Fungal Biology 127 (2023) 1491–1504

Contents lists available at ScienceDirect

Fungal Biology

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

Review

Ascus function: From squirt guns to ooze tubes

Nicholas P. Money [a,*], Jessica Stolze-Rybczynski [b], B. Eugene Smith [a], Dragana Trninić [a], Diana J. Davis [c], Mark W.F. Fischer [c]

[a] Western Program and Department of Biology, Miami University, Oxford, OH, 45056, USA
[b] Biomedical Sciences Department, Grand Valley State University, Allendale, MI, 49401, USA
[c] Department of Chemistry and Biochemistry, Mount St. Joseph University, Cincinnati, OH, 45233, USA

ARTICLE INFO

Handling editor: Prof. G.M. Gadd

Keywords:
Ascomycetes
Biomechanics
Dispersal
Spores
Turgor pressure

ABSTRACT

Unlike the mechanism of ballistospore discharge, which was not solved until the 1980s, the operation of asci as pressurized squirt guns is relatively straightforward and was understood in the nineteenth century. Since then, mycologists have sought to understand how structural adaptations to asci have allowed the ascomycetes to expel spores of different shapes and sizes over distances ranging from a few millimeters to tens of centimeters. These modifications include the use of valves at the tips of asci that maintain ascus pressure and expel spores at the highest speeds, and gelatinous appendages that connect spores after release and create larger projectiles with greater momentum than single spores. Clever experiments in the twentieth century coupled with meticulous microscopic studies led investigators to understand how asci with complicated apical structures worked and mathematical models produced estimates of launch speeds. With the recent application of high-speed video microscopy, these inferences about ascus function have been tested by imaging the motion of spores on a microsecond timescale. These experiments have established that ascospore discharge is the fastest fungal movement and is among the fastest movements in biology. Beginning with the history of the study of asci, this review article explains how asci are pressurized, how spores are released, and how far spores travel after their release. We also consider the efficiency of ascospore discharge relative to the mechanism of ballistospore discharge and examine the way that the squirt gun mechanism has limited the morphological diversity of ascomycete fruit bodies.

1. Preface

Asci are sporangia that produce and release ascospores. Some ascomycetes use them to propel their spores into the air, whereas others extrude spores slowly from their asci so that they emerge *en masse* as a sticky cirrus from the fruit body. These squirt guns and ooze tubes are pressurized by osmosis and the motion of the spores is coupled to the release of the surrounding ascus sap. The asci of truffles and many of the ascomycete yeasts work differently from these active devices, functioning instead as passive bags whose spores are exposed when their walls dissolve.

Among the asci that discharge spores into the air, we find a spectacular array of launch mechanisms that differ in ballistic performance. At this finer level of inquiry, the study of the ascus becomes an exploration of the structural diversification within the largest phylum of fungi and the evolution of the fastest propulsive mechanisms in nature. This biomechanical analysis also highlights an inherent limitation of the ascus as a device for launching spores from macroscopic fruit bodies and the exquisite solution offered by the basidium.

2. Studies on asci in the eighteenth and nineteenth centuries

Anton Micheli published the earliest drawings of ascospores in his *Nova Plantarum Genera* (1729; Fig. 1A–C). The spores were pictured as globules grouped in apothecia of lichenized fungi (Fig. 1A), and as dots arranged in single files, some in groups of eight, separated from the perithecia of species of Xylariaceae (Fig. 1B). Micheli did not show the ascus wall surrounding the spores in these drawings, but unequivocal sketches of walled asci appear in a truffle figure in the *Nova Plantarum* and in his notes on a lichenized fungus in an unpublished manuscript (Ainsworth 1976). Micheli also illustrated the mass discharge or puffing of ascospores from apothecia of a fungus that he named *Fungoides*

* Corresponding author.
E-mail address: moneynp@miamioh.edu (N.P. Money).

https://doi.org/10.1016/j.funbio.2023.11.001
Received 21 October 2023; Received in revised form 31 October 2023; Accepted 3 November 2023
Available online 18 November 2023

scutellatum, which may have been a species of *Scutellinia*, and described the way that its "very small seeds" were "thrown into the air like sparks" and "dispersed by the slightest breeze" (Fig. 1C).

The puffing phenomenon observed by Micheli struck later investigators, including Albrecht von Haller who heard "a sort of hissing noise" produced by the simultaneous discharge of thousands of asci from apothecia in the 1740s (Ramsbottom 1941). Jean Baptiste François Pierre Bulliard (1791) described how blowing on apothecia caused the release of jets of dust, writing that "the seeds rise as a vapor," and Christiaan Hendrik Persoon (1801) saw "seminal powder [fly] forth like smoke" from the perithecial stroma of *Rhytisma salicinum*.

Johannes Hedwig (1789) published the first clear views of asci in multiple ascomycetes (Fig. 1D–G). Even with his access to more powerful microscope objectives, Hedwig's illustrations are remarkable for their accuracy in the century before optical innovations corrected the blurring and discoloration caused by spherical and chromatic aberration (Ainsworth 1976; Ratliff 2009). At a time of considerable confusion about the relationship between fungi and plants, Hedwig thought that asci were floral organs which were fertilized by their surrounding paraphyses. He described the asci as membranous *thecae* (capsules) that contained eight seeds, using the Latin terms *seminales* or *seminibus*, which were synonymous with semen. The noun ascus was not used until 1816, when Gottfried Daniel Nees von Esenbeck (1816–1817) replaced thecae with asci, from the Greek *askos* for a bag or sac, and German *Schläuche*, meaning tube (Lepp 2021).

Surveying advances in mycological research in the nineteenth century, Anton de Bary (1887) provided definitive descriptions of ascospore discharge from individual asci and their relationship to puffing. His simple observation that asci deflated when they were immersed in solutions of sodium saccharin or glycerol showed that the turgidity of the ascus that powered the discharge of spores was generated by osmosis. De Bary described many other features of ascus function including the successive elongation of asci within perithecia and protrusion through the ostiole before discharge, the release of needle-shaped ascospores from *Cordyceps*, and operation of fissitunicate asci of *Pleospora*. The Crouan brothers were the first to illustrate the operculate asci of *Ascobolus* species, showing how the mechanism of ascus dehiscence worked in these apothecial fungi (Crouan et al., 1857). The observations and spectacular illustrations by the more famous French mycologist brothers, Charles and Louis-René Tulasne, in their *Selecta Fungorum Carpologia* (Tulasne and Tulasne, 1861–1865) were another critical source of information for De Bary and his contemporaries (Fig. 2).

3. Experiments by Buller and Ingold

A. H. R. Buller (1874–1944) is better known for his elucidation of ballistospore discharge in the first half of the twentieth century, but his work on "the physics of the ascus jet" was also significant (Buller 1909). He argued that the discharge of operculate asci of *Peziza varia* followed the loosening of the attachment between the lid and the subtending ascus wall, rather than an increase in ascus turgor pressure. Viewing the ascospores shot into a beam of light directed above the apothecia, he noted that the eight spores from each ascus separated within a fraction of a second after discharge. Without being able to see the launch of the ascospores, Buller reasoned that the spores were ejected with the surrounding ascus sap as single cylindrical projectile and were separated into droplets by the action of surface tension. This mechanism is called fluid thread breakup, which is an important field of research in fluid mechanics (Château et al., 2018; Lauga 2022). Ascospores of other species with operculate asci, including *Ascobolus immersus*, cling to one another within a common mucilaginous matrix after discharge. This matrix is elastic and resists separation so that most of the ascospores tend to fly in groups of two or more spores. Buller measured the ranges of operculate asci, showing that the heavier projectiles of multiple

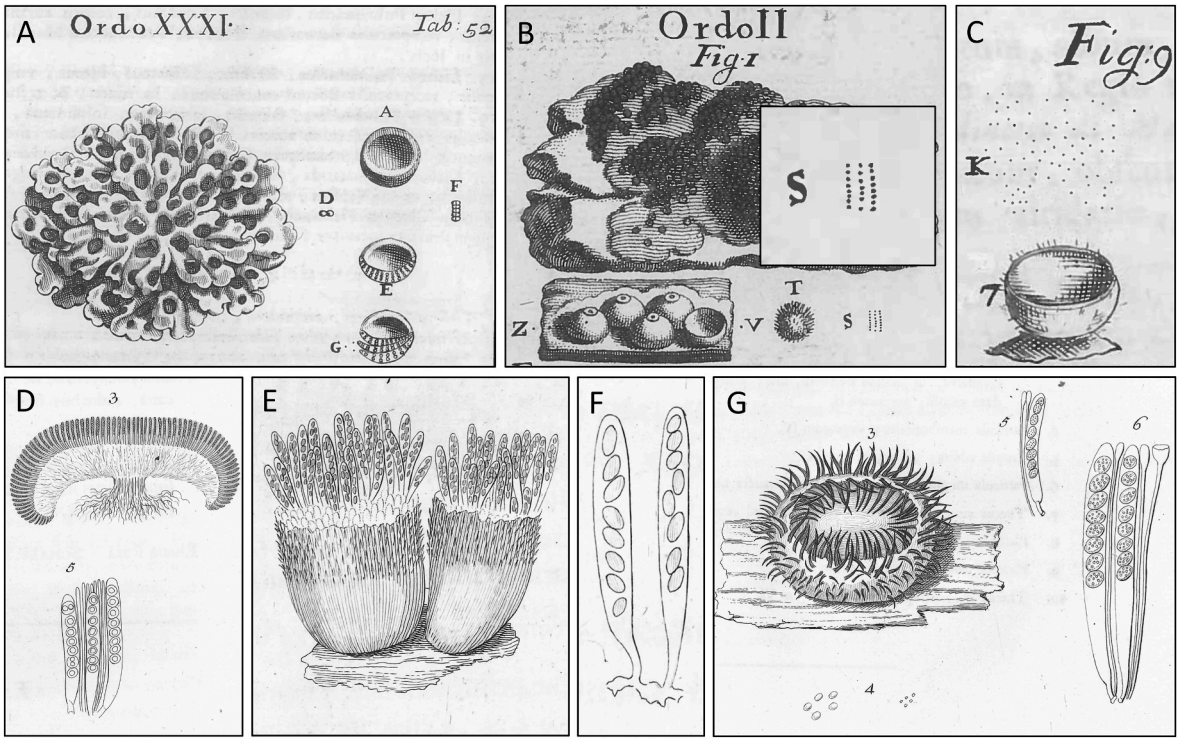

Fig. 1. Eighteenth century illustrations of asci and ascospores from (A–C) Micheli (1729) and (D–G) Hedwig (1789). (A) Foliose lichen with apothecia that contain groups of spores. Micheli cut fine sections of apothecia to expose the asci ("the flowers") lining the cup. A row of four of the spores is also shown in the detail from Tab (Plate): 52. (B) Species of Xylariaceae whose perithecia contain single files of spores. Inset shows enlarged view of groups of 8, 7, and 8 spores. (C) Apothecium puffing ascospores. (D, E, G) Hedwig's figures of apothecia containing asci. (F) Enlarged illustration of asci containing eight ascospores from apothecium shown in (E). Hedwig's apothecium in (G) may be a species related to the hirsute fruit body shown by Micheli in (C). From the Collection of The Lloyd Library and Museum.

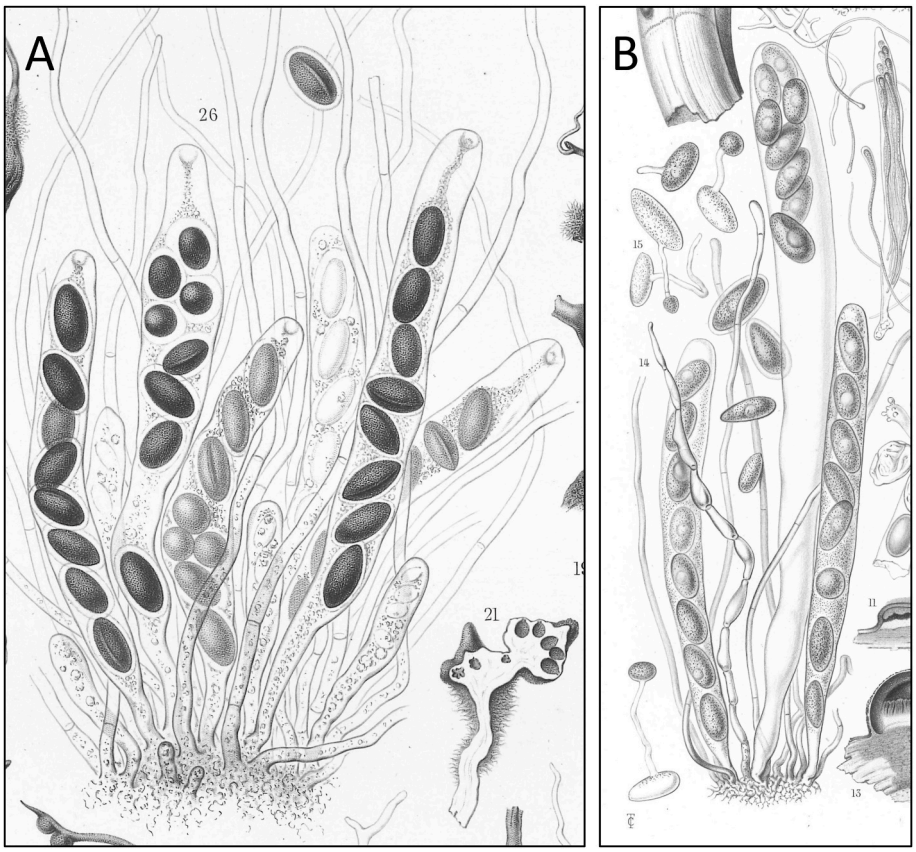

Fig. 2. Nineteenth century illustrations of asci of (A) *Podosordaria pedunculata* and (B) *Morchella semilibera* by Charles Tulasne (Tulasne and Tulasne 1863, 1865). From the Collection of The Lloyd Library and Museum.

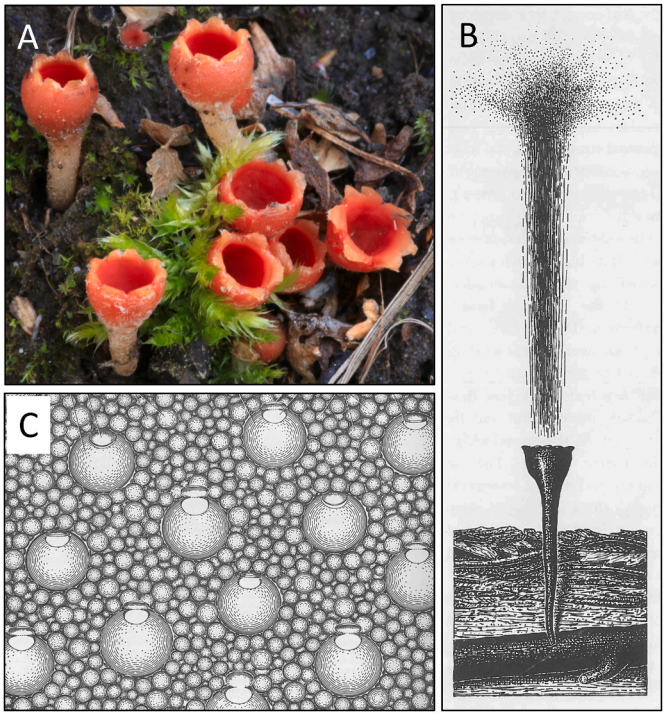

Fig. 3. *Microstoma protractum.* (A) Group of stalked apothecia. Source: Shutterstock. (B) Simultaneous ascus discharge or puffing illustrated by Buller (1934). (C) Surface of the hymenium immediately after puffing, showing empty asci with open opercula and surrounding paraphyses (Buller 1934).

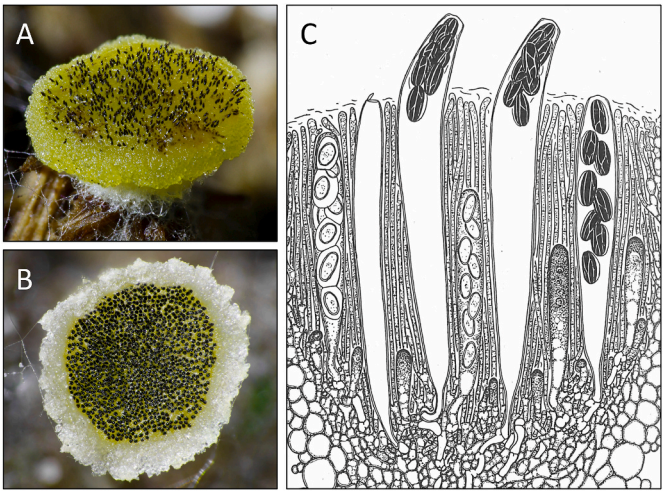

Fig. 4. Heliotropic asci of *Ascobolus stercorarius.* (A, B) Macroscopic views of the apothecia of this coprophilous ascomycete showing fields of asci containing black ascospores. (C) Asci at various stages of development including a pair of mature asci whose tips have bent toward the incident light and a shrunken ascus that has discharged its ascospores through its open hinged operculum. (A, B) Photo credit Chris Johnson (https://www.outerhebridesfungi.co.uk/identification.php). (C) Adapted from Corner (1929) with permission.

spores were shot much farther than single ascospores. He found that single spores of *Peziza* were shot only 1.0 cm from their apothecia compared with 2.5 cm for multiple attached spores. Masses of eight spores of *Ascobolus* were propelled up to 35 cm from the fruit body, which is the longest discharge distance measured for any ascus. Buller

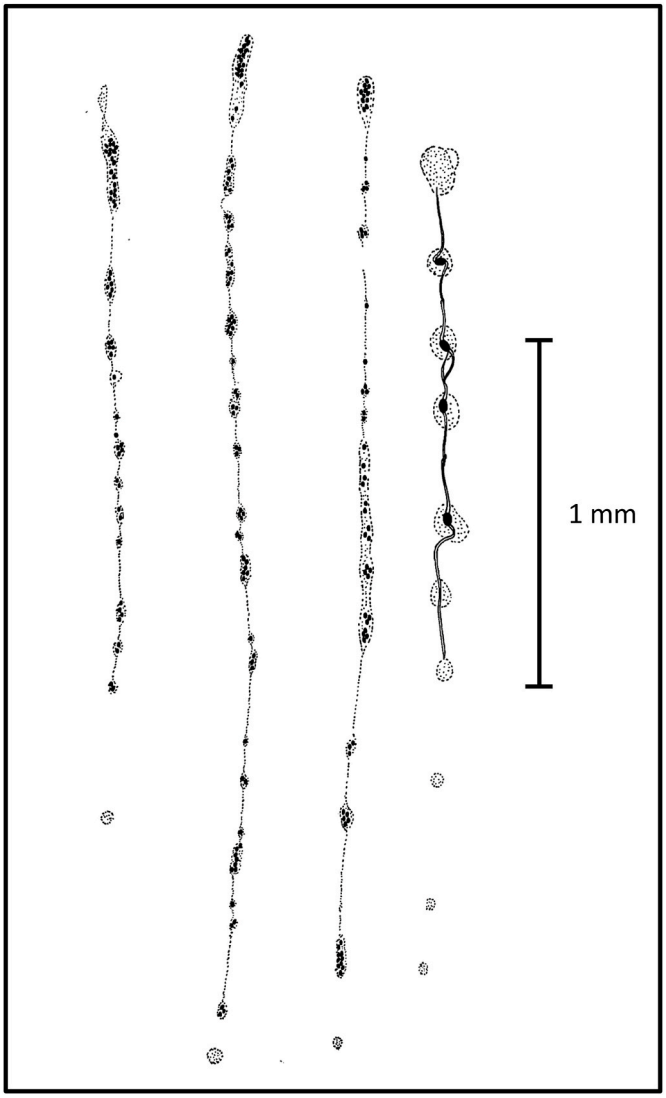

Fig. 5. Strings of ascospores captured on a plastic disc rotating above perithecia at a speed of 20 m s^{-1}. The three deposits on the left were produced by *Triangularia setosa* whose groups of ascospores are connected by thin threads of ascus sap. The single string of four ascospores on the right was discharged by a species of *Podospora*. The spores of this species are equipped with mucilaginous appendages that connect the spores to form a single projectile. Spread along a length of 1 mm, the spores of *Podospora* must have been expelled from the ascus in 60 μs (1×10^{-3} m ÷ 20 m $s^{-1} = 5 \times 10^{-5}$ s). The spores occupy a column in the ascus that is 250 μm long (Griffiths 1901), which means that the projectile must have been ejected at a minimum launch velocity of 5 m s^{-1} (2.5×10^{-4} m ÷ 5×10^{-5} s). From Ingold (1966).

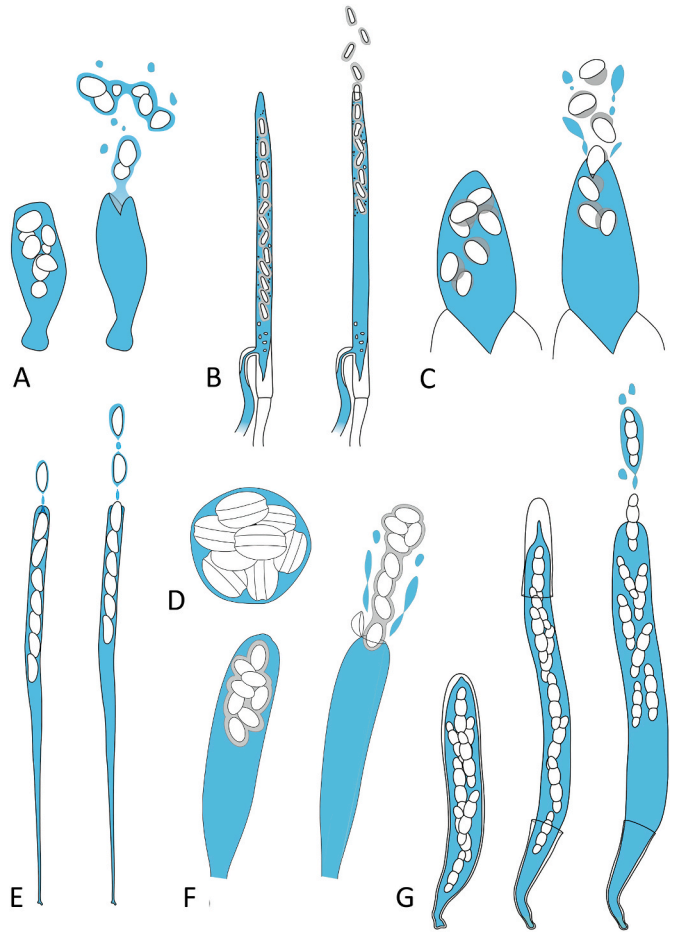

Fig. 6. Diversity of ascus morphology and ascospore discharge mechanisms. (A) *Taphrina deformans* (sub-division *Taphrinomycotina*). Asci of this plant pathogen are exposed on the surface of peach leaves. They split open at the tip and discharge multiple spores in a single shot. (B) *Dipodascus macrosporus* (*Saccharomycotina*) produces ascospores with a mucilage coating. The spores are extruded slowly through the torn apex of the ascus. This fungus forms exposed asci on surfaces. (C) Single asci of the powdery mildew *Podosphaera pannosa* (*Pezizomycotina*, *Erysiphales*) form in chasmothecia (one ascus per ascoma) and open via a slit at the apex. (D) Multiple non-explosive asci of *Emericella nidulans* (*Pezizomycotina*, *Eurotiales*) form inside cleistothecia. The ascospores of this fungus are ornamented with a double flange. (E) Asci of *Xylaria hypoxylon* (*Pezizomycotina*, *Xylariales*) discharge spores through constricting ring (apical apparatus). Ascomata of this fungus are perithecia. (F) Operculate asci of *Ascobolus immersus* (*Pezizomycotina*, *Pezizales*) expel spores when lid or operculum flips open. Ascomata of this species are apothecia. (G) Ascospore discharge from fissitunicate asci of *Pleospora herbarum* (*Pezizomycotina*, *Pleosporales*) occurs after outer wall of ascus ruptures to allow expansion of the inner wall. The fruit body of this species is a pseudothecium. From Watkinson et al. (2016).

estimated that a launch speed of 10 m s^{-1} was necessary to shoot the spores of *Ascobolus* over this distance.

In the sixth volume of his *Researches on Fungi*, Buller (1934) examined puffing in the bright red cup fungus *Microstoma protractum* (Fig. 3). He showed that the vertical discharge of the asci from the funnel-shaped apothecium of this species is assured by the accurate positioning of the operculum of each ascus toward the opening of the cup. This permits asci located on the sloping interior of the apothecium to shoot their spores directly upwards rather than in the direction of their central axes toward the opposite side of the cup. Each operculum flips open along a hinge formed by a small arc of its circumference that resists the fracture that initiates discharge. Other apothecial ascomycetes shift their aim through the curvature of the ascus apex rather than the positioning of the operculum (Fig. 4). In either case, sunlight provides the directional cue. Everything about the mechanism of ascospore discharge speaks to an astonishing level of biomechanical finesse.

Buller's experimental and theoretical studies on fungi marked a significant change from the chiefly observational work of previous investigators. As his intellectual heir, C. T. Ingold (1905–2010) spent a good deal of his career communicating and extending the results of Buller's research. Ingold's experiments on asci included the inventive use of a spinning disc as a target, or rather a trap, for discharged ascospores (Ingold and Hadland 1959). The plastic disc was rotated on the spindle of a motor like a record turntable, but at higher speed, above cultures of *Sordaria fimicola*. Asci extending from the perithecia shot

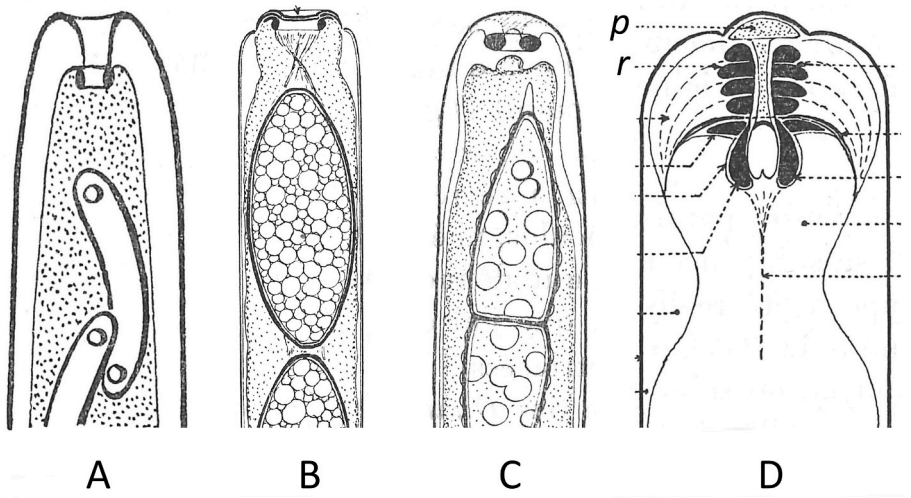

Fig. 7. Drawings of ascus tips by Marius Chaudefaud based on light microscopy. (A) *Eutypa lata*, family *Diatrypaceae*. (B) *Neurospora tetrasperma*, fam. *Sordariaceae*. In Chadefaud and Nicot (1957), Marius wrote that he stained the ascus tip of this species with Waterman black fountain pen ink, which speaks to the resourcefulness of this patriotic French mycologist (Waterman being a Parisian company). (C) *Hypomyces aurantius*, fam. *Hypocreaceae*. (D) Composite of ascus tip structures of the annellascus type (annellascé) with multiple rings (*r*) associated with the plug (*p*) produced by species belonging to several orders of ascomycetes. Sources: Chadefaud, 1957, 1958, 1964), Chadefaud and Nicot (1957). From the Collection of The Lloyd Library and Museum.

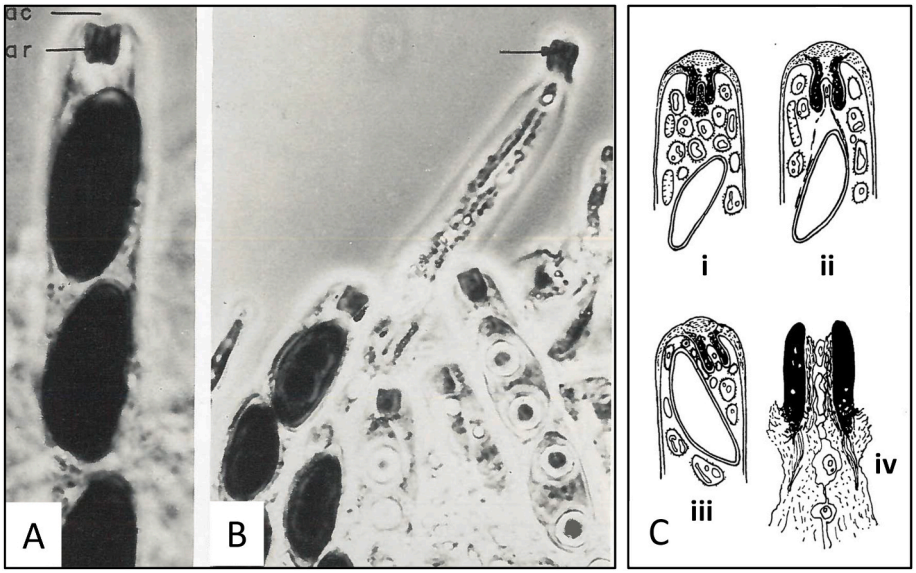

Fig. 8. Structure of the ascus apex in *Xylaria longipes* from Beckett and Crawford (1973). (A, B) Light micrographs showing ascus before and after ascospore discharge. Labels in (A) indicate apical cushion (ac) and apical ring (ar). (C) Drawings of ascus tip maturation (i-ii) and ascus tip following spore discharge (iv) with everted apical ring, traced from transmission electron micrographs shown in the original publication. Drawing (iii) shows that the apical ring is displaced by the uppermost ascospore shortly before it is everted and the spores are released.

their spores onto the bottom of the disc creating deposits of single spores and groups of two to eight spores depending upon their separation after discharge. The length of the deposits from individual asci (4×10^{-4} m) divided by the speed of disc rotation above fungal culture (20 m per second) provided an estimate of 2×10^{-5} s for the time taken for the exit of the eight spores from the ascus. To get to the launch speed, the length of the eight ascospores packed inside the ascus (2×10^{-4} m) is divided by the exit interval determined from the spread of spores on the disc (2×10^{-5} s) = 10 m s^{-1}. These estimates are based on rounding the measurements given by Ingold and Hadland (1959) to single significant figures, but these authors made a calculation error that reduced their estimated launch speed to 4 m s^{-1}. The revised velocity of 10 m s^{-1} matches Buller's 1909 estimate (see above), as well as direct measurements from high-speed video recordings (Sections 6, 7).

Walkey and Harvey (1966) augmented Ingold's studies by measuring the discharge distances of more than a dozen ascomycetes whose spores ranged in size from 1.7×10^{-17} m^3 (*Thelebolus nanus*) to 2.1×10^{-14} m^3 (*Podospora fimiseda*) with a corresponding three order-of-magnitude range of mass from 20 pg to 25 ng (assuming a density of 1.2×10^3 kg m^{-3}). The ascospores of some of the species in this study are equipped with mucilaginous appendages that act as tethers that keep groups of the spores together after they are launched. Discharge distances measured in this study varied from a few millimeters to half a meter and the larger projectiles tended to cover the longest discharge distances. The number

of spores per ascus was another variable: with four ascospores per ascus, most of the spores of *Podospora tetrasperma* were shot as quartets and travelled 7 cm, whereas an average of 256 tiny ascospores were shot as a single projectile from the asci of *T. nanus* and covered a distance of 3 cm. Ingold (1966) studied ascomycetes whose spores are discharged as a single projectile using his spinning disc method. Spores tethered via gelatinous appendages were deposited on the rotating target as strings resembling beaded necklaces (Fig. 5). These extended deposits are unlikely to form in nature when ascospores are shot onto stationary surfaces.

4. Ascus structure and evolution

Ascus structure shows considerable variation between and within groups of ascomycetes and serves as a useful characteristic for differentiating taxa (Fig. 6). At the level of classes, we find the simplest ascus structures in some of the yeasts classified in the *Saccharomycetes* that release spores by deliquescence. Asci formed by species of *Taphrinomycetes* and *Neolectomycetes* split at their apices and spurt masses of embedded in ascus sap. These groups of fungi are thought to have diverged before the evolution of the dozen or so extant classes within the subdivision *Pezizomycotina* in which we find asci with greater structural complexity (Schoch et al., 2009; Prieto and Wedin 2013; Li et al., 2021). Many of the asci in the basal groups of ascomycetes are rounded, whereas asci with elongated shapes fitted with apical lids, pores, and elastic rings that control the explosive discharge of spores are produced by the *Pezizomycotina*.

Marius Chadefaud (1900–1984) examined the apical structures of asci in the *Pezizomycotina* in unprecedented detail using light microscopy and electron microscopy (Parguey-Leduc et al., 1994). He described species whose asci lacked any obvious preformed openings at their tips and others with lids (opercula), cell wall thickenings, single or double rings, and apical plugs (Fig. 7). Beckett and Crawford (1973) published superb images of ascus development in *Xylaria longipes* and showed that the ring or annulus at the tip of the ascus everts during ascus discharge and works as a short tube through which the spores squeeze before they escape into the air (Fig. 8). The separation of spores during their transit through the everted apical apparatus accounts for the deposition of single spores of this species rather than groups which was reported by Walkey and Harvey (1966).

It is unclear how many species of perithecial ascomycetes utilize this mechanism of annular eversion, but the assumption that the ring at the tip of the ascus works as a fixed aperture is questionable unless the fungi in question have been studied with electron microscopy or high-speed video. This limits the usefulness of the analysis of the annulus as an elastic O-ring by Fritz et al. (2013), who assumed that the size and shape of the annulus before spore discharge in multiple species matched the dimensions of the sphincter through with the spores are expelled. Eversion and elongation of the cell wall material in the annulus of some of these fungi reduces the minimum diameter of the opening and makes for a longer and tighter squeeze. With or without eversion, the constriction at the ascus tip tends to be smaller than the ascospores, or creates a tight fit, and functions as a valve that slows the loss of ascus sap and turgor pressure until all of the spores are discharged (Greenhalgh and Evans 1967). This was discussed by Ingold (1954), who pointed out that operculate asci tend to release all of their spores as a single projectile, whereas inoperculate asci with some form of apical apparatus release spores one-at-a-time.

In her review article on ascospore discharge, Trail (2007) recognized three types of asci that forcibly discharge spores: single-walled asci (unitunicate) with and without lids (operculate and inoperculate), and double-walled asci (bitunicate or fissitunicate). Asci with apical rings are examples of unitunicate inoperculate asci. This structural classification is useful, but it elides the amazing diversity of discharge mechanisms in the ascomycetes: mycologists have documented as many as twenty mechanisms of ascospore release (Kirk et al., 2008). Here are

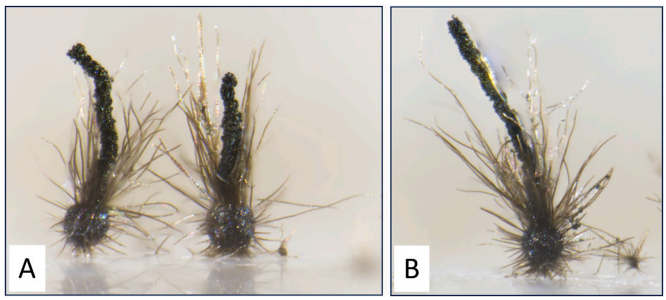

Fig. 9. Nonexplosive ascospore discharge. (A, B) Two views of perithecia of *Amesia atrobrunnea* extruding tall cirri of ascospores. From Wang et al. (2016) courtesy of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands.

three examples of subcategories of ascus function: (i) fissurate dehiscence of an ascus whose apex tears open along irregular lines leaving a ragged opening (Minter and Cannon 1984); (ii) rostrate dehiscence in which the inner wall of a bitunicate ascus extends to form a beak-like structure through which the spores are extruded (Honegger, 1978); (iii) suboperculate dehiscence involving the ejection of a stopper of cell wall material at the ascus tip (Van Brummelen 1975; Samuelson 1975).

These modifications to ascus structure and dehiscence have profound effects on the mechanics of spore discharge. Changes in ascus size, shape, and nozzle geometry affect the launch speed of the spores, whether they travel in groups, the timing of the successive discharge of individual spores, and so on. These variables affect the discharge distance of the spores, providing an explicit link between biomechanics and ecology that has molded ascus evolution. Broadly speaking, then, the ascus is a squirt gun whose cellular morphology has been crafted to propel spores of different sizes over distances of millimeters to centimeters to promote dispersal by wind or animal vectors. Changes in ascus turgor pressure are probably secondary in importance to these structural changes.

For asci that develop in ascomata, these structural modifications must be coupled with the evolution of fruit body morphology. Enlargement of a perithecium, for example, must be matched by coincident changes the development of the cell walls of the asci so that they can extend through the neck of the ascoma and discharge their spores into the air. These reciprocal adaptations are comparable to the iterative changes to the mechanism of ballistospore discharge and gill separation in mushrooms (Money 2023a). Rather than operating as the kind of evolutionary seesaw envisaged for mushrooms, however, there is no impetus for a give and take in ascus evolution. Asci either elongate through the ostiole or fail to do so. There is no penalty of ascus over-extension comparable to the cessation of dispersal when ballistospores are shot too far and hit the opposing gill. On the other hand, the failure of asci to elongate through the ostiole means that spores will be trapped in the perithecial chamber. If enough spores accumulate in this manner, the resulting pressure will force them through the ostiole. This results in the formation of a sticky cirrus from which ascospores are distributed when they become attached to the exoskeleton of insects or are consumed without digestion (Fig. 9). It should be noted that the dispersal of ascomycetes that produce cirri by insects is presumed more often than it has been documented. The loss of explosive ascospore discharge is comparable to the loss of ballistospory and the adoption of insect dispersal of basidiospores (Money 2023a).

The complete loss of ascospore discharge in favor of spore retention within the ascus and sequestration within buried ascomata, or truffles, has occurred independently in multiple groups of ascomycetes (Bonito et al., 2013). This appears to be a rapid evolutionary transition involving the mutation of small sets of genes and is often associated with the formation of mycorrhizal symbioses. Truffles deploy volatile aromatic compounds to attract animals that consume their fruit bodies and serve as vectors for the dispersal of truffle spores (Splivallo et al., 2011). The

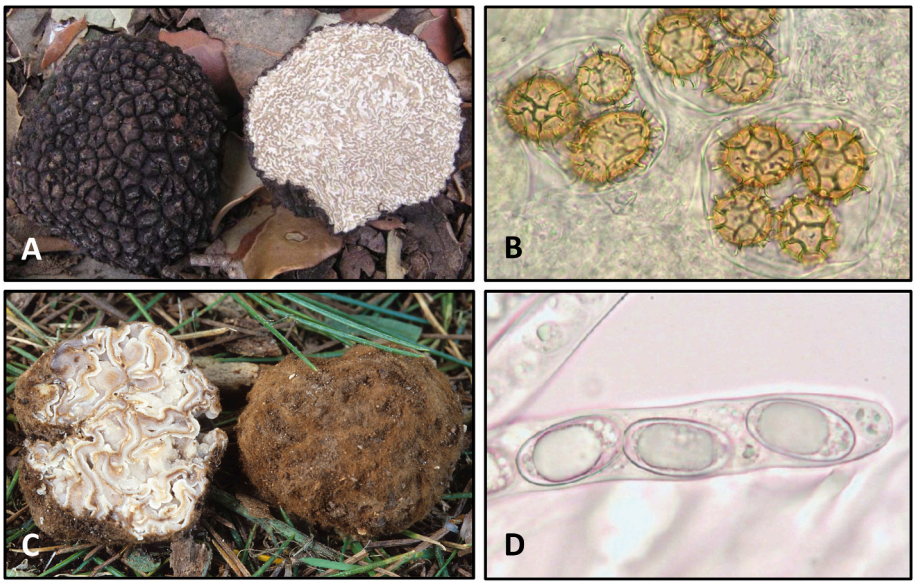

Fig. 10. Truffle asci. (A, B) The globular shape of the asci of the summer truffle, *Tuber aestivum*, is associated with the absence of the active mechanism of ascospore discharge. (C, D) Active ascospore discharge is preserved in the pine truffle, *Geopora cooperi*, whose asci are elongated and open via an operculum. (A, B) Antonio Rodriguez (https://www.trufamania.com/). (C) Michael Wood (https://www.mykoweb.com/). (D) Aziz Türkoğlu (Türkoğlu et al., 2015) with permission.

Table 1
Ascospore ballistics.

Species	Spore dimensions (μm): length x width (n)[a]	Number of spores released per ascus	Initial velocity (m s^{-1}): range, mean ± s.e.m. (n)	Calculated discharge distance (cm): range, mean
Ascobolus immersus	50 × 25 (12)	8	5–18, 14 ± 1.3 (12)	9–33, 26
Podospora anserina	34 × 20 (17)	4	10–25, 21 ± 1.1 (17)	7–17, 14
Morchella semilibera	35 × 20 (7)	8	16–25, 19 ± 1.9 (4)	17–27, 21 (5 cm for spores flying singly)
Neurospora tetrasperma	31 × 17 (5)	4	4–32, 16 ± 0.6 (58)	2–16, 8
Sordaria macrospora	27 × 18 (17)	8	5–30, 15 ± 0.1 (43)	4–24, 12
Sporormiella australis	34 × 6 (25)	one at a time	15–16, 16 ± 0.1 (5)	0.80–0.85, 0.85
Leptosphaeria acuta	54 × 7 (20)	one at a time	4–5, 4 ± 0.5 (3)	0.24–0.31, 0.24
Neolecta vitellina	7 × 3[b]	stream of multiple spores	13–19, 16 ± 1.6 (3)	0.12–0.18, 0.15
Macrospora scirpicola[c]	60 × 25 (9)	one at a time	3–7, 5 ± 0.5 (10)	0.02–0.05, 0.04
Leptosphaeria acuta[c]	54 × 7 (20)	one at a time	1–2, 1 ± 0.3 (4)	0.001–0.004, 0.003

[a] Spore dimensions measured from video recordings including surrounding ascus sap.
[b] Size of spores impossible to measure from video, so taken from Healy et al. (2013).
[c] Ascospores discharged into water. *L. acuta* is a terrestrial fungus that grows on the stems of nettles and normally discharges its spores into air.
Sources: Data from Yafetto et al. (2008) and Watkinson et al. (2016), and selected unpublished measurements from Money lab using the methods detailed by Yafetto et al. (2008).

Pezizomycetes includes many species with wrinkled and partly enclosed or secotioid fruit bodies whose structure is intermediate between open, cup-shaped apothecia and the fully sequestrate truffle morphology. *Geopora cooperi*, the pine truffle, is an example of a species with highly convoluted ascomata that develop below the soil surface. Surprisingly, however, its operculate asci will puff if the fruit body is cut open and exposed to the air (Burdsall 1965). This preservation of active ascospore discharge is emblematic of a transitional stage in the evolution of truffles. After the asci of truffles lose their elongated shape and explosive mechanism, there is no evidence for the resurrection of active ascospore discharge in any lineage of the Ascomycota (Fig. 10). Like the deactivation of ballistospory in basidiomycetes, the loss of ascospore discharge is irreversible (Money 2023a).

5. Ascus turgor pressure

The ascus is pressurized by the net influx of water across the plasma membrane lining the inner surface of the ascus cell wall. Following the early experiments by De Bary on ascus turgor pressure (Section 2), Ingold (1939) suggested that sugars derived from the breakdown of glycogen reserves in the ascus sap were responsible for increasing the osmotic pressure (or depressing the osmotic potential) of the sap. He inferred this mechanism from the chestnut-brown staining of young asci with iodine, which indicates glycogen, and the loss of this staining pattern in mature asci. Glycogen inclusions called rosettes have been identified in young asci of several lichenized and non-lichenized ascomycetes, which suggests that these are an important source of the osmolytes that generate ascus turgor (Walker and Andersen 1925; Honegger 1983). Following a highly conserved biochemical pathway, a debranching enzyme and glycogen phosphorylase act on stored glycogen to release glucose-1-phosphate and subsequent reactions convert the glucose to glycerol (Wilson et al., 2010). Many fungi accumulate cytoplasmic glycerol to support turgor pressure, including the iconic appressoria of the rice blast fungus that force their way through

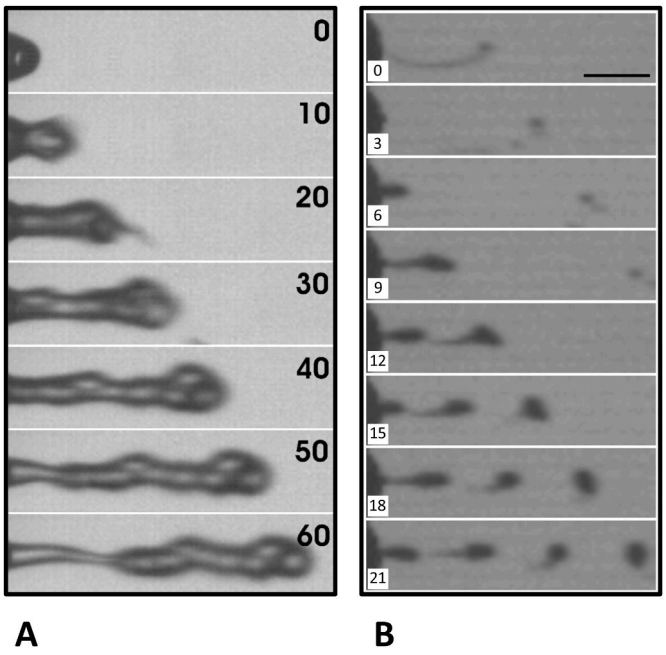

Fig. 11. Series of frames from high-speed video clips of ascospore discharge in (A) *Ascobolus immersus* and (B) *Neurospora tetrasperma*. Camera speeds (A) 100,000 frames per second (fps) (B) 1 million fps. Time intervals on images refer to microseconds. Scale bars (A) 50 μm (B) 20 μm.

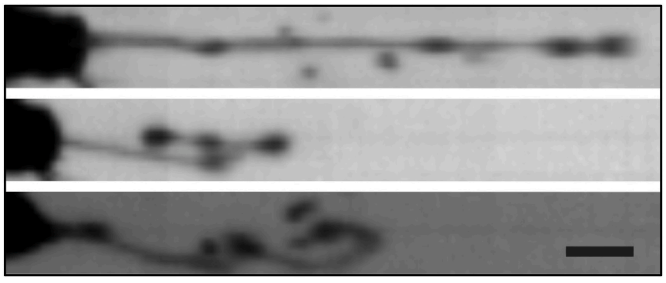

Fig. 12. Single frames from high-speed video recordings of ascospore discharge in *Podospora anserina* showing four spores released from each ascus connected via mucilaginous appendages. Camera speed 250,000 frames per second. Scale bar = 50 μm. See Fig. 5 for drawing of an ascospore deposit from a related species of *Podospora*.

the host leaf surface (Bourett and Howard 1990; Foster et al., 2017; Davis et al., 2000a). Glycerol, the 3C polyol, generates a similar osmotic pressure to 4C, 5C, and 6C polyols at concentrations below 1 M (Davis et al., 2000b). This property makes glycerol a carbon thrifty osmolyte for turgor generation, but the high permeability of the cell membrane to glycerol favors the use of larger uncharged polar molecules in some cell types.

With these physiological considerations in mind, it is interesting that glycerol has been detected in ascus sap of *A. immersus* (Fischer et al., 2004), but appears to be replaced by mannitol in *Gibberella zeae* (Trail et al., 2002, 2005). Proline was another osmolyte found in *A. immersus* and estimates of sap volume suggest that the combination of glycerol and this amino acid generate an osmotic pressure of 0.10 MPa (Fischer et al., 2004). The turgor pressure of the large operculate asci of this fungus, measured with a microprobe attached to a miniature strain gauge, was 0.31 MPa. This suggests that two-thirds of the ascus osmotic pressure was generated by inorganic ions. Trail et al. (2005) found that mannitol accounted for only 0.03 MPa of ascus osmotic pressure (and turgor) and that potassium chloride dominated the osmotic pressure of the inoperculate asci of *G. zeae*. Estimates of ascus turgor pressure ranging from 1.0 to 3.0 MPa by Ingold (1939, 1966) were made using the incipient plasmolysis technique and freezing point depression osmometry. These methods are unreliable and overestimated turgor by an order of magnitude.

6. High-speed video recordings of ascospore discharge

The availability of high-speed digital video cameras in the early 2000s, offered researchers the first view of the explosive release of ascospores (Yafetto et al., 2008, Table 1). Using camera speeds of up to 250,000 frames per second, Yafetto et al. (2008) measured launch speeds of 5–18 m s^{-1} in *A. immersus* and 10–25 m s^{-1} in *Podospora anserina* with corresponding accelerations approaching 2 million m s^{-2} (Figs. 11A and 12; Supplementary Video 1). Video recordings showed that the spores of these fungi often remain connected after release, either in their shared coating of ascus sap (*Ascobolus*) or via linkages between gelatinous appendages (*Podospora*). In some cases, the individual spores separated during flight producing deposits with varying numbers of spores immersed in blobs of ascus sap. Other examples of fast ascospore discharge include *Morchella semilibera* (16–25 m s^{-1}), *Sordaria macrosperma* (5–30 m s^{-1}; Supplementary Video 2), and, speediest of all, *Neurospora tetrasperma*, whose spores are launched at a top speed of 32 m s^{-1} (115 km h^{-1}; Fig. 11B; Supplementary Video 3). These experiments have established that ascospore discharge is the fastest fungal movement and is one of the fastest movements in biology. Indeed, the nematocysts of hydrozoans, which are often cited as the fastest natural explosive devices, cannot match the muzzle velocities of asci. Barbs are fired from nematocysts at a speed of 19 m s^{-1}, but stop short within a microsecond (Nüchter et al., 2006). Their distinction comes from the remarkable acceleration of the dart over this short timeframe, which reaches 5 million *g*.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.funbio.2023.11.001

Supplementary Video 1; High-speed video clip of ascospore discharge in *Ascobolus immersus* recorded at 100,000 frames per second. The eight ascospores remain together during the first microseconds of their trajectory.

Supplementary Video 2; High-speed video clip of ascospore discharge in *Sordaria macrospora* recorded at 1 million frames per second. Note that a plug of material from the ascus apex is discharged before the spores are released.

Supplementary Video 3; High-speed video clip of ascospore discharge in *Neurospora tetrasperma* recorded at 1 million frames per second. This species has the fastest ascospore discharge mechanism that has been recorded of up to 32 m s^{-1} or 115 km h^{-1}.

The launch of *N. tetrasperma* was clocked using a camera speed of one million frames per second. An earlier estimate of launch speed for this fungus of only 1.2 m s^{-1} by Roper et al. (2008) seems to have been measured without imaging the neck of the perithecium in a horizontal orientation to capture ascospore motion in a single plane. This interpretation is supported by the discharge distances of less than 2 mm reported by Roper and colleagues, compared with the much longer flights based on the unambiguous velocity measurements in our studies (Section 7). This reflects the difficulty in obtaining high-quality video footage of spore discharge mechanisms and their rarity in the mycological literature (Money 2023b). Another interesting feature of ascospore discharge in *N. tetrasperma* is the end-over-end rotation of the spores during flight, which exceeds 40,000 revolutions per second (or 2 million revolutions per minute). Although the difference in size renders the following physical comparison frivolous, we note that the discharged spores spin faster than neutron stars.

Although the fastest launch speeds seem to be produced by asci with apical rings (*Sordaria* and *Neurospora*), spore release from the large operculate asci of *A. immersus* is almost as fast and this species propels its spores over longer distances (Table 1). There is no inherent reason that the opening of a ring versus a lid at the tip of a pressurized ascus will

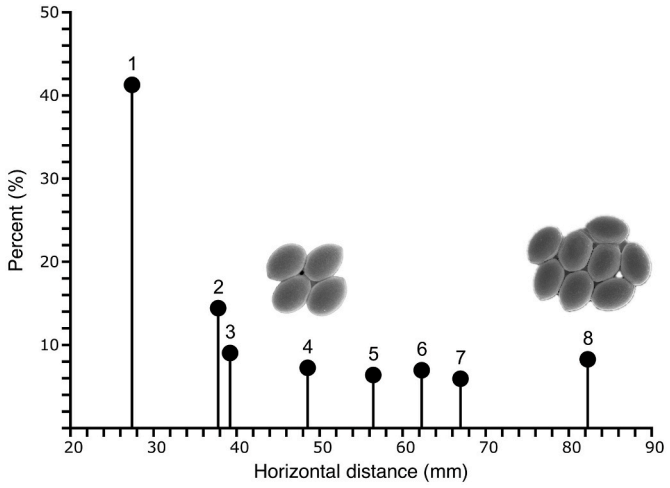

Fig. 13. Effect of projectile size (number of ascospores) on discharge distance in *Sordaria macrospora*. Culture dishes were positioned vertically to align the perithecial necks horizontally and spores were deposited on glass slides arranged at different distances from the cultures over a period of 12 h. Horizontal axis shows the average position of the different projectile sizes, ranging from single spores to groups of 8 spores; vertical axis shows percentage of total deposits for each projectile size, e.g., single spores represented 41 % of all the deposits. The positions of 3818 spore deposits were scored in this unpublished experiment from the Money lab.

produce major differences in the initial velocity. Both types of asci operate on the biomechanical principle of power amplification, in which a latch opens very swiftly to release the energy stored in the pressurized ascus sap. This is an example of a latch-mediated spring actuation (LaMSA) mechanism (Longo et al., 2019).

Ascospore launches are also very fast in fungi with fissitunicate asci, with a mean exit velocity of 16 m s^{-1} in *Sporormiella australis*. This speed is matched by *Neolecta vitellina* that produces a simple ascus without any specialized tip structure that ruptures as an irregular slit and sprays multiple tiny spores into the air. The absence of a reinforced aperture that controls the loss of ascus pressure limits the discharge distance of the spores of this fungus to about 1 mm. The launch speeds of aquatic ascomycetes whose fissitunicate asci discharge into water is much lower, varying from 1 to 7 m s^{-1} (Table 1).

7. Measured and predicted discharge distances

For high-speed imaging of spore discharge, a 10X or 20X objective lens is needed to capture the individual spores. At these magnifications, the footage of spore discharge shows less than 1 % of the complete transit of spores over a distance of a few centimeters. It would be impossible to capture the flight of the spores of *Ascobolus*, for example, launched at a speed of 10 m s^{-1}, as they travel over a distance of 20 cm in approximately 20 ms. In principle, this movement could be captured in 5000 frames at a camera speed of 250,000 frames per second, but the technical challenges of maintaining focus on the microscopic spores during their macroscopic flight are insurmountable. For this reason,

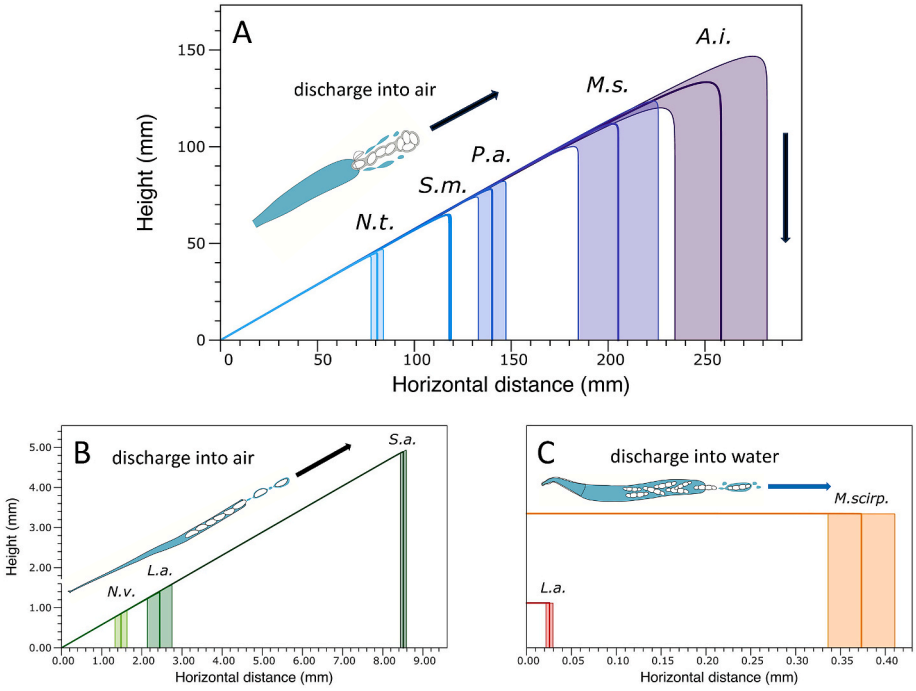

Fig. 14. Examples of trajectories of discharged ascospores over three distance intervals based on velocity measurements from high-speed video recordings. (A) Long-range discharge from 5 to 30 cm for spores shot into air: *N.t.*, *Neurospora tetrasperma* (Class *Sordariomycetes*, Order *Sordariales*), *S.m.*, *Sordaria macrospora* (*Sordariomycetes*, *Sordariales*), *P. a.*, *Podospora anserina* (*Sordariomycetes*, *Sordariales*), *M.s.*, *Morchella semilibera* (*Pezizomycetes*, *Pezizales*), *A. i.*, *Ascobolus immersus* (*Pezizomycetes*, *Pezizales*). Distances are much shorter for *Morchella* when the spores fly separately rather than in groups (Table 1). (B) Medium-range discharge from 1 to 9 mm for spores shot into air: *L. a.*, *Leptosphaeria acuta* (*Dothideomycetes*, *Pleosporales*) over a longer distance than spores of same species shot into water in (C), *N. v.*, *Neolecta vitellina* (*Neolectomycetes*, *Neolectales*), *S. a.*, *Sporormiella australis* (*Dothideomycetes*, *Pleosporales*). (C) Short-range discharge of spores into water over distances less than 0.5 mm: *L. a.*, *Leptosphaeria acuta*, and *M. scirp.*, *Macrospora scirpicola* (*Dothideomycetes*, *Pleosporales*). Methods: Ascospore trajectories calculated from the influences of viscous drag and gravity. Each projectile was treated as a sphere whose volume equaled the volume of the launched spore(s) plus an equivalent volume of ascus sap if present. The mass of the projectile was calculated assuming the density of spores and sap to be 1200 kg m^{-3} and 1000 kg m^{-3}, respectively. The viscosity of air was assumed to be 1.8×10^{-5} kg m^{-1} s^{-1}. The viscosity of water was assumed to be 1×10^{-3} kg m^{-1} s^{-1} for spores launched into water. The launch angle of 30° was arbitrarily chosen. Initial launch speeds were measured from high-speed video recordings. Finally, trajectories were calculated assuming a simple Stokes' Law model for viscous drag. This has been shown to correctly reproduce empirically determined spore launch speeds and discharge distances over a wide range of projectile sizes and launch conditions (Fischer et al., 2010).

measurements of the range of the discharge mechanisms in different fungi have been made from spore deposits on microscope slides or coverglasses positioned at specific distances from the asci (e.g., Walkey and Harvey 1966; Yafetto et al., 2008).

Viscous drag is the pivotal issue in predicting the discharge distance from the measured launch speed launch speed and vice versa. When smaller ascospores are launched, their motion is dominated by viscous drag and they travel the same distance from the ascus whether they are shot upwards or are propelled along a more horizontal vector. The effect of gravity is imperceptible until the viscosity of the air drags these spores to a halt and they fall toward the ground at a sedimentation rate of a few millimeters per second. Larger spores follow more parabolic tracks after discharge, with the gravitational force shaping the pronounced downward curve of the spores toward the end of their trajectory. For the same launch speed, larger spores travel farther than smaller ones because they have greater momentum and because the braking effect of air viscosity diminishes as spore size increases. This effect is evident in the greater distances traveled by ascospores that remain connected during flight versus spores that become singled after discharge (Fig. 13).

There have been different approaches to calculating the drag force in models of ascospore discharge. Beginning with Buller (1909), Stokes' law for viscous drag on small particles has been applied to estimating launch speeds from measured discharge distances, and vice versa (Fischer et al., 2004). This method assumes that the Reynolds number (R_e), which is a measure of the ratio of inertial forces to viscous forces, remains relatively constant for a spore from discharge to deposition. More complex interpolation models, in which the motion of the spore is calculated in an iterative fashion as it moves through the air, have been promoted in other ballistic studies and are explained by Vogel (2005). Now that we have direct measurements of launch speeds from high-speed video microscopy, we can assess the effectiveness of these competing models for the fluid mechanics of spore release. Critical measurements of ascus turgor pressure are also useful for testing these models.

Using the interpolation approach to modeling ascospore motion, Trail et al. (2005) estimated a launch speed of 35 m s^{-1} for *Gibberella*, and a corresponding ascus turgor pressure of 1.5 MPa. This launch speed is a little higher than the measured top speeds from *Sordaria* and *Neurospora*, whose spores are discharged through similar apical ring structures. However, the ascus turgor pressure estimated by Trail et al. (2005) is unnecessarily high when more modest pressures have been shown to launch spores at similar speeds (Table 1). Trail et al. (2005) also predicted a much faster launch speed for *Ascobolus* than the Stokes' prediction by Fischer et al. (2004). This discrepancy was resolved when the launch speed for this fungus was measured using high-speed video (Yafetto et al., 2008), demonstrating the superiority of Stokes' law for predicting discharge distance over more complex mathematical models (Fischer et al., 2010). The interpolation calculations tend to overestimate the launch speed (from measured discharge distances) and underestimate the discharge distance (from measured launch speeds). Incidentally, these more complex models championed by Vogel (2005) are even worse for modeling the flights of the larger projectiles launched by *Pilobolus* (Fischer et al., 2010).

In a theoretical study of ascospore morphology, Roper et al. (2008) showed that the ellipsoidal spores of selected species have drag-minimizing profiles that they related to maximizing discharge distances. High-speed imaging of spore discharge reveals a fundamental fallacy with this analysis, namely that ascospores are ejected with variable amounts of ascus sap that transform the shape of the projectiles from these idealized symmetrical shapes into irregular blobs of varying size and shape that are often connected to other spores. It seems more likely that Ingold (1971) was correct in his suggestion that ascospore shape has a lot to do with the mechanics of release. Spores with ellipsoidal shapes have the advantage of stoppering the opening of the ascus during 50 % of their transit before they are propelled into the air. This conserves ascus pressure until the moment of the launch and the ascus apex is immediately plugged in the same fashion by the spore next in line until it empties. This process is shown by the video of ascospore discharge in an aquatic fungus in Supplementary Video 4. Discharging into water, even at relatively high speeds, limits the distance travelled by the spores to less than 0.5 mm (Table 1; Fig. 14C).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.funbio.2023.11.001

Supplementary Video 4; Video clip of ascospore discharge in *Macrospora scirpicola* recorded real time. Note that each spore is gripped by the open ascus tip before release, maintaining ascus pressure until the last spore is ejected. This fungus is an aquatic species that grows on submerged culms of the lakeshore bulrush, *Schoenoplectus lacustris*.

8. Ascomata-level effects on spore dispersal

The near simultaneous discharge of hundreds or thousands of asci from single apothecia, first described in the eighteenth century (Section 2), was examined by Buller (1934) and has been revisited by several researchers over the last 90 years. Buller showed that puffing resulted in the displacement of air above the apothecium and suggested that this carried the spores over a longer distance than spores released from asci asynchronously. Ingold and Oso (1968) were first to demonstrate this experimentally, with the lofting of the puffed spores of *Ascobolus crenulatus* over 5 cm compared with 1 cm without puffing. Interestingly, Ingold and Dam used high intensity blue light to stimulate puffing in apothecia that they had maintained in darkness before the experiment. Rather than activating puffing via a blue light photoreceptor, illumination may have worked through the simple effect of heating and drying the apothecia.

Roper et al. (2010) took this inquiry further by measuring the velocities of spores at different positions within the jets of air from apothecia of *Sclerotinia sclerotiorum* using high-speed imaging. They detailed the relationship between the synchronous discharge of the microscopic asci and creation of the macroscopic plume of spores in "a cooperatively generated wind." The local fluid environment is modified by this process of mass ejection so that the spores flow around any obstacles above the apothecia, rather than striking these surfaces, and disperse in the prevailing air currents.

Questions remain about the impulse for puffing, but consensus among the different ideas is within reach. Ascus development within apothecia is a continuous process that produces successive arrays of mature asci interspersed with immature asci and sterile paraphyses. Blowing on a fresh fruit body or removing the lid of a Petri dish of cultured apothecia are very effective triggers. Both stimuli cause an immediate drop in the humidity of the air above the fruit body, which causes water to evaporate from the exposed tips of the asci. In operculate species, this will cause the rapid drying of the connection between the operculum and subtending cell wall of each ascus. This is the probable stimulus for the operculum to flip open under the force exerted by the pressurized ascus sap. The operculum is primed for fracture by thinning of the ring of ascus wall that circumscribes the operculum, presumably by enzyme action on the cell wall polymers, which is observed just before discharge (Van Brummelen 1975). These operations at the level of the single cell are linked to puffing by the disturbance to adjacent asci when a small group of asci begins firing. As the first asci discharge, the surrounding asci relax as their elastic walls bulge slightly into the space created by the collapsing cells. This results in a dip in ascus pressure, disturbing the tip shape which, we hypothesize, destabilizes and fractures the fragile lid connection. One or a few shots lead to a volley and a domino effect plays out across the apothecium, emptying the ripest asci.

Puffing can be transmitted from one spot as a wave across a large apothecium or may occur in separate puffs from multiple locations. In either case, we observe a cloud of spores expelled from the fungus. Puffing in inoperculate ascomycetes has not received much attention, but certainly occurs in some species including *Rhytisma acerinum* (Persoon 1801; Jones 1925). This probably works in the same fashion as

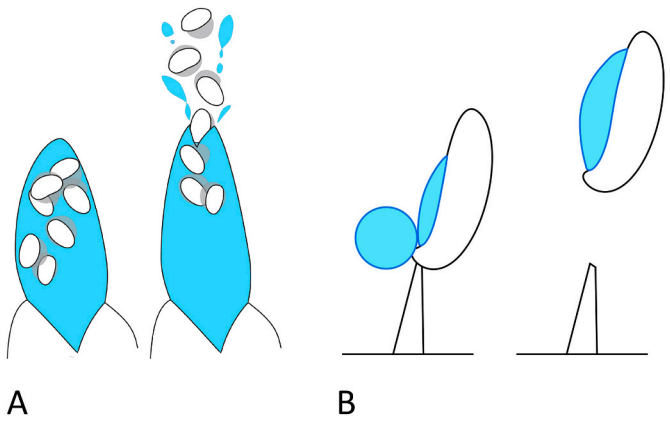

Fig. 15. Side-by-side comparison of (A) ascospore discharge and (B) ballistospore discharge, showing the role of fluid movement as the source of momentum for the spores. Diagrams are not to the same scale.

operculate asci, with the weakening of cell wall material in the ascus tips preceding mass discharge.

Studying the small fruit bodies of an *Ascobolus*, Roper et al. (2010) favored the idea that ascus discharge proceeds as a circular wave over the hymenium like the ripples on a pond surface produced by the impact of a pebble. Videoing the much larger apothecia of *Sarcoscypha austriaca*, Dam (2020) discounted the wave model and concluded that large numbers of asci fired simultaneously in association with the deformation of the fruit body. This idea was expressed by Ziegenspeck (1926) who challenged the more conventional view held by Anton de Bary (1887) that dry air caused asci to fire and resulted in puffing. It is difficult to choose between these ideas. Deformation of larger apothecia occurs whether it causes or follows the high-speed emptying of asci and will precede the slower appearance of the smoke of spores above the fruit body. One simple observation that supports the De Bary model, namely that deformation follows discharge, is that puffing is not stimulated by prodding or squeezing an apothecium. Puffing from the inflexible perithecial stroma of *Rhytisma* is another.

9. Comparisons between ascospore and ballistospore discharge

The life cycles of ascomycete and basidiomycete fungi share the formation of sporangia in which the fusion of nuclei (karyogamy) is followed by meiosis, to produce haploid nuclei containing recombined chromosomes that are packaged in spores. Asci are the meiosporangia of ascomycetes and basidia are the meiosporangia of basidiomycetes. Spore formation takes place inside asci and outside basidia; ascospores are discharged from the interior of asci and basidiospores are discharged from pegs called sterigmata that project from the surface of basidia (Fig. 15).

The location of meiosis in asci and basidia in the larger life cycles of these fungi is consistent with the hypothesis that these are homologous structures that originated from the same type of ancestral sporangium. The details are obviously obscure, but it seems more likely that basidia evolved from asci, rather than vice versa, as proto-basidiomycetes diverged from proto-ascomycetes. This is logical because the protrusion of the ascus interior to form external basidiospores seems more straightforward than the involution of the extruded portions of a basidium to form internal ascospores. The development of ascospores and basidiospores are topologically similar in the sense that the ascus and basidium sequester nuclei and small portions of cytoplasm that will become spores. These developmental programs diverge when the ascospores are separated from the surrounding cytoplasm by their own membranes and cell walls, and the cytoplasm of the basidium is squeezed into the tips of the sterigmata that swell into basidiospores. Speculation about evolutionary pathways is no longer fashionable, for many excellent reasons, but it is useful to offer this limited argument in the present case pending declarative phylogenetic studies. Such clarity is a big ask given the limited resolution of molecular phylogenies resulting from the estimated 400-million-year divergence of these clades, pitiful fossil record for calibration, and overwhelming extinction that has occurred during the immense history of these fungi.

The actions of asci and basidia have had a profound effect on the evolution of the ascomycete and basidiomycete fungi. Asci that develop inside perithecia protrude from the fruit body just before discharge, and fields of exposed asci fire directly into the air above apothecia as they mature. Asci could not work within the gilled or poroid fruit body morphologies that have evolved in the basidiomycetes because they fire their spores too far. The “genius” of ballistospore discharge lies in the way it can be tweaked to match the launch of the spores to the short distances between gills and radii of tubes (Money 2023a, 2023b). This has allowed basidiomycetes to release billions of spores from single mushrooms, whereas the largest ascomycete fruit bodies are limited to dispersing millions of spores. These differences in launch mechanism have also limited the diversification in fruit body morphology among the ascomycetes. Ascomycetes lack the flexibility afforded to ballistosporic fungi to increase the surface area for spore production by extending an exposed hymenium over gills or spines, or within tubes.

This mechanistic difference does not mean, of course, that one method of spore discharge is superior to the other. Instead, we find an incredible range of reproductive strategies among the fungi in these phyla, with variations in spore size and numbers, and discharge distance, and manifold strategies for long-distance dispersal. Coprophilous ascomycetes fire individual spores or groups of spores from single asci over the greatest distances. This mechanism has evolved to throw spores onto vegetation beyond the zone of repugnance that is avoided by the herbivores that serve as their vectors (Van Asperen et al., 2022). Ballistospore discharge cannot achieve these feats of athleticism, but non-ballistosporic basidiomycetes include the artillery fungi (*Sphaerobolus* spp.) that launch glebas (sporangia) containing 10 million spores over a distance of up to 6 m, and the larger peridioles of bird’s nest fungi containing 100 million spores are splashed 2 m from their fruit bodies.

Energetic comparisons between ascospore and ballistospore discharge are informative. Sugar alcohols or polyols generate some of the propulsive force for these mechanisms by increasing the osmotic pressure of the ascus sap, and by lowering the vapor pressure of Buller’s drop on the surface of the ballistospore (Fig. 15). Other osmolytes including hexose sugars and inorganic ions also contribute to these mechanisms, pressurizing the ascus sap by osmosis, and causing water to condense on the surface of Buller’s drop (Money 2023b). Because polyols have been measured in ascus sap and Buller’s drops, we can use their concentrations as proxies for the metabolic investment in powering these discharge mechanisms. Fungi use polyols in energy metabolism (Klein et al., 2017), so their emission during spore discharge represents one of the costs of these reproductive methods.

The following calculations refer to the 37 mM concentration of glycerol measured in *A. immersus* (Fischer et al., 2004), and 58 mM concentration of mannitol measured in *Itersonilia perplexans* (Webster et al., 1995). With an estimated sap volume of 1 nL per ascus, each ascus of *Ascobolus* contains 4 ng of glycerol (0.04 mol L^{-1} x 92 g mol^{-1} x 10^{-9} L). Extrapolating from a single ascus to the 5 to 10 million asci produced by a morel (Money, unpublished data), this rough estimate suggests that the fruit body uses 20–40 mg glycerol to expel 40 to 80 million ascospores. Rounding to single significant figures, these quantities of glycerol have an energy value of 90–200 millicalories (for 4.3 kcal g^{-1}) or 380–840 mJ (mJ). Applying the same logic to Buller’s drop with a radius of 5 μm and volume of 0.5 pL, the launch of each ballistospore of *Itersonilia* utilizes 22 pg of mannitol (0.06 mol L^{-1} x 182 g mol^{-1} x 2 × 10^{-12} L). Extrapolating from these numbers, we estimate that a meadow mushroom that releases 2 billion spores over a 2-d period (Buller 1909), will utilize 40 mg of mannitol with an energy value of 70 millicalories (1.6 kcal g^{-1}) or 290 mJ.

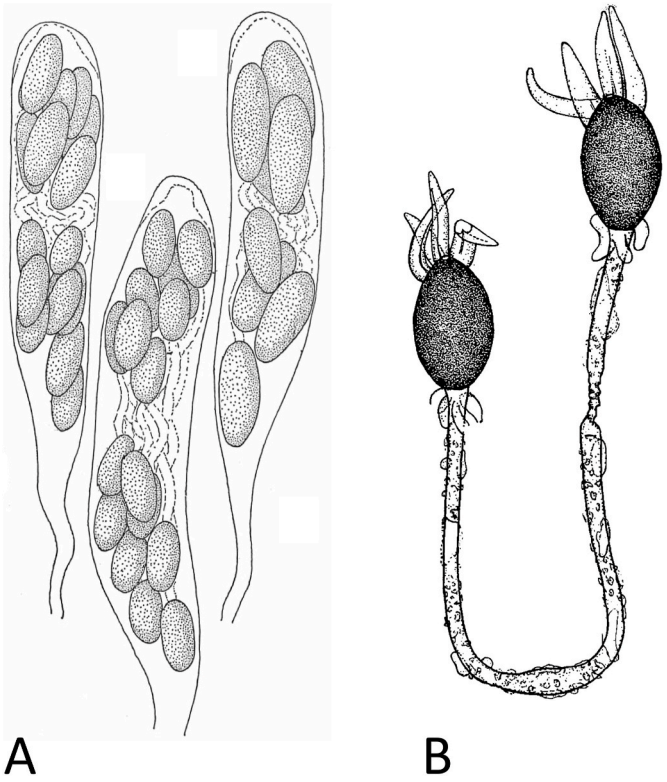

Fig. 16. (A) Asci and (B) tethered pair of discharged ascospores of *Zygopleurage zygospora*. From (A) Boedijn (1962). (B) Lundqvist (1969).

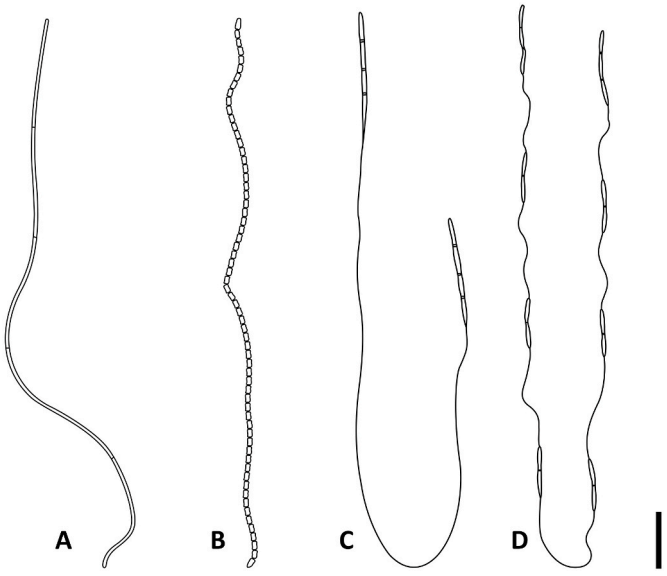

Fig. 17. Filamentous ascospores of *Cordycipitaceae*. (A) Filiform morphology with septa. (B) Ascospore composed of multiple part-spores. (C) Bola-shaped ascospore with single connecting thread between terminal groups of part-spores. (D) Ascospore with multiple groups of part-spores connected by sterile threads. Scale: 10 μm (A, B), 20 μm (C, D). Redrawn from Mongkolsamrit et al. (2022).

We conclude that spore release from ascomata and basidiomata of roughly the same size involves the accumulation of polyols that carry no more energy than a single garden pea. This energetic equivalency illustrates the way that a single primary metabolic pathway, namely polyol synthesis, has been channeled into very different launch mechanisms. The efficiency of polyol usage by ascomycetes and basidiomycetes allows both groups of fungi to disperse huge numbers of spores with a minuscule expenditure of chemical energy.

10. Future research

The diversity of ascus and ascospore structures leaves us with a tantalizing view of the ways in which a simple explosive discharge mechanism has been adapted in the largest fungal phylum. Coprophilous species are particularly interesting (see above) and very few of them have been studied experimentally. More extensive use of high-speed video is certain to reveal novel details of spore release in these uncharted species. Species of *Zygopleurage* seem fruitful experimental subjects, whose spores are yoked in pairs via gelatinous appendages that are coiled within the ascus and extend upon release (Fig. 16). The function of these yokes may be related to the capture of the spores on vegetation in a manner comparable to the funicular cords in some of the bird's nest fungi (Hassett et al., 2013).

The discharge of the elongated ascospores of *Cordyceps* and related fungi is another fascinating mechanism, in which highly bendable filaments are pushed through the apical ring of the ascus by the scalar force of turgor without becoming entangled. Using a hand lens, we can see the tip of each ascus protrude from the neck of its perithecium and discharge its spores one by one over the course of a few seconds before collapsing (Ingold 1971). The release of each spore is visible with this 10–20X magnification when it appears as a glassy fiber reflecting the light as it floats in the air 0.5 mm from the surface of the fruit body. Filamentous spores produced by spider pathogens in the Cordycipitaceae are particularly elaborate (Mongkolsamrit et al., 2022). Some of these are described as bola-like and resemble skipping ropes, others have multiple groups of part spores joined by interconnecting threads and look like strings of Christmas lights (Fig. 17). One wonders how filamentous spore shapes factor in the dissemination of the infections caused by these tropical pathogens. The taxonomic literature is filled with similarly unexamined spore morphologies awaiting biomechanical study.

Although the introduction of high-speed video and other modern experimental methods has resulted in a deeper understanding of the mechanics of ascospore discharge, it is important to recognize that earlier investigators made great progress without these tools. As we have seen in this article, measurements of ascus pressure in *Ascobolus* using a strain gauge were consistent with the composition of ascus sap, and mathematical modeling using these data predicted a launch speed of 9 m s^{-1} (Fischer et al., 2004). Later experiments showed that the predicted speed fell within the range measured with high-speed video microscopy (Yafetto et al., 2008). While we can feel pleased with the internal consistency of these results, it is worth remembering that A. H. R. Buller estimated that the launch speed of *Ascobolus* was 10 m s^{-1} using a simple calculation in 1909 (Section 3). In a similar vein, Ingold and Oso (1968) showed that apothecial puffing increased the discharge distance of ascospores relative to asynchronous spore release decades before the study by Roper et al. (2010) discussed in Section 8. As T. S. Eliott wrote, "We shall not cease from exploration/And the end of all our exploring/Will be to arrive where we started/And know the place for the first time" (Eliot 1943).

Acknowledgement

We thank Patricia Van Skaik, Executive Director, and librarians, Erin Campbell and Alex Herrlein, at The Lloyd Library and Museum, Cincinnati, Ohio, for access to their collection of mycological works and for scanning the historical images reproduced in this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funbio.2023.11.001.

References

Ainsworth, G.C., 1976. Introduction to the History of Medical and Veterinary Mycology. Cambridge University Press, Cambridge.

Bary, A. de, 1887. Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria. Clarendon Press, Oxford trans. Garnsey, H. E. F.

Beckett, A., Crawford, R.M., 1973. The development and fine structure of the ascus apex and its role during spore discharge in *Xylaria longipes*. New Phytol. 72, 357–369.

Boedijn, K.B., 1962. The sordariaceae of Indonesia. Persoonia 2 (3), 305–320.

Bonito, G., Smith, M.E., Nowak, M., Healy, R.A., Guevara, G., Cázares, E., Kinoshita, A., et al., 2013. Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. PLoS One 8 (1), e52765.

Bourett, T.M., Howard, R.J., 1990. *In vitro* development of penetration structures in the rice blast fungus *Magnaporthe grisea*. Can. J. Bot. 68, 329–342.

Buller, A.H.R., 1909. Researches on Fungi, vol. I. Longmans, Green, & Co., London.

Buller, A.H.R., 1934. Researches on Fungi, vol. I. Longmans, Green, & Co., London.

Bulliard, P., 1791. Histoire des Champignons de la France. Chez l'auteur, Barrois le jueune, Belin, Crouillebois, Bazan, Leblanc, Paris.

Burdsall, H.H., 1965. Operculate asci and puffing of ascospores in *Geopora* (Tuberales). Mycologia 57 (3), 485–488.

Chadefaud, M., 1957. Les asques des Diatrypales. C. R. Hebd. Seances Acad. Sci., Paris 244, 1813–1815.

Chadefaud, M., 1958. Sur les asques des Nectriales, et l'existence de *Pléosporales nectrioïdes*. C. R. Hebd. Seances Acad. Sci., Paris 247, 1376–1379.

Chadefaud, M., 1964. Sur l'origine de la structure das asques du type annellascé. C. R. Hebd. Seances Acad. Sci., Paris 258, 299–301.

Chadefaud, M., Nicot, J., 1957. Les asques de Sordariales. C. R. Hebd. Seances Acad. Sci., Paris 244, 2415–2418.

Château, J., Guazzelli, É., Lhuissier, H., 2018. Pinch-off of a viscous suspension thread. J. Fluid Mech. 852, 178–198.

Corner, E.J.H., 1929. Studies in the morphology of Discomycetes: II. The structure and development of the ascocarp. Trans. Br. Mycol. Soc. 14, 275–291.

Crouan, M.M., messieurs for brothers, P.L., H. M, 1857. Note sur quelques *Ascobolus* nouveaux et sur une espèce nouvelle de *Vibrissa*. Ann. Sci. Natur. (Bot.) Sèr. 4 (7), 173–178.

Dam, N., 2020. "Puffing" in *Sarcoscypha austriaca*: back to Ziegenspeck. Mycologia 112 (2), 224–229.

Davis, D.J., Burlak, C., Money, N.P., 2000a. Biochemical and biomechanical aspects of appressorial development in *Magnaporthe grisea*. In: Tharreau, D., Lebrun, M.H., Talbot, N.J., Notteghem, J.L. (Eds.), Advances in Rice Blast Research. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 248–256.

Davis, D.J., Burlak, C., Money, N.P., 2000b. Osmotic pressure of fungal compatible osmolytes. Mycol. Res. 104, 800–804.

Eliot, T.S., 1943. Four Quartets. Harcourt Brace and Company, New York.

Fischer, M., Cox, J., Davis, D.J., Wagner, A., Taylor, R., Huerta, A.J., Money, N.P., 2004. New information on the mechanism of forcible ascospore discharge from *Ascobolus immersus*. Fungal Genet. Biol. 41, 698–707.

Fischer, M.F.W., Stolze-Rybczynski, J.L., Davis, D.J., Cui, Y., Money, N.P., 2010. Solving the aerodynamics of fungal flight: how air viscosity slows spore motion. Fungal Biol. 114 (11–12), 943–948.

Foster, A.J., Ryder, L.S., Kershaw, M.J., Talbot, N.J., 2017. The role of glycerol in the pathogenic lifestyle of the rice blast fungus *Magnaporthe oryzae*. Environ. Microbiol. 19, 1008–1016.

Fritz, J.A., Seminara, A., Roper, M., Pringle, A., Brenner, M.P., 2013. A natural O-ring optimizes the dispersal of fungal spores. J. R. Soc. Interface 10, 20130187.

Greenhalgh, G.N., Evans, L.V., 1967. The structure of the ascus apex in *Hypoxylon fragiforme* with reference to ascospore release in this and related species. Trans. Br. Mycol. Soc. 50, 183–188.

Griffiths, D., 1901. The North American sordariaceae. Mem. Torrey Bot. Club 11, 1–134.

Hassett, M.O., Sugawara, Z.T., Stolze-Rybczynski, J., Fischer, M.W.F., Money, N.P., 2013. Splash and grab: biomechanics of peridiole ejection and function of the funicular cord in bird's nest fungi. Fungal Biol. 117, 708–714.

Healy, R.A., Arun Kumar, T.K., Hewitt, D.A., McLaughlin, D.J., 2013. Functional and phylogenetic implications of septal pore ultrastructure in the ascoma of *Neolecta vitellina*. Mycologia 105, 802–813.

Hedwig, J., 1789. Descriptio et Adumbratio Microscopico-Analytica Muscorum Frondosorum. Tomus secundus. I. G. Mülleriano, Lipsiae.

Honegger, R., 1978. The ascus apex in lichenized fungi I. The *Lecanora*-, *Peltigera*- and *Teloschistes*-types. Lichenologist 10 (1), 47–67.

Honegger, R., 1983. The ascus apex in lichenized fungi IV. *Baeomyces* and *Icmadophila* in comparison with *Cladonia* (Lecanorales) and the non-lichenized *Leotia* (Helotiales). Lichenologist 15, 57–71.

Ingold, C.T., 1939. Spore Discharge in Land Plants. Clarendon Press, Oxford.

Ingold, C.T., 1954. Ascospore form. Trans. Br. Mycol. Soc. 37, 19–21.

Ingold, C.T., 1966. Aspects of spore liberation: violent discharge. In: Madelin, M.F. (Ed.), The Fungus Spore. Butterworths, London, pp. 113–132.

Ingold, C.T., 1971. Fungal Spores. Their Liberation and Dispersal. Clarendon Press, Oxford.

Ingold, C.T., Hadland, S.A., 1959. The ballistics of *Sordaria*. New Phytol. 58, 46–57.

Ingold, C.T., Oso, B.A., 1968. Increased distance of discharge due to puffing in Ascobolus. Trans. Br. Mycol. Soc. 51, 592–594.

Jones, S.G., 1925. Life-history and cytology of *Rhytisma acerinum* (pers.) fries. Ann. Bot. 39 (153), 41–75.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. Ainsworth and Bisby's Dictionary of the Fungi, tenth ed. CABI International, Wallingford, U.K.

Klein, M., Swinnen, S., Thevelein, J.M., Nevoigt, E., 2017. Glycerol metabolism and transport in yeast and fungi: established knowledge and ambiguities. Environ. Microbiol. 19 (3), 878–893.

Lauga, E., 2022. Fluid Mechanics: A Very Short Introduction. Oxford University Press, Oxford.

Lepp, H., 2021. Snippets of Mycological History. Privately published, Canberra.

Li, Y., Steenwyk, J.L., Chang, Y., Wang, Y., James, T.Y., Stajich, J.E., Spatafora, J.W., Groenewald, M., Dunn, C.W., Hittinger, C.T., Shen, X.X., Rokas, A., 2021. A genome-scale phylogeny of the kingdom Fungi. Curr. Biol. 31 (8), 1653–1665.e5.

Longo, S.J., Cox, S.M., Azizi, E., Ilton, M., Olberding, J.P., St Pierre, R., Patek, S.N., 2019. Beyond power amplification: latch-mediated spring actuation is an emerging framework for the study of diverse elastic systems. J. Exp. Biol. 222 (15), jeb197889.

Lundqvist, N., 1969. *Zygopleurage* and *zygospermella* (sordariaceae s. lat., pyrenomycetes). Bot. Not. 122, 353–374.

Micheli, A., 1729. Nova Plantarum Genera. Bernardi Paperinii, Florence.

Minter, D.W., Cannon, P.F., 1984. Ascospore discharge in some members of the Rhytismataceae. Trans. Br. Mycol. Soc. 83 (1), 65–92.

Money, N.P., 2023a. Goldilocks mushrooms: how ballistospory has shaped basidiomycete evolution. Fungal Biol. 127, 975–984.

Money, N.P., 2023b. The fastest short jump in nature: progress in understanding the mechanism of ballistospore discharge. Fungal Biol. 127, 835–844.

Mongkolsamrit, S., Noisripoom, W., Tasanathai, K., Kobmoo, N., Thanakitpipattana, D., Khonsanit, A., Petcharad, B., Sakolrak, B., Himaman, W., 2022. Comprehensive treatise of *hevansia* and three new genera *jenniferia, parahevansia* and *polystromomyces* on spiders in Cordycipitaceae from Thailand. MycoKeys 91, 113–149.

Nees von Esenbeck, C.G., 1816-1817. Das System der Pilze und Schwämme. Stahelschen Buchhandlung, Würzburg.

Nüchter, T., Benoit, M., Engel, U., Ozbek, S., Holstein, T.W., 2006. Nanosecond-scale kinetics of nematocyst discharge. Curr. Biol. 16 (9), R316–R318.

Parguey-Leduc, A., Janex-Favre, M.C., Letrouit-Galinou, M.A., Bellemère, A., 1994. M. Chadefaud and ascomycete systematics. In: Hawksworth, D.L. (Ed.), Ascomycete Systematics: Problems and Perspectives in the Nineties, NATO Sci. Series A, 269. Springer, Boston, MA, pp. 37–41.

Persoon, C.H., 1801. Synopsis Methodica Fungorum. Hendricum Dieterich, Gottingen.

Prieto, M., Wedin, M., 2013. Dating the diversification of the major lineages of Ascomycota (Fungi). PLoS One 8 (6), e65576.

Ramsbottom, J., 1941. The expanding knowledge of mycology since Linnaeus. Proc. Linn. Soc. 151, 280–367.

Ratliff, M.J., 2009. The Quest for the Invisible. History of Microscopy in the Enlightenment. Ashgate, Farnham, Surrey, U.K.

Roper, M., Pepper, R.E., Brenner, M.P., Pringle, A., 2008. Explosively launched spores of ascomycete fungi have drag-minimizing shapes. Proc. Natl. Acad. Sci. USA 105 (52), 20583–20588.

Roper, M., Seminara, A., Bandi, M.M., Cobb, A., Dillard, H.R., Pringle, A., 2010. Dispersal of fungal spores on a cooperatively generated wind. Proc. Natl. Acad. Sci. USA 107 (41), 17474–17479.

Samuelson, D.A., 1975. The apical apparatus of the suboperculate ascus. Can. J. Bot. 53 (22), 2660–2679.

Schoch, C.L., Sung, G.H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., de Hoog, G.S., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D. M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P. S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst. Biol. 58 (2), 224–239.

Splivallo, R., Ottonello, S., Mello, A., Karlovsky, P., 2011. Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol. 189, 688–699.

Trail, F., Xu, H., Loranger, R., Gadoury, D., 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae*. Mycologia 94, 181–189.

Trail, F., Gaffoor, I., Vogel, S., 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genet. Biol. 42, 528–533.

Trail, F., 2007. Fungal cannons: explosive spore discharge in the Ascomycota. FEMS Microbiol. Lett. 276 (1), 12–18.

Tulasne, L.R., Tulasne, C., 1863. Selecta Fungorum Carpologia, vol. II. Imperiali Typographeo, Paris.

Tulasne, L.R., Tulasne, C., 1865. Selecta Fungorum Carpologia, vol. III. Imperiali Typographeo, Paris.

Türkoğlu, A., Castellano, M.A., Trappe, J.M., Güngör, M.Y., 2015. Turkish truffles I: 18 new records for Turkey.". Turk. J. Bot. 39, 359–376.

Van Asperen, E., Brennan, J., Reid, A., 2022. Dispersal distances of dung fungal spores: an in vivo experimental setup. Curr. Res. Environ. Appl. Mycol. 12, 220–237.

Van Brummelen, J., 1975. Light and electron microscopic studies of the ascus top in *Sarcoscypha coccicea*. Persoonia 8 (3), 259–271.

Vogel, S., 2005. Living in a physical world II. The bio-ballistics of small projectiles. J. Biosci. 30 (2), 167–175.

Walker, L.B., Andersen, E.N., 1925. Relation of glycogen to spore-ejection. Mycologia 17, 154–159.

Walkey, D.G.A., Harvey, R., 1966. Studies of the ballistics of ascospores. New Phytol. 65, 59–74.
Wang, X.W., Houbraken, J., Groenewald, J.Z., Meijer, M., Andersen, B., Nielsen, K.F., Crous, P.W., Samson, R.A., 2016. Diversity and taxonomy of *Chaetomium* and chaetomium-like fungi from indoor environments. Stud. Mycol. 84, 145–224.
Watkinson, S.C., Boddy, L., Money, N.P., 2016. The Fungi, third ed. Academic Press, Amsterdam.
Webster, J., Smirnoff, N., Fricke, W., Hinde, P., Tomos, D., Turner, J.C.R., 1995. Mannitol and hexoses are components of Buller's drop. Mycol. Res. 99, 833–838.
Wilson, W.A., Roach, P.J., Montero, M., Baroja-Fernandez, E., Munoz, F.J., Eydallin, G., Viale, A.M., Pozueta-Romero, J., 2010. Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol. Rev. 34, 952–985.
Yafetto, L., Carroll, L., Cui, Y., Davis, D.J., Fischer, M.W.F., Henterly, A.C., Kessler, J.D., Kilroy, H., Shidler, J.B., Stolze-Rybczynski, J.L., Sugawara, Z., Money, N.P., 2008. The fastest flights in nature: high-speed spore discharge mechanisms among fungi. PLoS One 3 (9), e3237.
Ziegenspeck, H., 1926. Schleudermechanismen von Ascomyceten. Bot. Arch. 13, 341–381.