



British Mycological
Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio



Splash and grab: Biomechanics of peridiole ejection and function of the funicular cord in bird's nest fungi

Maribeth O. HASSETT^a, Mark W. F. FISCHER^b, Zachary T. SUGAWARA^a,
Jessica STOLZE-RYBCZYNSKI^a, Nicholas P. MONEY^{a,*}

^aDepartment of Biology, Miami University, Oxford, OH 45056, USA

^bDepartment of Chemistry and Physical Science, College of Mount St. Joseph, Cincinnati, OH 45233, USA

ARTICLE INFO

Article history:

Received 15 May 2013

Received in revised form

15 July 2013

Accepted 30 July 2013

Available online 14 August 2013

Corresponding Editor:

Steven Harris

Keywords:

Basidiomycota

Dispersal

High-speed video

Nidulariaceae

Spore discharge

ABSTRACT

The bird's nest fungi (Basidiomycota, Agaricales) package millions of spores into peridioles that are splashed from their basidiomata by the impact of raindrops. In this study we report new information on the discharge mechanism in *Crucibulum* and *Cyathus* species revealed with high-speed video. Peridioles were ejected at speeds of 1–5 m per second utilizing less than 2 % of the kinetic energy in falling raindrops. Raindrops that hit the rim of the basidiome were most effective at ejecting peridioles. The mean angle of ejection varied from 67 to 73° and the peridioles travelled over an estimated maximum horizontal distance of 1 m. Each peridiole carried a cord or funiculus that remained in a condensed form during flight. The cord unravelled when its adhesive surface stuck to a surrounding obstacle and acted as a brake that quickly reduced the velocity of the projectile. In nature, this elaborate mechanism tethers peridioles to vegetation in a perfect location for browsing by herbivores.

© 2013 The Authors. Published by Elsevier Ltd on behalf of The British Mycological Society.

Open access under [CC BY license](http://creativecommons.org/licenses/by/4.0/).

Introduction

The unusual fruit bodies of the bird's nest fungi (Basidiomycota, Agaricales) were described by Carolus Clusius in 1601 and attracted the interest of pioneering mycologists in the eighteenth and nineteenth centuries (Brodie 1975). Surprisingly, the mechanism of splash dispersal was not recognized until the 1920s, when Martin (1927) deduced that raindrops propelled peridioles from their fruit bodies and that they stuck to surrounding vegetation. Harold Brodie, who studied with A.

H. R. Buller, dedicated his research career to the bird's nest fungi (Savile 1989). Brodie's experiments on peridiole discharge included studies on the relationship between basidiome morphology and the splash patterns formed by water ejected from the cups and the distance of peridiole ejection. He also looked at the structure and function of the funicular cord carried by peridioles of *Crucibulum* and *Cyathus* species (Brodie 1975).

The funicular cord is condensed within a structure called the purse that is connected to the inner surface of the

* Corresponding author. Tel.: +1 513 529 2140; fax: +1 513 529 4243.

E-mail addresses: moneynp@miamioh.edu, moneynp@muohio.edu (N. P. Money).

1878-6146 © 2013 The Authors. Published by Elsevier Ltd on behalf of The British Mycological Society. Open access under [CC BY license](http://creativecommons.org/licenses/by/4.0/).

<http://dx.doi.org/10.1016/j.funbio.2013.07.008>

basidiome (Fig 1). Manipulation of the peridioles with fine forceps is instructive: peridioles can be removed from their basidiomata without opening the purse; the purse ruptures when the hyphae that attach it to the wall of the basidiome are pulled, and this exposes the funicular cord which can be unravelled to a length of a few centimetres. The free end of the funicular cord is widened to form an adhesive pad called the hapteron. In nature, elongated funicular cords are seen wrapped around the stems and petioles of plants to which peridioles are tethered. From these observations, Brodie concluded that the funicular cord remains in its condensed form during the flight of the peridiole, and unravels when the peridiole hits an obstacle. In the absence of high-speed cameras, however, this hypothesis could not be tested. In the present study, we have used high-speed video to examine the details of peridiole ejection and funicular cord function in the bird's nest fungi.

Methods

Specimens

Mature basidiomata of *Crucibulum laeve*, *Cyathus olla*, *Cyathus stercoreus*, and *Cyathus striatus* were collected from landscaping mulch and wood chips on the campus of Miami University, Oxford, Ohio, USA.

Models

Models of *Crucibulum* and *Cyathus* basidiomata of various sizes were crafted by cutting plastic microfuge tubes (1.5 mL volume) with a heated scalpel to heights of 8–15 mm and mouth diameters of 5–8 mm. The base of the tubes was melted and fused to the base of inverted Petri dishes to provide a stable platform. Models resembling the more open types of fruit body produced by *Mycocalia* and *Nidularia* were made with modelling clay (ShurTech Brands, Avon, OH). Mucilage in these fruit bodies was modelled using 0.8 % agar (w/v). Peridioles were modelled with nylon spheres (3/32 inch diameter; Small Parts Manufacturing, Portland, OR); 5–6 of these plastic beads were placed in the models of *Crucibulum* and *Cyathus*; 10–15 beads were placed in the soft agar inside the models of *Mycocalia* and *Nidularia*.

High-speed video

Fresh specimens of basidiomata were pinned to squares of corkboard to maintain an upright orientation during the splash experiments. The corkboard was placed on a rack inside a glass enclosure that protected the camera and lenses from water. The same enclosure was used for experiments with the model fruit bodies. Water drops were released from a burette positioned 1.2 m above the basidiomata to simulate water drops. The diameter of these drops was 6 mm and they hit the fruit bodies with a mean velocity of $4.4 \pm 0.1 \text{ m s}^{-1}$ ($n = 41$). Most freefalling raindrops are 1–2 mm in diameter (Marshall & Palmer 1948; Lamb & Verlinde 2011). The larger drops used in our experiments are characteristic of water drops shed from wet vegetation. Because the drops were released from a height of 1.2 m they did not reach their terminal velocity (approx. 9 m s^{-1}), nor did they fall long enough to deform and fragment (Gunn & Kinzer 1949; Villermaux & Bosa 2009). In order to study the mechanics of peridiole attachment, basidiomata were surrounded by 5 cm lengths of floral wire. Video recordings of splash discharge were captured at frame rates between 3000 and 6000 frames per second (fps) and minimum shutter speed of 0.17 ms using a tripod-mounted FASTCAM 1024 PCI camera (Photron, San Diego, CA) fitted with a macro lens.

Image analysis and mathematical modelling

For analysis, video clips compiled from 70 to 200 individual image files edited from recordings of tens of thousands of images captured in a few seconds (e.g., 42 000 frames in 7 s at 6000 fps). Analysis of video clips was performed using Video-Point v.2.5 (Lenox Softworks, Lenox, MA), Image-Pro Plus 6.2 (Media Cybernetics, Bethesda, MD), and proprietary software from Photron. For calculations of peridiole kinetic energy ($\frac{1}{2} mv^2$) and trajectories after discharge, wet weight of peridioles was measured with an accuracy of 0.1 mg. Models of peridiole trajectories were created using MATHEMATICA 6 (Wolfram Research, Champaign, IL). To generate equations for the x- and y-positions of spore mass as functions of time, the software was used to integrate Newton's Second Law ($\Sigma F = ma$), where the forces were taken to be gravity (mg) in the y-direction and Stokes Law drag opposing motion according to the following equation:

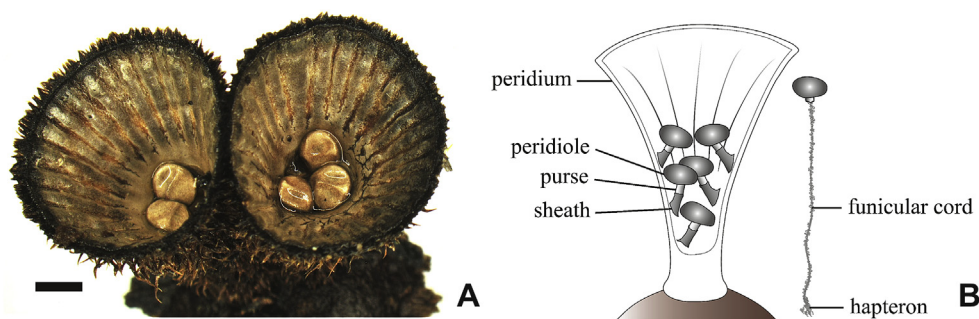


Fig 1 – Structure of the bird's nest fungus basidiome. (A) Pair of fruit bodies of *Cyathus striatus* showing peridioles glistening with surrounding fluid at the bottom of the fruit body. Scale bar, 2 mm. (B) Diagram of sectioned fruit body showing structure of peridioles before discharge and single peridiole following splash discharge. Adapted from Money et al. (2013).

$$F_{\text{drag}} = -6\pi r\eta v$$

where, r = effective radius of the peridiole (radius of sphere with same volume as pip-shaped peridiole plus any attached water),

η = air viscosity = 1.81×10^{-5} Pa s (or $\text{kg m}^{-1} \text{s}^{-1}$), and
 v = peridiole velocity (m s^{-1}).

It follows, that $-6\pi r\eta v_x = ma_x$, and $-6\pi r\eta v_y - mg = ma_y$ can be solved for the x - and y -positions as functions of time (Fischer et al. 2010). This pair of equations was used to model the flight of the peridiole based upon measurements of peridiole size, mass, and the launch speed and angle of the projectile. The resulting predictions were compared with video observations and provided estimates of maximum height and maximum range for each species.

Scanning electron microscopy

Mature peridioles were removed from basidiomata with forceps and the funicular cord was teased from the purse. Peridioles were immersion fixed in 2 % paraformaldehyde, 2.5 % glutaraldehyde in 0.05 M sodium cacodylate buffer for 1 h at room temperature. Samples were rinsed 4×15 min at room

temperature with 0.05 M sodium cacodylate buffer, followed by dehydration in ethanol (20 min each in 25 %, 50 %, and 75 %; 30 min in 95 %; 60 min in 100 %). After critical point drying, samples were mounted on stubs and sputter coated with gold 20 nm (once untilted, then tilted on opposing sides to ensure even coating of all sides). Samples were examined with a Zeiss Supra 35 VP FEG SEM.

Results

Splash discharge of peridioles and plastic beads

High speed video recordings of splash discharge revealed details of drop impact upon the basidiome and peridiole ejection (Fig 2; Supplementary movies S1–S4). Mean ejection speeds of peridioles varied from 1.5 m s^{-1} in *Cyathus olla* to 3.6 m s^{-1} (13 km h^{-1}) in *Cyathus striatus* (Table 1). Ejection angles ranged from 18 to 89° , with the shallowest discharges observed in *C. olla*; mean ejection angles did not vary significantly between the four species in this study ($67\text{--}73^\circ$; one-way ANOVA $P = 0.78$). Comparisons between the kinetic energy of the water drops as they hit the rims of the basidiomata and the kinetic energy of the peridioles at the moment of ejection

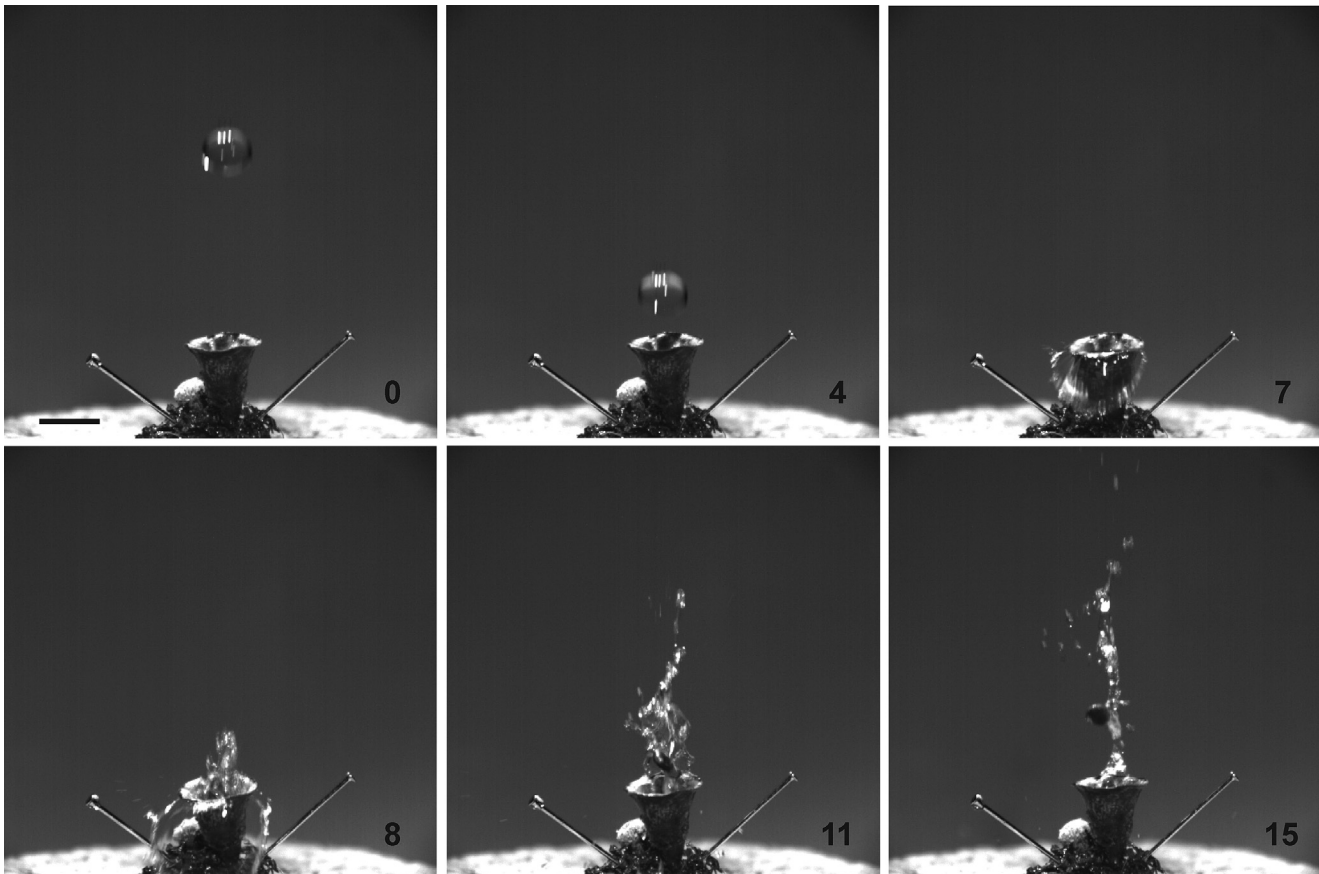






Fig 2 – Selected frames from high-speed video (3000 fps) of splash discharge in *Cyathus olla*. Peridiole is ejected by the upward displacement of water from the interior of the basidiome when water drop hits the rim of the peridium. Capture times in milliseconds shown in bottom right of each image. Scale bar = 3 mm. A selection of videos is provided in the Supplementary movies.

Table 1 – Peridiole mass, ejection speeds and angles, and energy usage for four species of bird's nest fungus.

| Species ^a | Peridiole mass (mg): mean ± standard error, sample size | Peridiole ejection speed (m s ⁻¹): mean ± standard error, range, sample size | Peridiole ejection angle: mean ± standard error, range | Fraction of drop energy consumed in launch |
|--|---|--|--|--|
| <i>Crucibulum laeve</i>  | 1.2 ± 0.1, n = 7 | 3.1 ± 0.2, 1.6–4.5, n = 15 | 67 ± 4°, 30–88° | 0.4 % |
| <i>Cyathus olla</i>  | 8.6 ± 0.3, n = 14 | 1.5 ± 0.2, <0.1–3.0, n = 20 | 72 ± 5°, 18–89° | 1.0 % |
| <i>Cyathus stercoreus</i>  | 1.3 ± 0.1, n = 6 | 2.9 ± 0.3, 1.1–4.1, n = 12 | 69 ± 5°, 36–89° | 0.6 % |
| <i>Cyathus striatus</i>  | 2.1 ± 0.1, n = 4 | 3.6 ± 0.3, 1.7–5.5, n = 16 | 73 ± 3°, 50–87° | 1.9 % |

a Scale bars, 2 mm.

showed that less than 2 % of the drop energy was transferred to each peridiole.

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.funbio.2013.07.008>.

If the basidiome was relatively dry before the experiments and the peridioles were not bathed in water, the first or second water drops entering the basidiome did not discharge peridioles. Once the basidiome was filled with water, the impact of subsequent water drops was associated, invariably, with the discharge of one or more peridioles. Off-centre, or non-axisymmetric drops that hit the rim of the basidiomata divided, with some portion of the fluid shedding away from the basidiome, and the remainder merging with the pool of water in the cup, propelling water and one or more peridioles into the air. Axisymmetric drops hitting the centre of the basidiomata shed water in all directions around the rim and were ineffective at peridiole discharge.

Plastic beads were discharged from model splash cups with a conical shape at a mean velocity of 1.3 m s⁻¹, which was not significantly different from the ejection speeds of peridioles from *C. olla* ($P = 0.42$; Fig 3A). This was slower than the ejection speeds for the other species examined using high-speed video (Table 1). Ejection angles for the models matched those of the

genuine reproductive organs ($P = 0.54$). Model splash cups resembling the more open types of fruit body produced by *Mycocalia* and *Nidularia* showed much slower bead ejections at much shallower launch angles (Fig 3B).

Mathematical modelling

According to our mathematical model (Methods), the peridioles followed parabolic arcs during their 200–700 ms of flight, reaching a maximum height of 0.5 m and maximum horizontal distance of 1.0 m (Fig 4). The largest and slowest peridioles, launched from *Cyathus olla*, reached the lowest heights (maximum of 0.1 m) and travelled the shortest horizontal distances (maximum of 0.2 m). Analysis of trajectories for all species according to launch angle showed that maximum horizontal distances were covered by peridioles ejected at the lowest angles. This is interesting given the high proportion of discharges at the steepest angles: 58–74 % of launches in all species were at 70–90°.

Funicular cord structure and function

The funicular cord is composed of thousands of hyphae, aligned in a longitudinal direction, twisted around one

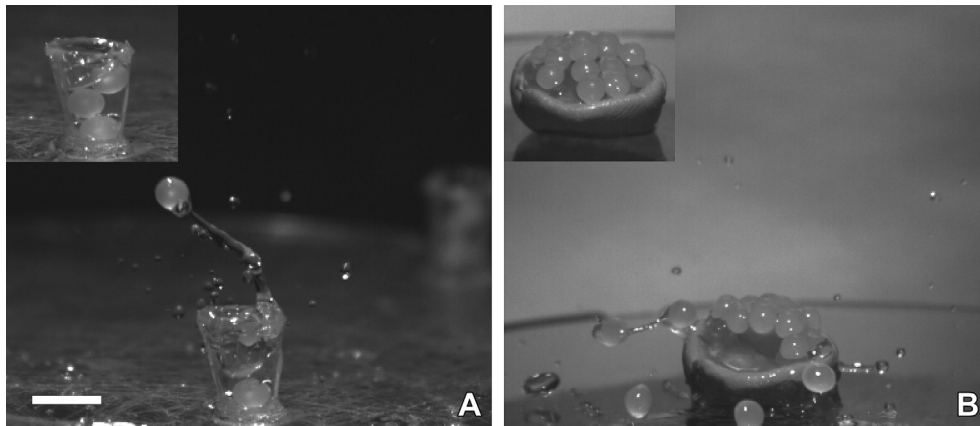


Fig 3 – Splash experiments using models of bird's nest fungi basidiomata. (A) Discharge of nylon bead from cup resembling basidiome shape characteristic of *Crucibulum*, *Cyathus*, and *Nidula*. (B) Discharge of multiple nylon beads from cup resembling basidiome shape characteristic of *Mycocalia* and *Nidularia*. Insets show models before drop impact. Scale bar = 5 mm.

another to form a strand with a stretched length of up to 12 cm and diameter of 0.1 mm (Fig 5A). The cord emerges from the underside of the tunica of the peridiole and the hyphae at the other end are more diffuse, terminating in a sticky pad, or hapteron. Hyphae within the cord are inflated around both of the septa within clamp connections. Scanning electron micrographs suggest that the cell wall is thickened in circumferential bands around these septa (Fig 5B). High speed videos of peridiole discharge showed that the funicular cord remained in condensed form within the purse during flight and unravelled only when the hapteron hit an obstacle. In experiments with floral wires surrounding fruit bodies, the

funicular cord played out after the hapteron stuck to the wire, and the momentum of the peridiole wound the cord around the wire (Supplementary movie S5). The entire discharge process is diagrammed in Fig 6.

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.funbio.2013.07.008>.

Discussion

Peridiole ejection from the basidiomata of bird's nest fungi utilizes a tiny proportion of the kinetic energy in falling

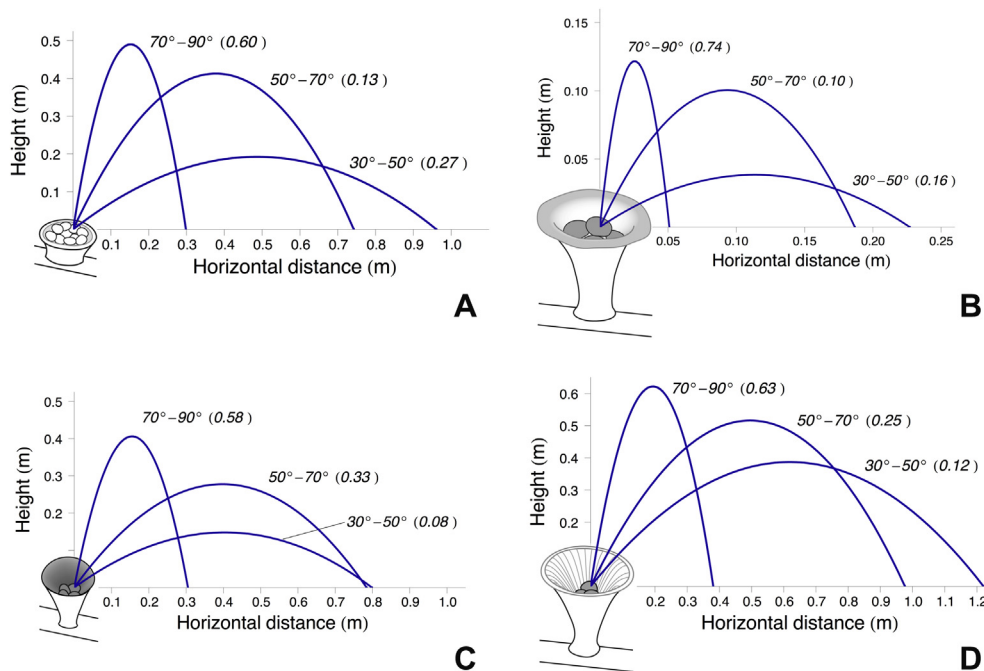


Fig 4 – Predicted trajectories of peridioles from basidiomata of four species of bird's nest fungi based upon launch speeds measured from high-speed video recordings. (A) *Crucibulum laeve*, (B) *Cyathus olla*, (C) *Cyathus stercoreus*, and (D) *Cyathus striatus*. Each plot shows trajectories calculated for three ranges of launch angles; figures in parenthesis indicate proportion of measured discharges that fell within these ranges.

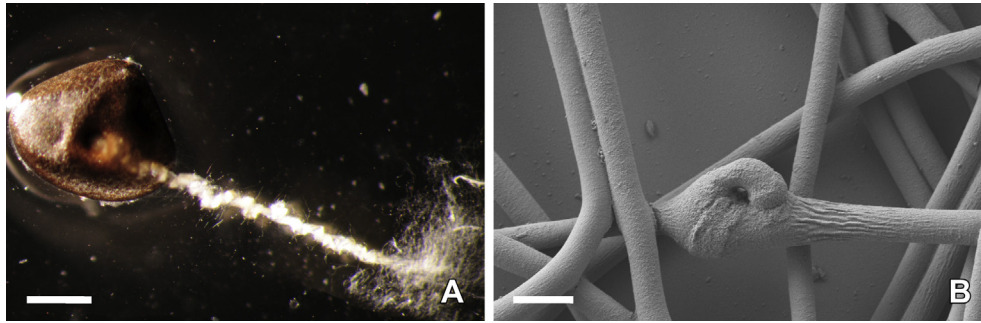


Fig 5 – Funicular cord structure. (A) Complete cord connecting peridiole (on left) to the hapteron. (B) Scanning electron micrograph showing unusual structure of clamp connections within the cord. Scale bars (A) = 1 mm, (B) = 2 μ m.

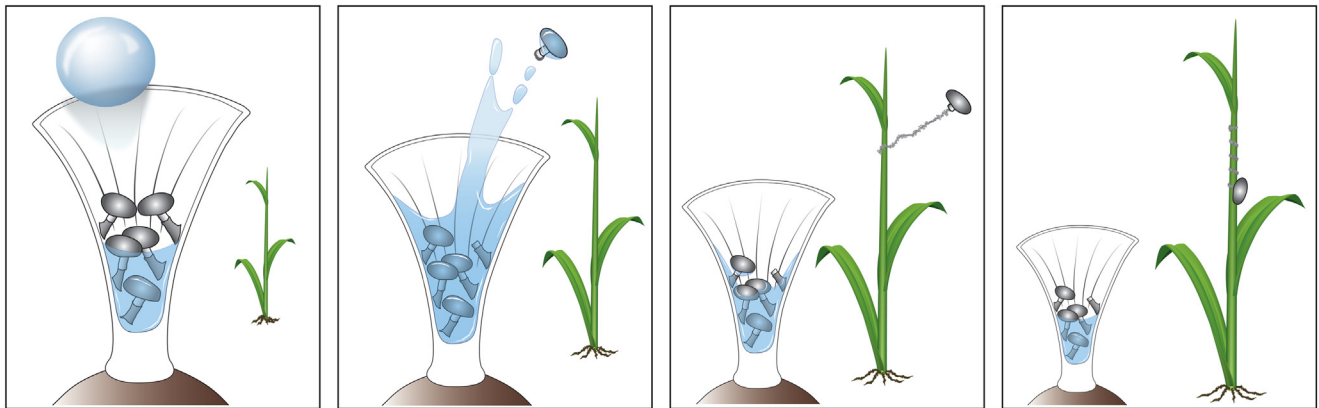


Fig 6 – Diagram showing splash discharge of peridiole and its mechanism of attachment to vegetation. The funicular cord is packed within the purse before discharge. The force of the raindrop fractures the purse, leaving the sticky end of the cord exposed during the flight of the peridiole. Deployment of the funicular cord occurs when the hapteron contacts an obstacle. The process is completed in less than 700 ms. Adapted from Money et al. (2013).

raindrops. Raindrops that hit the rim of the basidiomata (non-axisymmetric impacts) are more effective at discharging peridioles than axisymmetric drops that hit dead-center. The same effect has been observed in the splash-discharge of seeds from plants (Amador et al. 2013). Seeds dispersed by the splash mechanism are very small (<0.3 mm), with masses ranging from 5 to 180 ng, compared with peridiole masses of 1.2–8.6 mg (Table 1). Splash-dispersed seeds are three orders of magnitude smaller than the raindrops in which they are discharged; peridioles can match the size of smaller raindrops that launch them into the air.

Mean launch angles did not vary significantly between the four species of bird's nest fungi examined in our experiments. The greatest range of ejection angles was measured in *Cyathus olla* that produced the largest and heaviest peridioles. The species with smaller peridioles and more steep-sided basidiomata showed narrower ranges of discharge angles and were adapted for maximizing vertical height rather than horizontal distance in their launches. Peridioles of *C. olla* were launched to a predicted maximum height of 0.1 m and a horizontal distance of only 5 cm; the other species reached predicted heights of 0.4–0.6 m and horizontal distances of 0.3–0.4 m (Fig 4). Splash-discharged seeds are scattered over horizontal distances of up to 1 m (Amador et al.

2013). These distinctions in biomechanical behaviour correspond to differences in reproductive strategy between splash-dispersed plants and the bird's nest fungi. Seeds are splashed from the parent plants and germinate in the surrounding soil. In the bird's nest fungi, optimization of splash discharge for vertical travel accords with their putative coprophilous life cycles (Brodie 1975).

REFERENCES

- Amador GJ, Yamada Y, Hu DL, 2013. Splash-cup plants accelerate raindrops to disperse seeds. *Journal of the Royal Society Interface* 10: 20120880.
- Brodie HJ, 1975. *The Bird's Nest Fungi*. University of Toronto Press, Toronto.
- Fischer MWF, Stolze-Rybczynski JL, Davis DJ, Cui Y, Money NP, 2010. Solving the aerodynamics of fungal flight: how air viscosity slows spore motion. *Fungal Biology* 114: 943–948.
- Gunn R, Kinzer GD, 1949. The terminal velocity of fall for water droplets in stagnant air. *Journal of Meteorology* 6: 243–248.
- Lamb D, Verlinde J, 2011. *Physics and Chemistry of Clouds*. Cambridge University Press, Cambridge.
- Marshall JS, Palmer WMcK, 1948. The distribution of raindrops with size. *Journal of Meteorology* 5: 165–166.

-
- Martin GW, 1927. Basidia and spores of the Nidulariaceae. *Mycologia* 19: 239–247.
- Money NP, Hassett MO, Fischer MWF, 2013. Circus fungorum: the aesthetics of the invisible and their movements. *Philosophy Activism Nature* (in press).
- Savile DBO, 1989. Harold Johnston Brodie, 1907–1989. *Mycologia* 81: 832–836.
- Villermaux E, Bosa B, 2009. Single-drop fragmentation determines size distribution of raindrops. *Nature Physics* 5: 697–702.