeds decrease more slowly with height and the jet broadens, also as predicted by our theory (Fig. 2A, B).

Cooperative benefits are shared unequally among spores. If spores are ejected randomly across the apothecium over the duration of the puff, creating a uniform and homogenous spore jet, then the first spores to be ejected—between 25% and 85% of spores according to our simulations of randomly ejected spores—set the air into motion but travel less far than later ejected spores (*SI Appendix*). It is generally accepted (4, 10) that shorter ranges decrease spore fitness by increasing the probability of either falling back onto the parent fungus or onto already exploited resources. In this sense the first spores to be ejected are sacrificed to benefit the ensemble of spores. Because of the sensitivity of a spore's range to the timing of its ejection within the puff, it is natural to ask what local cues or signals trigger the ejection of individual spores, and therefore control their placement within the puff. We used high-speed imaging to determine how ejection is coordinated among asci.

The synchronized ejection of spores is self-organized. Imaging of wild isolates of *Ascobolus* cf. *furfuraceus* at 1,000 fps (frames per second) shows that ejection begins when a small group of nearby asci discharge at nearly the same time, and proceeds in a wave that expands across the apothecium (Fig. 4A–D) at a speed $v_w \approx 1.5 \text{ cm}\cdot\text{s}^{-1}$. All spores are ejected after a time $t_{\text{puff}} \sim D/v_w$. In fact we measured this signature scaling of puff duration with apothecium size for many different genera and these data suggest that ejection is self-organized in many apothecial fungi (*Materials and Methods* and *SI Appendix*). It is likely that after a small group of asci are triggered, e.g., by a localized change in air pressure, neighboring asci are triggered by elastic stresses within the apothecium. We documented apothecia shrinking proportionately to the number of spores ejected (Fig. 4E), strongly suggesting that apothecia are prestrained. Asci are separated by a bed of paraphyses, which become turgid as the fruit body ripens (11, 12). Although their function has been hitherto mysterious (12–14), we saw paraphyses reorganizing following nearby spore discharges, suggesting that turgid paraphyses provide the requi-

boundary layer flow induced by the spores. Neglecting variations in the sheet span wise direction, we can describe the sheet dynamics using a single variable z for variation in the direction of spore travel, and one-dimensional fields c_s , u_s , and u_j for, respectively, the number of spores per unit area of sheet, the speed of these spores, and the speed of the air in the sheet. Conservation of mass and momentum within the sheet then give:

$$c_s u_s = Q_s \quad [6]$$

$$\frac{d}{dz}(Q_s u_s) = \frac{c_s}{\tau}(u_j - u_s), \quad [7]$$

where Q_s is the flux of spores per unit width of sheet, and τ is the Stokes time scale. Through the quantity u_j the dynamics of the spores in the sheet are coupled to the dynamics of the surrounding air. Air within the sheet resists being accelerated by the spores, because a finite thickness of air on either side of the sheet must also be accelerated with the air in the sheet. Quantitatively, the viscous stress from this layer of air balances the drag from the spores:

$$-2\mu \left. \frac{\partial u_j^s}{\partial x} \right|_{x=0} = \zeta c_s (u_s - u_j), \quad [8]$$

where we have written $u_j^s(x, z)$ for the velocity of the surrounding air, x for the distance from the sheet, μ for the viscosity of the air, and ζ for the Stokes drag coefficient for a single spore. On the sheet: $u_j^s(x = 0, z) = u_j(z)$.

In the main text we derive scaling relations for the thickness and speed of the layer of entrained air. These scalings are well supported by our DNS (SI Appendix). For quantitative comparison with the range differences that we compute for cheating and cooperating spores, we determine the full profile of the jet, and thereby the prefactors in our expressions for the thickness and speed of the entrained air layer by introducing scaled variables first proposed by Görtler (see SI Appendix for expanded discussion). If $u_j \equiv u_j^{(1)} \left(\frac{\zeta^2 Q_s^2 z}{\mu^2 \nu^2} \right)^{1/3} + O(z^{2/3})$ for some constant $u_j^{(1)}$, to be determined, then natural variables for analyzing dynamics of velocity variation parallel and perpendicular to the sheet are (respectively):

$$\xi = \frac{3u_j^{(1)}}{4} \left(\frac{\zeta^2 Q_s^2}{\mu^2 \nu^2} \right)^{1/3} z^{4/3} + O(z^{5/3}) \quad [9]$$

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$$\eta = x \left(u_j^{(1)3/4} \left(\frac{\zeta^2 Q_s^2}{3\mu^2 \nu^2} \right)^{1/4} + O(1) \right). \quad [10]$$

Similarly to our analysis of the circular jet (see SI Appendix), we define a stream function using the ansatz: $u_j^s = u_j(z)F(\eta, \xi)$, and expand the function F as a power series in powers of $\xi^{1/4}$. Keeping only the first term of the expansion $F(\eta, \xi) = \xi^{1/4} f_1(\eta) + O(\xi^{1/2})$, and substituting into the steady boundary layer equations ((29), SI Appendix), we see that f_1 must solve the Falkner-Skan equation:

$$f_1'' + f_1 f_1'' - \frac{1}{2} f_1'^2 = 0, \quad [11]$$

subject to boundary conditions $f_1(0) = 0$, $f_1'(0) = 1$, and $f_1' \rightarrow 0$ as $\eta \rightarrow \infty$. We solve this third order ordinary differential equation by integrating from $\eta = 0$, and numerical shooting on the unknown initial condition $f_1''(0)$ (34). We find $f_1''(0) = -0.8299$ and obtain the value of the constant $u_j^{(1)}$, by substituting the similarity form of the velocity gradient into Eq. 8:

$$-2\sqrt{2} u_j^{(1)3/2} f_1''(0) = 1 \Rightarrow u_j^{(1)} = 0.566. \quad [12]$$

Finally we determine the coefficient for the boundary layer thickness from the asymptotic behavior of f_1 as $\eta \rightarrow \infty$. From our integration of Eq. 11 we find $f_1 \rightarrow 1.0628$, $\Rightarrow f_1' \sim \exp(-1.0628\eta)$, so that for $x \gtrsim \delta$: $u_j^s \sim e^{-x/\delta}$ with $\delta = 1.443 \left(\frac{\mu^2 z}{\zeta Q_s} \right)^{1/3}$. On taking these values for the thickness and center line speed the self-similar profile of the jet agrees almost exactly with the results of our DNS (Fig. 5B).

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