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# Short-range splash discharge of peridioles in *Nidularia*

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ABSTRACT

The distinctive shapes of basidiomata in the bird's nest fungi reflect differences in the mechanism of splash discharge. In the present study, peridiole discharge was examined in *Nidularia pulvinata* using high-speed video. *Nidularia pulvinata* produces globose basidiomata that split open at maturity to expose 100 or more peridioles within a gelatinous matrix. Each peridiole contains an estimated 7 million spores. The impact of water drops splashed the peridioles horizontally from the fruit body, along with globs of mucilage, at a mean velocity of 1.2 m $s^{-1}$. Discharged peridioles travelled for a maximum horizontal distance of 1.5 cm. This launch process contrasts with the faster vertical splashes of peridioles over distances of up to one metre from the flute-shaped fruit bodies of bird's nest fungi in the genera *Crucibulum* and *Cyathus*. Peridioles in these genera are equipped with a funicular cord that attaches them to vegetation, placing them in an ideal location for ingestion by browsing herbivores. The absence of cords in *N. pulvinata* and its use of a sloppy discharge mechanism suggest that it is more likely to be dispersed by animals feeding on the forest floor.



## Introduction

Bird's nest fungi (Agaricales, Nidulariaceae) are saprotrophic microorganisms that grow on woody plant debris and herbivore dung (Brodie 1975). The basidiomata of bird's nest fungi function as splash cups, from which sporangia (peridioles) are discharged by raindrops. Peridioles of the different species range from 1 to 3 mm in diameter and contain up to 50 million non-ballistosporic basidiospores. Fruit body shapes in the Nidulariaceae range from rounded cups with a smooth wall, or peridium, in *Crucibulum* and *Nidula*, to grooved flutes in *Cyathus striatus*, and blobs of varying size in *Mycocalia* and *Nidularia* (Table 1). These morphological differences affect the launch speed and trajectory of peridioles splashed by raindrops (Hassett *et al.* 2013).

Our previous research on *Crucibulum* and *Cyathus* demonstrated that peridiole launches at angles of 30 to 50° to the horizontal resulted in the longest horizontal flights. Launches at these relatively low angles were observed in these fungi, but the mean launch angle in the four species included in these experiments was approximately 70°. This tendency toward a steeper ascent favours the collision of peridioles with grass culms and their attachment via the deployment of a tethering device called the funicular cord. The present study concerns

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**Table 1 – Morphology and launch parameters of splash discharge mechanism in *Nidularia pulvinata* (data obtained in present study) compared with two of the species of bird's nest fungi examined by Hassett *et al.* 2013. Scale bars, 2 mm.**

| Species | Image of basidiomata | Morphological details | Mean ejection speed (m $s^{-1}$) | Mean ejection height (m) | Mean horizontal range (m) |
|---|---|---|---|---|---|
| *Nidularia pulvinata* |  | Globular shape<br>Lacks funiculus<br>100 or more peridioles | $1.2 \pm 0.5$<br>$n = 14$ | $0.005 \pm 0.002$<br>$n = 22$ | $0.008 \pm 0.003$<br>$n = 11$<br>(Model prediction = 0.038) |
| *Crucibulum laeve* |  | Rounded cup shape<br>Simple funiculus<br>Up to 15 peridioles | 3.1 | 0.49 | 0.30 |
| *Cyathus striatus* |  | Flute shape<br>Complex funiculus<br>Up to 20 peridioles | 3.6 | 0.62 | 0.38 |

the mechanics of splash discharge in *Nidularia pulvinata*, whose peridioles are not equipped with funicular cords and are discharged horizontally from globose basidiomata. This bird's nest fungus grows on rotting wood.

## Materials and methods

### *Culture*

Cultures of *Nidularia pulvinata* #156133B on nutrient straw agar were obtained from Carolina Biological Supply (Burlington, NC). These jar cultures contained a mixture of immature and mature basidiomata providing a source of fresh material for experiments over a 3-month period.

### *High-speed video*

Splash experiments were conducted using the experimental apparatus described by Hassett *et al.* (2013). Mature unopened basidiomata of *Nidularia pulvinata* were placed in the centre of inverted lids of Petri dishes in preparation for splashing. It was not necessary to secure the fruit bodies of this species with pins, because their compacted shape and sticky base kept them stable during the experiments. The Petri dish lids were placed on a rack inside a glass enclosure that protected the camera and lenses from water. Water drops were released from a burette positioned 1.2 m above the basidiomata to simulate raindrops. The diameter of these drops was 6 mm and they hit the fruit bodies with a mean velocity of $4.4 \pm 0.05$ m $s^{-1}$ ($n = 41$). The drops used in our experiments are characteristic of larger water drops shed from wet vegetation (Hassett *et al.* 2013). Video recordings of splash discharge were captured at frame rates of 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*

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 LoggerPro software (Vernier Software & Technology, Beaverton, OR), and proprietary software from Photron. Discharge distances were predicted using the mathematical model detailed in Hassett *et al.* (2013).

## Results and discussion

The intact peridium of mature basidiomata of *Nidularia pulvinata* was torn open by the impact of water drops, exposing hundreds of peridioles sitting in a gelatinous matrix. Subsequent drops splashed into this matrix, spreading the fluid toward the circumference of the fruit body, and dislodging several peridioles at a time (Fig 1; Supplementary Movie). This splash mechanism discharged peridioles horizontally at velocities ranging from 0.3 to 2.0 m $s^{-1}$ over a mean distance of 8 ± 3 mm from the basidiomata (Table 1). Water drops striking the centre of the basidiomata of *N. pulvinata* were most effective at discharging peridioles.

The horizontal shedding of peridioles recorded in the present study was anticipated from earlier experiments in which nylon beads embedded in agar were splashed from clay models of fruit bodies (see Fig 3 in Hassett *et al.* 2013). Applying a flat trajectory to the motion of the peridioles of *N. pulvinata*, and using a mean mass of 0.2 mg and launch speed of 1.2 m $s^{-1}$, the simple mathematical model described in the earlier research predicted a horizontal range of 38 mm. This is more than twice the maximum distance measured in our experiments (15 mm), which may be explained by the presence of mucilage strings connecting peridioles to the fruit body during and after the launch (Supplementary Movie). Nevertheless, the measured and predicted discharge distances are only a fraction of the 1.2 m range of peridiole splashes in *Cyathus striatus*.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2015.01.003.

The effectiveness of drop impact in the centre of the basidiomata of *N. pulvinata* in discharging peridioles contrasted with the greater success of drop strikes on the rim of the peridium in *Crucibulum* and *Cyathus* (Fig 2). The launch speed of the peridioles of *N. pulvinata* was one third of the mean velocity recorded from *Crucibulum laeve* and *Cyathus striatus* (Table 1). The fastest of the discharge mechanisms are observed in *C. striatus* that produces tall basidiomata (up to 15 mm in height) with a stiff peridium. Water droplets are propelled vertically from these structures. Grooves on the inner surface of the peridium in this species may help to break up the pool of water swirling inside the fruit body after the impact of the drop. This turbulence continues for 10 ms or so before the ejection of fluid and one or two peridioles is completed. It is possible that this contributes to the rapid launch of peridioles in this species.

Basidiomata of *N. pulvinata* have a very different structure, with a thin and fragile peridium that is ruptured by raindrops. The peridium of *N. pulvinata* does not play any role in splash discharge other than holding the peridioles together until

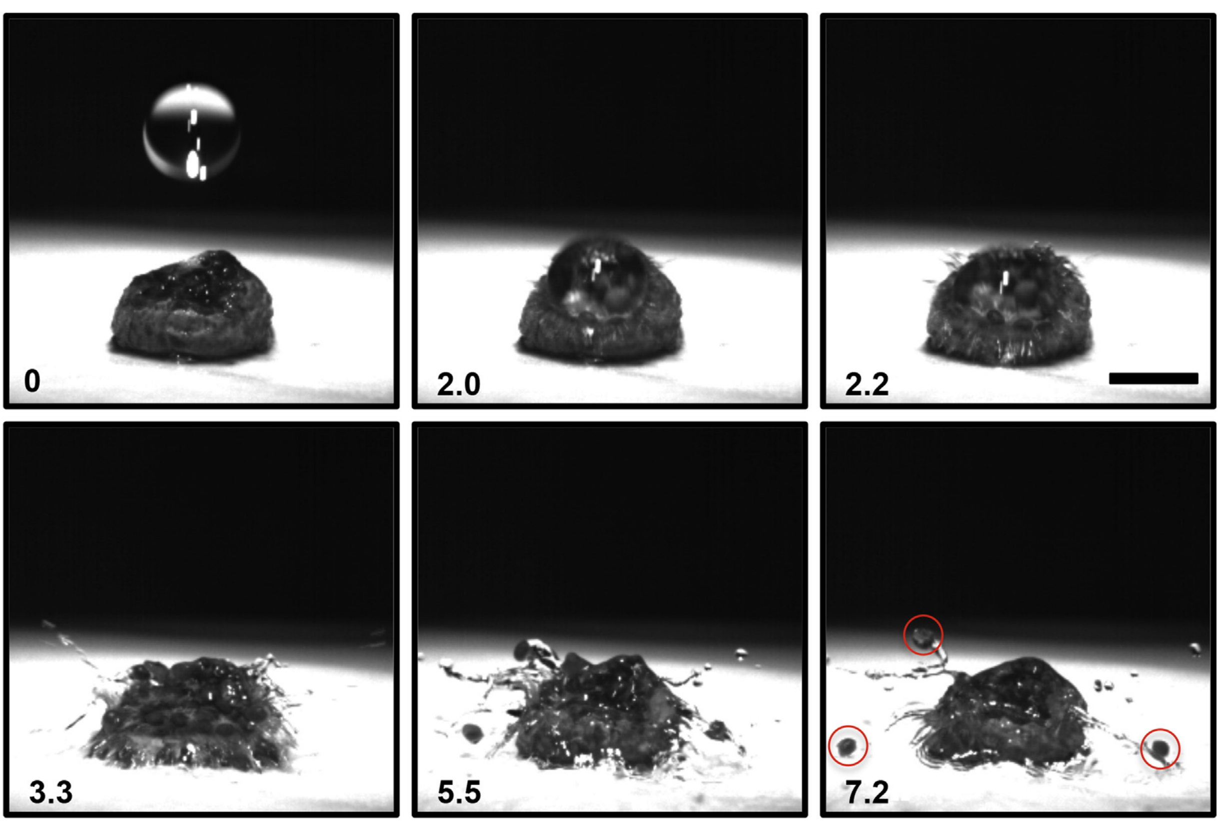


**Fig 1 – Selected frames from high-speed video recording (300 fps) of splash discharge in *Nidularia pulvinata*. Multiple peridioles ejected sideways over lip of this open fruit body 1 ms after drop impact. Capture times in ms shown at bottom left of each frame. Scale bar = 5 mm. Representative video recording is provided in Supplementary movie.**

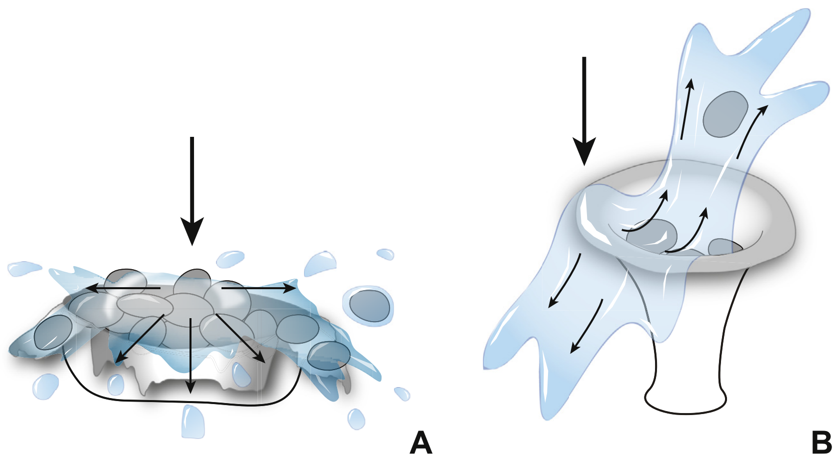


**Fig 2 – Diagram comparing mechanisms of splash discharge in *Nidularia pulvinata* and a bird's nest fungus with a flute-shaped basidioma.**

**Fig 3 – Diagram showing splash discharge of peridioles in *Nidularia pulvinata* based on high-speed video recordings. The process is completed in less than 10 ms.**

they are spread by the impact of raindrops (Fig 3). Additional morphological differences between *N. pulvinata* and other bird's nest species with flute-shaped basidiomata include the absence of a funicular cord, smaller size of peridioles, and larger number of peridioles per fruit body (Tables 1 and 2). It seems likely that the development of these features of the *Crucibulum* and *Cyathus* basidiomata necessitates a greater energy cost than the production of small peridioles without funicular cords inside a thin-walled peridium by *Nidularia*. Basidiomata of *Nidularia* contain an estimated 700 million basidiospores compared with 4 million spores in *Cyathus stercoreus*. Spore numbers in *Crucibulum* and other species of *Cyathus* fall between these extremes. The mean launch speed of peridioles of *Cyathus olla* is similar to the speed measured in *N. pulvinata*,

**Table 2 – Peridiole sizes and spore numbers for five species of bird's nest fungus.**

| Species | Mean peridiole dimensions (diameter × thickness, mm) | Mean peridiole mass (mg) | Maximum number of peridioles per basidioma[a] | Number of spores per peridiole[b] | Number of spores per basidioma |
|---|---|---|---|---|---|
| *Crucibulum laeve* | 1.5 × 0.5 | 1.2 | 15 | $3 \times 10^7$ | $5 \times 10^8$ |
| *Cyathus olla* | 3.0 × 1.3 | 8.6 | 10 | $5 \times 10^7$ | $5 \times 10^8$ |
| *Cyathus stercoreus* | 1.9 × 0.3 | 1.3 | 20 | $2 \times 10^5$ | $4 \times 10^6$ |
| *Cyathus striatus* | 2.0 × 0.4 | 2.1 | 15 | $2 \times 10^6$ | $3 \times 10^7$ |
| *Nidularia pulvinata* | 1.1 × 0.5 | 0.2 | 100+ | $7 \times 10^6$ | $7 \times 10^8$ |

a Sources: Brodie (1975); Ellis JB, Ellis MB (1990); Flegler SL, Hooper GR (1978). Other parameters from Hassett *et al.* (2013), or measured for present paper.
b Number of spores estimated from peridiole volume ÷ spore volume based on dimensions of peridioles and spores provided by Brodie (1975).

but the greater mass of *C. olla* peridioles (8.6 mg versus 0.2 mg) accounts for their discharge over a horizontal distance of up to 0.2 m (Table 2; Hassett *et al.* 2013).

The morphology of the bird's nest fungi has been altered in many ways during their evolution from gilled mushrooms in the Agaricaceae (Matheny *et al.* 2006). The resulting short flights of *Nidularia* peridioles may seem futile when we consider the presumed value of spore dispersal over longer distances, but at least they allow the fungus to spread from its parent basidiomata. After splash discharge, the peridioles may be distributed farther by continuing rainfall and water trickling over the surface of the rotting wood on which the fruit bodies develop. Although the peridioles of *Nidularia* do not become attached to vegetation by funicular cords, spore dispersal may also occur if animals consume the peridioles directly from the forest floor.

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