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Fungal artillery of zombie flies: infectious spore dispersal using a soft water cannon

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Dead sporulating female fly cadavers infected by the house fly-pathogenic fungus Entomophthora muscae are attractive to healthy male flies, which by their physical inspection may mechanically trigger spore release and by their movement create whirlwind airflows that covers them in infectious conidia. The fungal artillery of E. muscae protrudes outward from the fly cadaver, and consists of a plethora of micrometric stalks that each uses a liquid-based turgor pressure build-up to eject a jet of protoplasm and the initially attached spore. The biophysical processes that regulate the release and range of spores, however, are unknown. To study the physics of ejection, we design a biomimetic ‘soft cannon’ that consists of a millimetric elastomeric barrel filled with fluid and plugged with a projectile. We precisely control the maximum pressure leading up to the ejection, and study the cannon efficiency as a function of its geometry and wall elasticity. In particular, we predict that ejection velocity decreases with spore size. The calculated flight trajectories under aerodynamic drag predict that the minimum spore size required to traverse a quiescent layer of a few millimetres around the fly cadaver is approximately 10 µm. This corroborates with the natural size of E. muscae conidia (approx. 27 µm) being large enough to traverse the boundary layer but small enough (less than 40 µm) to be lifted by air currents. Based on this understanding, we show how the fungal spores are able to reach a new host.

1. Introduction

The fitness of living organisms is often measured as a number of lifetime progeny and relies critically on reproduction and dispersal of offspring to successfully colonize new habitats. Many fungal species use air-borne spores. Their discharge strategies vary widely [1,2] and involve either an active mechanism, such as forcefully propelling away spores using dynamic fluid processes [3–9], or a passive mechanism, such as splash dispersal of spores by rain [10,11] or discharge by wind [12]. The goal is to reach a vector—such as wind, precipitation, plants or animals—that can mediate long-distance dispersal [13]. Optimizing the spatial range of dispersal reduces competition with the parent and facilitates the colonization of suitable new micro-habitats [14].

Although many insect-pathogenic fungi are generalists some have very high host specificity. The natural primary host of the obligate pathogenic fungus Entomophthora muscae s. str. (Entomophthoraceae; Entomophthoromycotina; hereafter referred to as E. muscae) [15,16] is the house fly Musca domestica [17–19] (figure 1a: inset). Other populations of E. muscae represent genetically distinct host sub-types, such as those infecting cabbage flies (Delia radicum) and drosophilid fruit flies [20,21]. Dispersal of fungal spores thus requires reaching a new specific susceptible fly host species that provides an appropriate fresh nutrient source.
Most stages of the infection cycle of *E. muscae* have been studied intensively [21–23]: conidia (spores) germinate on the host surface and penetrate through the insect cuticle into the haemolymph; the fungus proliferates logistically as protoplast cells until all nutrients are depleted; hyphal bodies and hyphae with cell walls are formed just before the host dies; rigid-walled conidiophores (spore-bearing stalks) penetrate outwards through the intersegmental membranes in the fly’s abdomen; a single conidium is formed at the tip of each conidiophore (figure 1a).

The closing step in this infection circle, the forceful ejection of conidia to reach a new, susceptible host, is remarkably less studied, and often in terms of the resulting flight trajectories [19,24,25] and dispersal range rather than its actual ejection dynamics. *E. muscae* conidia are dispersed over a range of a few centimetres [16,19] amounting to 1000-fold the (equivalent) spore diameter of 20–30 µm [15,16,19]. Reaching this large spatial range in spite of aerodynamic drag requires the spores to be forcefully ejected. It has been suggested that conidia are ejected with a liquid jet of protoplasm originating from the conidiophore [16,26–28]. The increase of turgor pressure causes a rupture of a circumscissile weakness just below the septum that separates the matured conidium [25]. Only recently the ejection velocity of an *E. muscae* isolate (host: drosophilid fruit flies) was investigated using high-speed videography and estimated to be up to 10 m s$^{-1}$ [29].

However, a systematic study of the spore ejection mechanism is lacking due to experimental challenges associated with the biological system—the strong separation of timescales between individual ejection (milliseconds) and total conidial shower (hours)—and limited natural variation in conidiophore sizes and cell wall properties. We hypothesize that the conidiophore acts as a ‘soft’ cannon with elastic walls, shooting a liquid jet. To aid a systematic study, we first develop a millimetre-scale biomimetic cannon. We tune geometry and elasticity to control the break-through pressure required for projectile ejection, and thereby control the available elastic energy. Second, we study the resulting ejection velocity and identify energy losses during ejection. Finally, we predict a decrease of ejection velocity with system size, which has implications for the optimum dispersal of fungal spores under aerodynamic drag.

2. From fungal to soft water cannon

Figure 1b shows a high-speed sequence (see Methods) of the spore ejection by *E. muscae*. The conidium (blue) is ejected and out-of-frame within our time resolution of 67 µs. The ejection and atomization of a protoplasm jet are revealed both by a small approximately 2 µm satellite droplet that follows the conidium and a larger approximately 20 µm droplet that remains attached to the collapsing conidiophore. A recent visualization by Elya *et al.* [29] shows a (blurred) liquid jet during a single frame (19 µs at 54 000 fps) allowing them to roughly estimate the ejection velocity to be approximately 10 m s$^{-1}$. We estimate its volume to be at most approximately 4× the conidial volume (using the observations that ejection takes approximately 19 µs, and the width of the jet is approximately half the conidial diameter). This is an upper bound since it ignores the deceleration of the jet and motion blur. The ejected volume is thus small in comparison to the volume of liquid typically discharged with ascospores, while the acceleration is still among the fastest in nature [25].

The high-ejection velocity of 10 m s$^{-1}$ requires recording at 500 k fps to limit initial displacements to one spore diameter per frame—while each millisecond ejection event must be correctly predicted and recorded within the hours-long peak window for sporulation [30]. To overcome these challenges, and allow for variation of cannon geometry and elasticity,
we design a millimetric biomimetic soft cannon whose key characteristics resemble the fungal system (figure 1c). The mechanisms for pressure build-up and resisting force in the fungal cannon (left) are hypothesized to involve osmotic absorption of water [1] and cell cohesion in a circumsiccile rupture line [27,28], respectively. Our model system (right) consists of an elastomeric tube fitted with a cylindrical projectile with a slightly larger diameter that is inserted over a distance $h_0$. The liquid pressure is increased using a syringe pump while the resisting force is the static friction between the tube wall and the projectile. We stress here that the geometry and elasticity of the soft cannon system: wall thickness $T$ and Young’s modulus $E$; projectile (blue) with diameter $d$, length $l$ and mass $m$ and insertion distance $h_0$; and static friction coefficient between tube wall and projectile $\mu_s$. Right: set-up. The pressure is increased using a syringe pump (light grey = liquid or gas) and measured using a pressure sensor. A high-speed camera records the ejection at a frame rate up to 40 000 fps. (b) Typical curves of $(p, t)$ for incompressible liquids: (red) water and (blue) high-viscosity syrup (approx. 2 Pa s); and a compressible gas: (grey) air. The pressure drops steeply at $p_c$. (c) Projectile ejection. Three example cases with the projectile initially inserted $h_0 = 2.7$ mm into the cannon ($D = 4.0$ mm and $T = 1.0$ mm). (i) Silicone tube pressurized by air to $p_c \sim 121$ kPa; $m = 0.175$ g projectile; $v_0 \sim 3.6$ m s$^{-1}$. (ii) Silicone tube pressurized by water to $p_c \sim 121$ kPa; $m = 0.087$ g projectile; $v_0 \sim 1.3$ m s$^{-1}$. (iii) PVC tube pressurized by high-viscosity syrup to $p_c \sim 314$ kPa; $m = 1.050$ g projectile (attached stainless steel sphere); $v_0 \sim 0.1$ m s$^{-1}$ (arrow not to scale). Gravity is pointing upward. (Online version in colour.)

Figure 2. Experiments: soft water cannon. (a) Experimental set-up. Left: geometrical and material parameters. Elastomeric tube (grey) with diameter $D$, wall thickness $T$ and Young’s modulus $E$; projectile (blue) with diameter $d$, length $l$ and mass $m$ and insertion distance $h_0$; and static friction coefficient between tube wall and projectile $\mu_s$. Right: set-up. The pressure is increased using a syringe pump (light grey = liquid or gas) and measured using a pressure sensor. A high-speed camera records the ejection at a frame rate up to 40 000 fps. (b) Typical curves of $(p, t)$ for incompressible liquids: (red) water and (blue) high-viscosity syrup (approx. 2 Pa s); and a compressible gas: (grey) air. The pressure drops steeply at $p_c$. (c) Projectile ejection. Three example cases with the projectile initially inserted $h_0 = 2.7$ mm into the cannon ($D = 4.0$ mm and $T = 1.0$ mm). (i) Silicone tube pressurized by air to $p_c \sim 121$ kPa; $m = 0.175$ g projectile; $v_0 \sim 3.6$ m s$^{-1}$. (ii) Silicone tube pressurized by water to $p_c \sim 121$ kPa; $m = 0.087$ g projectile; $v_0 \sim 1.3$ m s$^{-1}$. (iii) PVC tube pressurized by high-viscosity syrup to $p_c \sim 314$ kPa; $m = 1.050$ g projectile (attached stainless steel sphere); $v_0 \sim 0.1$ m s$^{-1}$ (arrow not to scale). Gravity is pointing upward. (Online version in colour.)

3. Break-through pressure in a soft cannon

We measured the break-through pressure as function of insertion distance $h_0$ for a series of soft cannons with varying wall thickness, tube diameter, wall material, projectile mass and pressurizing liquid (figure 3a). For each tube, $p_c$ initially increases linearly with $h_0$ and then reaches a plateau value. In addition, we observe that $p_c$ depends on tube geometry and material: $p_c$ increases with increasing wall thickness (figure 3b(i)), decreases with increasing tube diameter (figure 3b(ii)), and varies with the tube material with approximately 3× higher $p_c$ obtained for the polyvinylchloride (PVC) tube compared to the silicone tube (figure 3b(iii): grey and green symbols). On the contrary, $p_c$ is independent of projectile mass (figure 3b(iii): marker types) and independent of the liquid used to pressurize the cannon (figure 3b(iv): marker types). To explain these results, we use a force-balance model.
with $\delta D$ the change in diameter of the tube due to uniform internal pressure $P$, and $E$ and $\nu$ the Young’s modulus and Poisson’s ratio of the tube material, respectively. Note that, we define the small parameter $\eta_{rl/T} = [(1 + (T/D))^{-1} + 2(1 + \nu)(T/D)]^{-1}$ that reduces to unity in the thin-wall approximation $T \ll D$, and to $[2(1 + \nu)(T/D)]^{-1}$ in the thick-wall approximation $T \gg D$. From the imposed radial stretch $\delta D = d - D$, we find the corresponding radial stress $\sigma_r$ (assuming the tube can freely slide over the projectile in the axial direction)

$$\sigma_r = P = E \frac{d - D}{D} \frac{2\eta_{rl/T} T}{P} \frac{T}{E}.$$  

The inset of figure 3b shows that break-through pressure $p_c$ is reached when the force $F_p$ of the liquid working on the lower face of the projectile (area $(\pi/4)d^2$) balances the maximum static friction force $F_{\mu_r}$ that follows from the normal force $N$ of the stretched elastomeric tube squeezing inward. Balancing liquid pressure and friction forces leads to

$$p_c \left( \frac{\pi}{4} d^2 \right) = F_{\mu_r} = \mu_r N,$$

where $\mu_r$ is the coefficient of static friction between the tube wall and the projectile. The radial stress is equal to the internal pressure needed to stretch the tube from its initial diameter $D$ to diameter $d = D$ imposed by the inserted projectile. The latter can be calculated from well-established elastostatic theory [32] that yields the displacement field for a uniformly pressurized unclamped tube. When evaluated at the inner wall

$$\delta D = \frac{D}{2T} \left[ \left( 1 + \frac{T}{D} \right)^{-1} + 2(1 + \nu) \frac{T}{D} \right] \frac{P}{E}$$

The inset sketch and solid black line: friction-dominated ejection regime. (iii) Projectile mass: $D = 1.5 \text{ mm}$ with metal projectile ($T = 1.0 \text{ mm}$). (iv) Pressurizing liquid: air (open squares), water (open triangles) and high-viscosity syrup (closed circles), for PVC tubes ($D = 4.0 \text{ mm}$, $T = 1.0 \text{ mm}$). (v) Pressurizing liquid: air (open squares), water (open triangles) and high-viscosity syrup (closed circles), for silicone tubes ($D = 2.0 \text{ mm}$). (vi) Pressurizing liquid: air (open squares), water (open triangles) and high-viscosity syrup (closed circles), for silicone tubes ($D = 2.0 \text{ mm}$).

$$\delta D = \frac{D}{2T} \left[ \left( 1 + \frac{T}{D} \right)^{-1} + 2(1 + \nu) \frac{T}{D} \right] \frac{P}{E}$$

$$= \frac{D}{2\eta_{rl/T} T} \frac{P}{E}$$

with $\delta D$ the change in diameter of the tube due to uniform internal pressure $P$, and $E$ and $\nu$ the Young’s modulus and Poisson’s ratio of the tube material, respectively. Note that, we define the small parameter $\eta_{rl/T} = [(1 + (T/D))^{-1} + 2(1 + \nu)(T/D)]^{-1}$ that reduces to unity in the thin-wall approximation $T \ll D$, and to $[2(1 + \nu)(T/D)]^{-1}$ in the thick-wall approximation $T \gg D$. From the imposed radial stretch $\delta D = d - D$, we find the corresponding radial stress $\sigma_r$ (assuming the tube can freely slide over the projectile in the axial direction)

$$\sigma_r = P = E \frac{d - D}{D} \frac{2\eta_{rl/T} T}{P} \frac{T}{E}.$$  

This stress exerts a normal force $N = \sigma_r(h_0 \pi d)$ on the inserted section of the projectile, and the corresponding break-through pressure can be obtained from equation (3.1)

$$p_c = \mu_r E \frac{d - D}{D} \frac{2\eta_{rl/T} T}{P} \frac{T}{E}$$

$$= 8\mu_r E \left\{ \frac{d - D}{D} \frac{\eta_{rl/T} T}{P} \frac{T}{E} \right\}.$$  

It is noted that two other forces are considered negligible in our analysis. First, the contribution of gravity is typically less

Figure 3. Geometric control of soft cannon ejection pressure. (a) Break-through pressure versus insertion distance with variation of system parameters. (i) Tube wall thickness: $T/D = 1/4$ (cyan), 1/2 (blue) and 1 (purple), for silicone tubes ($D = 2.0 \text{ mm}$). (ii) Tube diameter: $D = 2.0$ (cyan), 4.0 (green), 6.0 (yellow) and 8.0 mm (red), for silicone tubes ($T/D = 1/4$). (iii) Projectile mass: $m = 0.087$ (closed circles), 0.175 (open squares) and 1.050 g (open triangles), for silicone (green) and PVC (grey) tubes ($D = 4.0 \text{ mm}$, $T = 1.0 \text{ mm}$). (iv) Pressurizing liquid: air (open squares), water (open triangles) and high-viscosity syrup (closed circles), for PVC tubes ($D = 4.0 \text{ mm}$, $T = 1.0 \text{ mm}$). Measurement errors: $p_c \pm 3 \text{ kPa}$; $h_0 \pm 0.2 \text{ mm}$. (b) Normalized break-through pressure $p_c/(\mu_r E)$ versus normalized geometrical contributions $8\left\{ (d - D)/D \right\} \eta_{rl/T} T$ of the liquid working on the inner wall of the inserted projectile, and the corresponding radial stress $\sigma_r$ that reduces to unity in the thin-wall approximation $T \ll D$, and to $[2(1 + \nu)(T/D)]^{-1}$ in the thick-wall approximation $T \gg D$. From the imposed radial stretch $\delta D = d - D$, we find the corresponding radial stress $\sigma_r$ (assuming the tube can freely slide over the projectile in the axial direction)
than 10% for pressures greater than 50 kPa and can thus be neglected for our pressures of a few hundreds of kilopascal. Second, the stress field that is exerted by the friction force has an additional axial component in the deformed region of the tube near the tail end of the projectile (see inset of figure 3b). It leads to a push-out force pointing towards the tube exit. This push-out force can be estimated as \( \sigma_r \delta_{\text{push}} \sin(\theta) \) with \( \theta \) the widening angle of the tube near the tail end of the projectile (typically 5–10°) and \( \delta_{\text{push}} \), the characteristic length scale. The ratio between the push-out force and friction force is thus \( \sim (\delta_{\text{push}}/h_0)(\sin(\theta)/\mu_s) \). While the latter term is close to unity, the first term approaches zero since the axial component of the stress is integrated over an infinitely small area.

The push-out force is independent of insertion length, and thus provides a constant correction to the break-through pressure. In the absence of a systematic shift in our experiments, the push-out force should be negligible.

A few simple scaling laws now emerge: \( p_c \) increases linearly with both material parameters \( \mu_s \) and \( E \), and also increases linearly with two normalized geometrical parameters: the projectile insertion distance with respect to its diameter, \( h_0/d \) (the control parameter in figure 3a) and the tube strain \( (d - D)/D \). The dependence on wall thickness is linear in the thin-wall limit \((T/D \ll 1)\) while \( p_c \) is independent of wall thickness in the thick-wall limit \((T/D \gg 1)\). This nonlinear behaviour is observed in figure 3(a) for the two larger wall thicknesses.

For all experiments, we normalize \( p_c \) with material parameters: \( p_c/(\mu_s E) \), which is shown in figure 3b versus normalized geometrical contributions \( \eta_{E}T/D(h_0/d) \). All measured pressures collapse on the prediction in equation (3.4), up to reaching a plateau value (dashed lines). We find \( \mu_s = 0.20 \pm 0.07 \) (standard deviation, s.d.) for the static friction coefficient; it expectedly slightly varies with tube material and different iterations of the same experiment with a new tube or a new projectile (with possibly different macro- and microtexture).

The plateau value of the break-through pressure is explainable by the transition to the second ejection regime, sketched in the inset of figure 3c. The initially unstretched part of the tube is stretched due to the internal pressure. For sufficiently high-insertion distance \( h_0 \) the internal pressure increases sufficiently to stretch the tube to a diameter that approaches that of the clamped projectile, after which leakage in the gap will occur. In that case, the contact area between the tube and the projectile will be lubricated and the static friction vanishes. In first approximation, the break-through in this regime is then determined by the liquid pressure needed to widen the tube by \( \delta D \sim \alpha (d - D) \) with \( \alpha \approx 1 \). Using equation (2.2) this yields the maximum break-through pressure \( p_{c,\text{max}} \)

\[
p_{c,\text{max}} = aE \frac{d - D}{D} \frac{2 \eta_{E} T}{D} = 2aE \left( \frac{d - D}{D} \frac{\eta_{E} T}{D} \right) ,
\]

which is in independent of \( h_0 \) and captures the plateau observed in figure 3a. For the first regime dominated by wall friction, we thus obtain the scaling \( p_c/p_{c,\text{max}} \approx (4 \ h_0 \mu_s) / (a d) \). The factor \((4 \ h_0 \mu_s) / d \) originates from the projection of radial stress into resisting force acting normal to the projectile motion axis. The transition to the leakage-dominated regime is obtained when \( p_c/p_{c,\text{max}} = 1 \), which yields the critical insertion distance \( h_{0c}/d = \alpha / (4 \ \mu_s) \).

We can indeed fully collapse the experimental data by plotting \( p_c/p_{c,\text{max}} \) versus \( h_0/h_{0c} \), as shown in figure 3c. The experimental scatter is mainly set by inaccuracies in insertion distance, i.e. a slight tilt of the inserted projectile or skewed tube exit—and thus most apparent for small \( h_0 \). We find a coefficient \( \alpha = 0.7 \pm 0.2 \) (s.d.), reasonably close to unity as expected. For our numeric values of \( \mu_s \) and \( \alpha \), we find that the critical insertion distance should scale as \( h_{0c} \sim d \), which is confirmed in figure 3a. In conclusion, we have demonstrated that we can accurately predict the break-through pressure of a soft cannon based on its known geometrical and material properties.

4. Ejection velocity

The performance of a soft cannon is mainly determined by its ejection velocity \( v_0 \) at the exit (with respect to its system size). We will thus consider projectile motion up to the ejection point, after which the pressure in the tube quickly decays (but liquid continues to be ejected due to inertia). Figure 4a shows \( v_0 \) as a function of \( p_c \), determined above for a series of soft cannons: \( v_0 \) increases with \( p_c \) for all soft cannons. The air-pressurized silicone cannons have an asymptote (dashed lines) at their respective plateau pressure \( p_{c,\text{max}} \sim 100, 110 \) and 130 kPa. For the water-pressurized PVC cannon, the ejection velocity is significantly lower. Note that in this case no asymptote was obtained since measurements were only taken in the regime dominated by wall friction \((p_c < p_{c,\text{max}})\).

To understand the asymptote at \( p_{c,\text{max}} \), we consider the influence of the insertion distance \( h_0 \), which may vary \( \geq h_{0c} \) at constant pressure \( p_{c,\text{max}} \). The total elastic energy available for conversion into kinetic energy of the projectile, \((1/2) m v_0^2 \), plus other energy and dissipation terms considered as losses (discussed in a later section) is given by

\[
E_d = \int_0^{V_{\text{ej}}} p_d dV = p_c h_0 \left( \frac{m v_0^2}{4} \right) ,
\]

where \( \Delta V_{\text{ej}} = h_0 (m v_0^2/4) \) is the total ejected volume upon the exit of the projectile. The pressure during ejection \( p_{\text{ej}} \) is assumed to remain fixed at the ejection pressure \( p_c \) (figure 3). This is accurate for small \( \Delta V_{\text{ej}} \) (since \( p_{\text{ej}} \approx 1 - (B/p_c) (\Delta V_{\text{ej}}/V_0) \)) with \( V_0 \) the total volume of the system with bulk modulus \( B \) as well as negligible pressure leakage in the system. Equation (4.1) demonstrates that the available elastic energy scales linearly with the product \( p_c h_0 \). Thus, to include the dependence of elastic energy on \( h_0 \) (and not merely on \( p_c \)), we re-plot \( v_0 \) as a function of \( p_c h_0 \) in figure 4b. The observed square root dependency (dashed lines: best fit) indicates a constant energy conversion from stored elastic energy to projectile kinetic energy \((1/2) m v_0^2 \). In other words, for each system (tube geometry and pressurizing liquid), dissipation amounts to a constant percentage that is independent of the stored elastic energy. This is clearly shown in figure 4c. The ejection velocity—and energy conversion—is significantly larger for those cannons pressurized by air than for those cannons pressurized by water. This suggests that the liquid imposes a significant energy loss via either dissipation in or acceleration of the liquid phase.

Indeed, for the water-pressurized cannon \( v_0 \) is independent of projectile mass (figure 4b: grey solid circles and open triangles present a sixfold difference), with
corresponding higher energy conversion for the larger projectile mass (figure 4c: 8.4% versus 1.6%). The ejection dynamics are thus dominated by the mass of the accelerated liquid, rather than the projectile mass. Air-pressurized cannons do not suffer from these effects and exhibit a clear dependence of on projectile mass (red solid circles and open triangles). Air-pressurized silicone tubes (red) and (cyan) D = 4.0 mm, T = 1.0 mm tube with (solid circles) m = 0.177 g (sixfold heavier) and (open triangles) m = 1.053 g (sixfold heavier). Water-pressurized PVC tube: (grey) D = 4.0 mm, T = 1.0 mm tube with (solid circles) m = 0.177 g and (open triangles) m = 1.053 g (sixfold heavier). Dashed lines indicate . Measurement errors: pc = (2 ± 2) kPa; v0 = (0.12 ± 0.09) m s−1. (b) Ejection velocity v0 versus . Dashed lines: best fit for v0 ∝ pc using weighted nonlinear regression. Measurement error: pc, h0 = (30 ± 20) N m−1. (c) Projectile kinetic energy Еk versus stored elastic energy Еel (equation (4.1)). Dashed lines: best fit for Еk ∝ Еel using weighted linear regression. The black solid line indicates 100% energy conversion. Measurement error: Еel = (11 ± 7)%; Еk = (14 ± 13)%. Additional data: (blue) air-pressurized PVC tube (D = 4.0 mm, T = 1.0 mm) with (solid circles) m = 0.177 g and (open triangles) m = 1.053 g (sixfold heavier). (d) Energy conversion versus modified Reynolds number Re′ for five different liquids (see Methods). We set constant pc, h0 = 241 ± 18 N m−1, using silicone tubes (D = 4.0 mm, T = 1.0 mm). The grey triangle indicates a power 1/2. (Online version in colour.)

Figure 4. Ejection velocity and energy conversion. (a) Ejection velocity v0 versus break-through pressure pc. Air-pressurized silicone tubes: (red) D = 4.0 mm, T = 1.0 mm tube with (solid circles) m = 0.177 g and (open triangles) m = 1.053 g (sixfold heavier) and (cyan) D = 8.0 mm, T = 2.0 mm tube with (solid circles) m = 1.185 g. Water-pressurized PVC tube: (grey) D = 4.0 mm, T = 1.0 mm tube with (solid circles) m = 0.177 g and (open triangles) m = 1.053 g (sixfold heavier). Dashed lines indicate . Measurement errors: pc = (2 ± 2) kPa; v0 = (0.12 ± 0.09) m s−1. (b) Ejection velocity v0 versus . Dashed lines: best fit for v0 ∝ pc using weighted nonlinear regression. Measurement error: pc, h0 = (30 ± 20) N m−1. (c) Projectile kinetic energy Еk versus stored elastic energy Еel (equation (4.1)). Dashed lines: best fit for Еk ∝ Еel using weighted linear regression. The black solid line indicates 100% energy conversion. Measurement error: Еel = (11 ± 7)%; Еk = (14 ± 13)%. Additional data: (blue) air-pressurized PVC tube (D = 4.0 mm, T = 1.0 mm) with (solid circles) m = 0.177 g and (open triangles) m = 1.053 g (sixfold heavier). (d) Energy conversion versus modified Reynolds number Re′ for five different liquids (see Methods). We set constant pc, h0 = 241 ± 18 N m−1, using silicone tubes (D = 4.0 mm, T = 1.0 mm). The grey triangle indicates a power 1/2. (Online version in colour.)

and will introduce a loss factor scaling of approximately m/(m + m recoil) that is significant for small recoil mass.

To study the main source of energy loss in more detail, i.e. the pressurizing liquid, we performed experiments with air, water and various aqueous glycerol solutions (see Methods) at a constant pc, h0 = 241 ± 18 N m−1 such that the available elastic energy is the same: 4.0 ± 0.3 mJ (see equation (4.1)). Ideally, this energy is fully converted to the kinetic energy of the projectile. Energy losses are attributed to viscous dissipation in the ejection liquid, and its inertia. A full understanding of the energy loss would require a description of the flow field (obtained through PIV or finite-element analysis)—coupled to elastic deformation of the tube wall. Here, as a first approach, we study the energy conversion as a function of the viscous forces in the liquid, and the inertia of both the projectile and liquid (accelerated to the same velocity). Their balance defines a modified Reynolds number Re′:

\[
Re' = \frac{[\rho D^3 + m \omega^2 r/dt]}{\eta (dr/dt) D^3} = \frac{\rho D v_0}{\eta} \left[ 1 + \frac{m}{\rho D} \right] = Re_0 \left[ 1 + \frac{m}{\rho D} \right],
\]

with Re0, the Reynolds number of the liquid flow with typical length-scale D. Figure 4d demonstrates the energy conversion
from the source diophore speed recordings of fungal spore ejection demonstrate concluded previously for the conidia of 102 strength is a material property and amounts to approximately (for E. muscae 49). The black dashed arrow indicates the absolute travel distance from the source (s). Inset: zoom near the origin to highlight the trajectory of the 2 µm spore (magenta). (c) Travel distance as a function of time. Grey-shaded area: boundary layer of a few millimetres thickness (shading fades between 1 and 10 mm). (Online version in colour.)

as function of Re'. With increasing Re' the projectile inertia dominates, thus the energy conversion increases: the conversion tends to 100% for systems with large projectile inertia and small viscous forces.

5. Fungal spores: ejection velocity and dispersal range

We use an in-depth understanding of our biomimetic system to predict the break-through pressure and ejection velocity for the conidiophore system of E. muscae. While the resisting force in our model system is set by surface friction, in the conidiophore system it is set by the tensile strength of the conidiophore cell wall as sketched in figure 1c. The cell wall eventually ruptures along a circumcissile weakness below the conidium. The force balance at rupture then contains the critical force exerted by the protoplasm, still scaling as \( p_v d^2 \), and the (longitudinal) tensile strength (or peak stress) \( \sigma_t \) multiplied by the cross-sectional area of the wall scaling as the product of \( d \) and cell wall thickness \( \delta \). The balance \( p_v d^2 \sim \sigma_t \delta d \) yields a scaling for the break-through pressure: \( p_v \sim \sigma_t \delta / d \). This shows that the break-through pressure scales inversely with spore size. Tensile strength is a material property and amounts to approximately 10^5–10^6 MPa for bacterial and algal cell walls [33].

Assuming no size effect on dissipation (as will be discussed below), the kinetic energy scales directly with elastic energy: \( m v_0^2 \sim p_v \Delta V_{el} \). We assume that the ejected volume of protoplasm scales with spore volume \( \sim d^3 \) and insert the scaling for \( p_v \) into the energy balance: \( d^3 m v_0^2 \sim \sigma_t \delta d^2 \). We find that the exit velocity scales with spore size: \( v_0 \sim d^{-1/2} \) (figure 5a). Importantly, this is the weakest scaling that can be expected: the inverse scaling will be stronger if the ejected volume scales less than cubically with size (e.g. quadratically). Thus, the ejection speed \( v_0 \) increases for decreasing system size.

It is important to evaluate the possible influence of energy losses on the proposed scaling. For fungal spores, the protoplasm viscosity is very similar to that of water, approximately 2–3 mPa s [34], and the ejection volume is limited to the cell content of a single conidiophore. Indeed, our high-speed recordings of fungal spore ejection demonstrate that the spore detaches almost instantaneously from the conidiophore—with very little attached fluid. The same was concluded previously for the conidia of B. ranarium [25]. Thus, we expect the losses in viscous dissipation and liquid inertia to be minor. Rather we evaluate the role of surface tension, which may limit the conversion to spore kinetic energy through the creation of free surface area of the liquid jet. The surface energy depends on the break-up details of the jet, but we can estimate its magnitude in the energy balance. The length of the jet scales with \( d \), such that its surface energy scales with the surface area of the cylindrical jet as \( \gamma \pi d^2 \).

The flight trajectory of the spore is influenced by aerodynamic drag and gravitational forces

\[
\dot{x} = -\frac{F_{dx}}{m} \quad (5.1a)
\]

and

\[
\dot{y} = -\frac{F_{dy}}{m} - g \quad (5.1b)
\]

with \( m \) the mass of the spore and \( g \) the gravitational constant. The drag force is given by \( F_d = (\pi/8)C_{D,0} \rho \mu v^2 d^2 \) with drag coefficient \( C_{D,0} \). Previous works [24,25,35] have shown agreement between observed spore ejection distances and their prediction assuming Stokes drag in equation (4.2), using as a boundary condition the ejection velocity \( v_0 \) estimated from high-speed recordings for each fungus species. Here, we study whether optimum ejection conditions exist as a function of spore size \( d \). As the initial Reynolds number \( Re_0 \) increases with spore size (up to approx. 100 in our calculations), the drag force can no longer be assumed linear in the velocity. Thus, we use the Brown–Lawler model [36] for the drag coefficient, which has validity over an extended range of \( Re = \rho_d ud / \mu_d ) up to \( 2 \times 10^5 \) (\( \rho_d \): air density; \( \mu_d \): air viscosity). Equation (4.2) will be numerically integrated to calculate the trajectory of the spores.

From equation (4.2), it follows that the deceleration due to drag scales with \( d^{-1/2} \) (note that \( C_{D,0} \) varies only weakly with \( d \) for intermediate \( Re \)). In other words, the smaller the spore size, the larger the deceleration due to drag. Since smaller spores also have a higher ejection velocity, \( v_0 \sim d^{-1/2} \), the spore trajectory will display a maximum range for intermediate system size. To demonstrate this optimum, we solve the equation of motion numerically using the following boundary conditions:

Figure 5. Dispersal range. (a) Ejection velocity versus system size following \( v_0 \sim d^{-1/2} \) with \( v_0 = 10 \text{ m s}^{-1} \) for \( d = 20 \mu \text{m} \) to match the ejection velocity observed for E. muscae [29]. Coloured markers indicate spore sizes for which trajectories are plotted in (b,c) magenta: 2 µm; cyan: 20 µm; red: 200 µm; and blue: 2000 µm. (b) Trajectories of spherical projectiles under gravity and aerodynamic drag. The ejection angle is 45°.
the initial positions \( y(0) = x(0) = 0 \), and the initial velocities \( \dot{y}(0) = v_0 \sin(\theta) \) and \( \dot{x}(0) = v_0 \cos(\theta) \) with \( \theta \) the ejection angle with respect to the horizontal.

Figure 5b shows the results for an ejection angle of 45° and \( v_0 \sim d^{-1/2} \) with \( v_0 = 10 \text{ m s}^{-1} \) for the typical spore size of 20 µm (figure 5a, cyan). Indeed, the spatial distance initially increases with \( d \leq 200 \mu m \) and then decreases again \((d = 2000 \mu m, \text{ blue})\). Moreover, we see two distinct behaviours, with the small projectiles ‘hitting an aerodynamic wall’ [37]: they are decelerated to almost a full stop by drag only (gravity is initially negligible), and then fall straight down with a low steady-state velocity (balancing the gravitational force and aerodynamic drag). This is the typical behaviour for spores. By contrast, the large projectiles have fully symmetric trajectories dominated by gravity. The maximum range is obtained for a projectile size of approximately 500 µm in this simulation. For smaller ejection angles, a smaller fraction of kinetic energy is lost to the vertical component of drag, so this favours smaller projectile sizes (approx. 100 µm at 1°).

The above range calculations assume quiescent air conditions. In reality, the spore has to be ejected with sufficient velocity to traverse the thin boundary layer it is surrounded by. Fungal spores will then typically be dispersed by air currents [2,13,35] to a new micro-habitat. In the case of the host-specific \( E. \text{muscae} \), however, the conidium will exclusively germinate on a live housefly—which is a highly specific and moving target. To increase the likelihood of reaching a new suitable host, sporulating female cadavers have been shown to attract live male flies that inspect, touch and sometimes attempt to copulate with the dead female cadaver [38,39]. The latter might induce sporulation by mechanical stimulus. The proximity of a suitable host thus merely necessitates spore ejection out of the quiescent layer around the fly cadaver. The movement of the fly inspecting the cadaver may create small air currents that can lift the spores further and ensure they reach their attracted target. The boundary layer thickness \( \delta \sim \sqrt{v_{air} L_f}/U_{air} \) [35] \((v_{air}: \text{air kinematic viscosity}; \ L_f: \text{by characteristic length}; \ U_{air}: \text{wind velocity}) \) is typically 1 mm thick. Yet, the required traversing distance may be a few millimetres due to the presence of protrusions such as the fly’s wings, legs and hairs. Figure 5c shows travel distance from the source. While small projectiles (2 µm, cyan) have a high-initial velocity, they are completely decelerated by drag before reaching the edge of the boundary layer. Larger projectiles initially move slower but eventually reach a further distance from the source (up to the spore size that reaches optimum range—which decreases for stronger decay of \( v_0 \) with \( d \)). We observe that there is a minimum spore size \( \geq 10 \mu m \) to reach the edge of a boundary layer of a few millimetre thickness (only weakly varying with ejection angle).

Increasing the spore size above the above-predicted value needed to traverse the boundary layer is counter-productive since there is a maximum spore weight that can be lifted by air currents (which need to overcome the spore inertia). For a typical wind speed of 20 cm s\(^{-1}\), Koper et al. [35] have shown that the maximum spore size is approximately 40 µm.

With a typical mean conidia width and length of 25.9 ± 0.4 µm and 30.4 ± 0.5 µm, respectively [40], our model indicates that \( E. \text{muscae} \) conidia have an optimal size being large enough to traverse the boundary layer but small enough to still be lifted by wind currents. Our model is based on spherical conidia, while \( E. \text{muscae} \) conidia are round with a pointy apex and a wider base (figure 1a–c). This likely increases aerodynamic properties and reduces conidial drag even further, similar to other drag-optimizing shapes [35].

When landing on a surface—including a host cuticle—a conidium ejected by a conidiophore of \( E. \text{muscae} \) mycelium typically does not form a germ tube required to infect the host. Rather, the conidium forms a new conidiophore that grows a secondary conidium. Its development requires no additional nutrients, as a secondary conidium is readily formed from a primary conidium on sterile glass or plastic surfaces, for example. It has been shown that these secondary conidia are the infective unit [41] and typically for a germ tube unless they have landed on a surface unsuitable for their growth—in that case, they can form a tertiary conidium as the next step in the ‘cascade’.

Owing to the finite amount of available cell material the size of subsequent conidia in the cascade will decrease. We thus hypothesize that the comparatively large size of an \( E. \text{muscae} \) primary conidium (compared to most fungi within Ascomycota) facilitates the formation and ejection of a secondary conidium, from a newly developed conidiophore. The equivalent diameter of secondary conidia is 20 µm [42], which assuming the same ejection mechanism is consistent with secondary conidia traversing a boundary layer of a few millimetres (corresponding to the cyan curve in figure 5c). This two-step ‘cascade’ increases the dispersal potential of \( E. \text{muscae} \). By contrast, tertiary conidia are rare and reduced to roughly 10 µm in diameter [30]. Their smaller size would make them increasingly susceptible to aerodynamic arrest and less able to traverse even thin boundary layers (falling into the grey-shaded area in figure 5c). To confirm the role of aerodynamic arrest in the ‘cascade’ of \( E. \text{muscae} \) conidia, it would be valuable to directly compare \( v_0 \) of primary, secondary and tertiary conidia using high-speed videography.

6. Conclusion

We use a millimetric biomimetic ‘soft cannon’ and associated force-balance model to study the mechanism of forceful ejection of spores from a fungal cannon as a function of cannon geometry and elasticity (figure 1c). The soft cannon has a break-through pressure that scales linearly with tube wall elasticity, friction coefficient and three geometrical normalized parameters: the amount of stretch of the tube, the projectile insertion distance and the tube wall thickness (in the thin-wall limit). The pressure applied to eject the projectile can thus be predicted precisely (figure 3c) and sets together with insertion distance the elastic energy that scales with \( p_w h_0 \). The conversion of this energy into projectile kinetic energy is constant for a single cannon (figure 4c) and depends on the Young’s modulus of the tube (a stiffer cannon has lower energy loss in recoil and deformation) and in particular on the pressurizing liquid. Both fluid acceleration and dissipation in the flow field are a significant source of energy loss for cannons pressurized with viscous liquid rather than air (figure 4d).

Our model suggests that the resulting projectile ejection velocity scales negatively with system size, which for the fungal geometry with rupture of the spore along the conidiophore circumference leads to \( v_0 \sim d^{-1/2} \). Although smaller projectiles have higher ejection velocity, they also experience larger aerodynamics drag and we find that there is a minimum spore size \( \geq 10 \mu m \) that is able to traverse the quiescent layer (of a few millimetres) around the sporulating fly (figure 5c). This corroborates with natural primary conidia.
size of *E. muscae* being larger than this minimum spore size to avoid aerodynamic drag, and being small enough to be dispersed by moderate air currents towards nearby flies that are attracted to the sporulating cadaver. Moreover, it allows the formation of a secondary conidiophore that forcibly discharges a highly infective secondary conidium in the same way. This two-step ‘cascade’ is thought to substantially increase the dispersal potential of *E. muscae*.

7. Methods

7.1. Soft cannon properties

The soft cannon consists of an elastomeric tube fitted with a cylindrical projectile. We use a range of tubes (VWR) with varying diameters *D*, wall thickness *T* and wall material (table 1). The corresponding Young’s modulus *E* is determined from stress–strain curves of thin strips of tubing (five repeated experiments). The projectiles (if not stated otherwise: 3D-printed polypropylene) have a diameter *D* > *D* (with *d* = 0.6–0.8 mm) in order to control *p*<sub>c</sub> by the static friction force between tube wall and projectile. The pressurizing liquid is air, water, or an aqueous glycerol solution. For the latter, we carefully monitor the temperature in the experiment (27 ± 1 °C) in order to calculate the density and viscosity from the known weight percentage of glycerol. Liquid properties are listed in table 2.

7.2. Projectile ejection from millimetric model system

In each experiment, the elastomeric tube is connected to a syringe pump (KD Scientific) operating at a low pumping rate (20 μl/min) and pre-filled with degassed liquid. At the end of the tube, the projectile is inserted over a distance *h*<sub>0</sub> taking care that the cannon is fully filled with liquid without incorporating any air bubbles. Then, the pressure is increased quasi-statically using a syringe pump (KD Scientific) operating at a low pumping rate (2–50 ml min<sup>−1</sup>), while continuous measurements are taken using a pressure sensor (LabSmith upPS8000-T116-10; *d*<sub>f</sub> = 100 ms). We extract the break-through pressure *p*<sub>c</sub>. Simultaneously, we visualize the projectile ejection dynamics using a Krontech Chronos 1.4 high-speed video camera with the Nikon zoom lens (AF-S DX VR Zoom-Nikkor 55–200 mm f/4-5.6G IF-D), recording at frame rates up to 40 000 frames s<sup>−1</sup>. We determine *v*<sub>c</sub> from zoom-in videos, tracking the trailing side of the projectile inside the tube using a custom MATLAB code. The resulting distance–time curve is fitted to obtain the velocity when the projectile leaves the exit.

### Table 1. Geometric and material properties of elastomeric tubes.

<table>
<thead>
<tr>
<th>material</th>
<th><em>D</em> (mm)</th>
<th><em>T</em> (mm)</th>
<th><em>L</em> (mm)</th>
<th><em>E</em> (MPa)</th>
<th><em>d</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>silicone</td>
<td>2.0</td>
<td>0.4</td>
<td>5 ± 1</td>
<td>3.49 ± 0.09</td>
<td>2.6 ± 0.05</td>
</tr>
<tr>
<td>silicone</td>
<td>2.0</td>
<td>1.0</td>
<td>5 ± 1</td>
<td>4.10 ± 0.22</td>
<td>2.6 ± 0.05</td>
</tr>
<tr>
<td>silicone</td>
<td>2.0</td>
<td>2.0</td>
<td>5 ± 1</td>
<td>3.91 ± 0.09</td>
<td>2.6 ± 0.05</td>
</tr>
<tr>
<td>silicone</td>
<td>4.0</td>
<td>1.0</td>
<td>10 ± 1</td>
<td>3.51 ± 0.27</td>
<td>4.7 ± 0.05</td>
</tr>
<tr>
<td>silicone</td>
<td>6.0</td>
<td>1.5</td>
<td>15 ± 1</td>
<td>3.84 ± 0.16</td>
<td>6.8 ± 0.05</td>
</tr>
<tr>
<td>silicone</td>
<td>8.0</td>
<td>2.0</td>
<td>20 ± 1</td>
<td>4.28 ± 0.13</td>
<td>8.8 ± 0.05</td>
</tr>
<tr>
<td>PVC</td>
<td>4.0</td>
<td>1.0</td>
<td>10 ± 1</td>
<td>11.39 ± 0.46</td>
<td>4.7 ± 0.05</td>
</tr>
</tbody>
</table>

### Table 2. Properties of pressurizing liquids.

<table>
<thead>
<tr>
<th>liquid</th>
<th>ρ (kg m&lt;sup&gt;−3&lt;/sup&gt;)</th>
<th><em>η</em> (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>air</td>
<td>1.176 ± 0.004</td>
<td>0.01848 ± 0.00005</td>
</tr>
<tr>
<td>water</td>
<td>996.5 ± 0.3</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>glycerol, 60.6 wt%</td>
<td>1151.1 ± 0.6</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>glycerol, 85.7 wt%</td>
<td>1219.0 ± 0.6</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>glycerol, 96.2 wt%</td>
<td>1246.9 ± 0.6</td>
<td>360 ± 30</td>
</tr>
</tbody>
</table>

7.3. High-speed videography of spore ejection

The fungus *Entomophthora muscae* s. str. (isolate nos. ARSEF 13,376 and KVL-14-115) is obtained from a controlled growing cycle in the laboratory using its natural host *Musca domestica*. Infected young-adult flies are kept at room temperature (21 ± 1 °C) and exposed to illumination conditions that simulate natural day–night cycles (16:8 h). After 6 days, we select infected flies that show signs of critical illness. After death, flies are dissected and the thorax and abdomen with clearly visible fungal mycelium are fixated into a small glass housing that allows for careful monitoring of sporulation and subsequent microscopy, while containing the spores. Sporulation is studied using a Leica DM2500 inverted microscope and Krontech Chronos v. 1.4 high-speed video camera, recording spore ejection at up to 15 000 frames s<sup>−1</sup> and 6300× magnification. In each experiment, we focus on a single, mature conidium—with clearly visible septum—that is oriented parallel to the field of view.

Data accessibility. The data that support the plots within this paper and other findings of this study are available from the corresponding author upon request.

Authors’ contributions. J.D.R., H.H.D.F.L. and K.H.J. designed the research. J.D.R., S.F.A.-N., P.H. and F.H. performed the research. J.D.R., S.F.A.-N. and K.H.J. analysed the data. J.D.R. wrote the manuscript with contributions from H.H.D.F.L. and K.H.J.

Competing interests. We declare we have no competing interests.

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