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More *g*'s than the Space Shuttle: ballistospore discharge

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Abstract: Ballistospores of basidiomycete fungi form at the tips of spear-shaped projections called sterigmata that extend from basidia. At maturity, a spherical drop of fluid appears at the base of each spore, and a few seconds later the spore is propelled into the surrounding air. The development of the fluid drop was first reported in 1889, but a century of innovative research was necessary to solve the mechanistic link between the drop and spore discharge. Through an extraordinary series of experiments the composition of the drop has now been established, its development is explained, and an effective solution to the relationship between drop appearance and spore discharge has been proposed. Drop formation is initiated when a femtomole quantity of mannitol and hexoses is excreted from a specific site at the base of the spore, forming a hygroscopic nucleus upon which water condenses from the surrounding air. Discharge of the spore occurs when the drop fuses with a film of liquid that curves over the adjacent spore surface. This rapid coalescence results in a decrease in surface free energy within the liquid and displaces the center of mass of the spore. The change in weight distribution exerts a force that is opposed by the pressurized sterigma, and the spore is shot away from the basidium into the surrounding air. The mechanism is described as a surface-tension catapult. During discharge, ballistospores are subjected to an acceleration of 25 000 *g*, which is about ten thousand times the acceleration experienced by astronauts during the launch of the Space Shuttle! Even more impressive is the fact that while the Shuttle consumes 50% of its weight in fuel in the first 2 min of flight, ballistospore discharge is fueled by the mannitol and hexoses that cause water to condense on the spore surface, and these solutes represent only 1% of the mass of the spore.

Key Words: basidiomycete, basidiospore, biomechanics

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1. INTRODUCTION

Basidiomycete fungi are unified by the formation of basidiospores on cells called basidia. With the exception of the gasteromycete fungi, most basidiomycetes propel their spores away from the basidium. Such spores are called "ballistospores" referring to the apparently active nature of their discharge. In the case of the familiar agarics, boletes, and brackets, the ballistospores are projected from basidia that stud the gills or tubes of the fruit body (FIG. 1), and fall out from the undersurface of the cap whereupon they are dispersed by wind (Ingold, 1971). FIGURE 2 shows successive stages in the process of ballistospore discharge in the basidiomycete yeast *Itersonilia perplexans*. Between 30 and 45 s elapse between the first and last images in the sequence. The lemon-shaped ballistospore is attached asymmetrically to the tip of a spear-shaped projection called a sterigma (plural = sterigmata), with a bulge termed the hilar appendix above the point of contact. *Itersonilia* does not produce a fruit body, but forms its spores from single sterigmata that project from mycelial or yeast cells that can be grown on solid nutrient media. The first frame in FIG. 2 shows the mature spore primed for the beginning of the discharge process. A drop then expands from a point on the hilar appendix termed the *punctum lacrymans*, swells rapidly, and the spore disappears; in the final frame, the sterigma continues to project into the air, but the spore is no longer attached to its tip. The same process occurs in mushrooms with the complication of four discharges from a single basidium (FIG. 3). When observations are made from *Itersonilia* cultures (Webster et al., 1984a), or from slices of mushroom gills placed on the surface of an agar plate (Webster and Davey, 1985), discharged spores accumulate up to one mm away from their sterigmata. To explain the mechanism of discharge, two questions must be answered: (i) What is the nature of the drop formed at the hilar appendix, and how does it develop? (ii) How is the development of the drop linked to the process of discharge?

The aim of this article is to communicate a comprehensive answer to these questions founded on more than a century of experimental inquiry. While some of the alternative (and largely untenable) models of discharge are discussed, the reader is referred to previous papers for further analysis of mechanisms

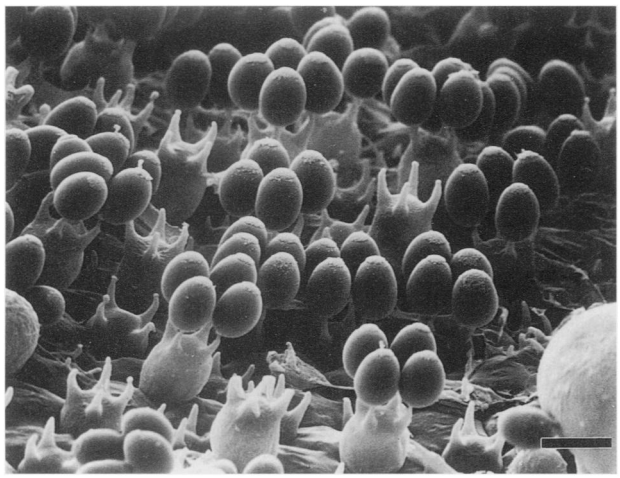

FIG. 1. Mature basidia of *Coprinus cinereus*. Some basidiospores dislodged during fixation, revealing sterigmata. Scanning electron micrograph of gill tissue prepared by semisimultaneous chemical fixation and critical-point drying. Scale bar = 10 μm. From McLaughlin et al. (1985).

involving flexing cell walls, squirting sterigmata, springboards, bursting bubbles, and electrostatic repulsion (Ingold, 1971; Gregory, 1979; McLaughlin et al., 1985; Webster et al., 1988; Webster and Chien, 1990). Significant contributions from a number of mycologists will be explored, but the discussion will center on the experiments and mathematical analyses of John Webster's group at the University of Exeter in England, who are to be credited with the most significant advances in unraveling the discharge mechanism since the work of this century's solitary mycological genius, A. H. Reginald Buller (1874–1944).

2. AN HISTORICAL PRELUDE

Mycologists interested in fungal mechanics usually turn to Buller's seven-volume work titled *Researches on Fungi* (1909–1950) to begin their quest for understanding the physical mechanisms that underlie the often extraordinary, sometimes bizarre microscopic behavior of fungi. So it is with ballistospore discharge that Buller (1909, 1922, 1924) is the foundation for almost all subsequent research. In 1910, Buller rediscovered the phenomenon of "drop-excretion" that was first described by Fayod (1889), and subsequently the drop has been referred to as Buller's drop or the Buller drop. Buller (1922) described and illustrated his observations on spore discharge in *Agaricus campestris* (FIG. 3), *Calocera cornea* (FIG. 4), and 20 other basidiomycetes. He became convinced that the discharge process with drop formation was a universal one among ballistosporic basidiomycetes, and went on to describe that the drop was carried on the spore surface when the spore was shot away from the sterigma. Buller (1922) even illustrated the spore a moment after discharge, carrying the drop on its surface (FIG. 4). This points to remarkable insight, and correct interpretation, because the discharged spore travels far too fast to be observed in flight (see Section 7).

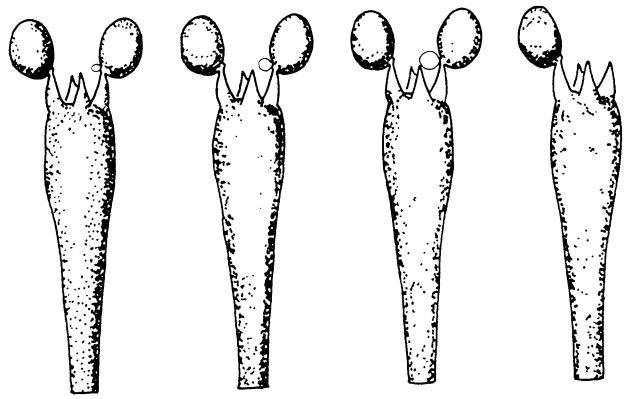

FIG. 3. Discharge of two spores from basidium of *Agaricus campestris*. Redrawn from Buller (1922).

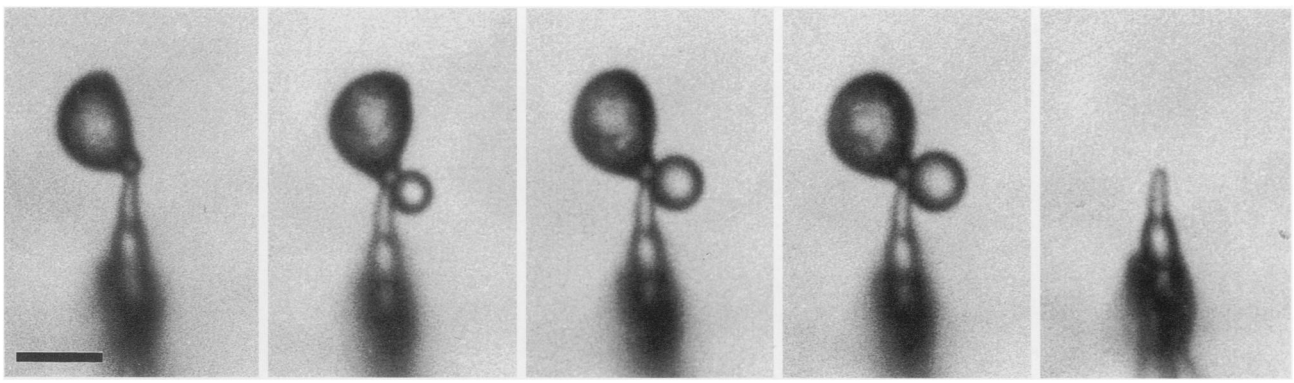

FIG. 2. Ballistospore discharge in *Itersonilia perplexans*. Note hilar appendix at base of spore, and change in the profile of the ballistospore caused by the accumulation of liquid on the spore surface. Scale bar = 10 μm. Photographs courtesy of John Webster.

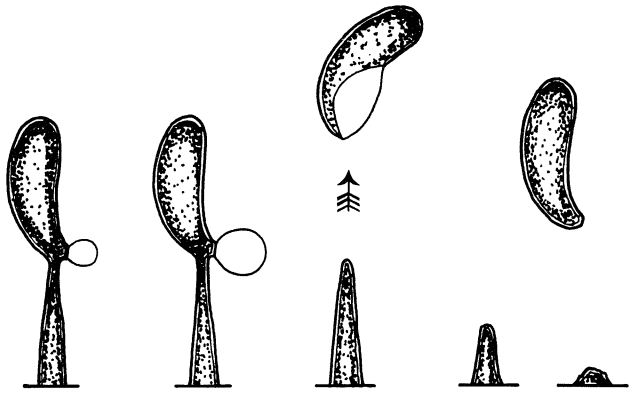

FIG. 4. Discharge of spore and fluid drop in *Calocera cornea* illustrated by Buller. Redrawn from Buller (1922).

In the early 1980s Terence Ingold, another champion of innovative mycological research, isolated *Itersonilia perplexans* (first described by Derx, 1948) during a search for fungi growing on the surface of the tiny fruit bodies of *Dacrymyces stillatus* (Ingold, 1983, 1991). Ingold had been captivated by the discharge mechanism for almost 50 yr (see Ingold, 1939), and recognized that because *Itersonilia* produced large spores on the surface of agar media it would be a superb subject for further studies on ballistospore discharge. The recognition of the utility of *Itersonilia* was a key stimulus for Webster's multidisciplinary assault on the discharge mechanism.

3. BULLER'S DROP OR BULLER'S BUBBLE?

Olive (1964) suggested that Buller's drop was a gas-filled bubble rather than a droplet of liquid, spawning a series of erroneous models of the discharge mechanism (e.g., Savile, 1965; van Neil et al., 1972). But many lines of evidence show that Buller's original interpretation of the liquid nature of the drop was correct. In *Itersonilia,* as in other basidiomycetes, the discharge process sometimes fails, and the spore remains attached to its sterigma until the whole apparatus collapses (Webster et al., 1984a). As we shall see, the humidity of the air surrounding the spore is absolutely critical to the discharge process, so microclimatic changes in water availability above the agar surface (especially in an open Petri dish) are the usual cause of such failures. But these natural failures provide useful information. When the drop reaches maximum size, it contacts the base of the spore and spreads over its surface, behaving like a film of liquid (Fayod, 1889, was the first to describe this process). This observation is obviously inconsistent with the gas-bubble hypothesis because a bubble would burst and disappear. If a growing drop is touched with the tip of a micropipet, it does not burst, but is dispersed over the glass surface of the pipet tip, and the refractive properties of the expanding drop viewed under the microscope also support the liquid-drop interpretation. Spores discharged onto glass are often bathed in a glistening film of liquid and it is logical to suggest that the liquid is derived from Buller's drop and carried with the spore to its resting place. Finally, authoritative evidence is afforded by the ultrastructural analyses of McLaughlin et al. (1985; Section 4.4), and by the fact that Webster et al. (1995) were able to collect Buller's drops with a micropipet and analyze the composition of these picoliter samples of fluid (Section 4.2). But where does the liquid in the drop come from?

4.1 THE SOURCE OF BULLER'S DROP

Van Neil et al. (1972) observed that Buller's drop would form at the hilar appendix of a ballistospore of *Sporobolomyces holsaticus* (a basidiomycete yeast) even after it was detached from its sterigma with a microneedle. They also reported that the drop disappeared, and when it vanished that the spore jolted against the needle; Webster et al. (1984a) repeated and videotaped this experiment using *Itersonilia* (FIG. 5). Van Neil et al. (1972) interpreted these events incorrectly as evidence of gas bubble formation and bursting, but their experiments were useful because they clearly demonstrated that Buller's drop was not derived from the sterigma. Discounting the sterigma, the spore would seem to be the most obvious source of liquid for the expanding drop. But exudation of liquid from the spore would result in significant shrinkage of the spore, unless its wall were capable of sustaining extremely negative hydrostatic pressures or cavitated during the development of Buller's drop. Analysis of photomicrographs of *Itersonilia* by Webster et al. (1984a, b) showed that there was no significant change in spore size concomitant with drop expansion, and in some cases that the diameter of the drop actually exceeded the diameter of the spore to which it was attached. Therefore, Webster and colleagues postulated that the liquid in the drop might be derived from water vapor in the air surrounding the spore.

The next stage of the odyssey was to engage the attention of a chemical engineer, Robin Turner, to further examine the source of the liquid in Buller's drop (Webster et al., 1989). Webster and colleagues extended their inquiry from *Itersonilia* to a variety of basidiomycetes by placing thin slices (0.1–0.2 mm) of fruit bodies on tap water agar. They reported a number of interesting observations. First, they verified that drop expansion was not linked to a decrease in spore volume in any of the fungi examined. They

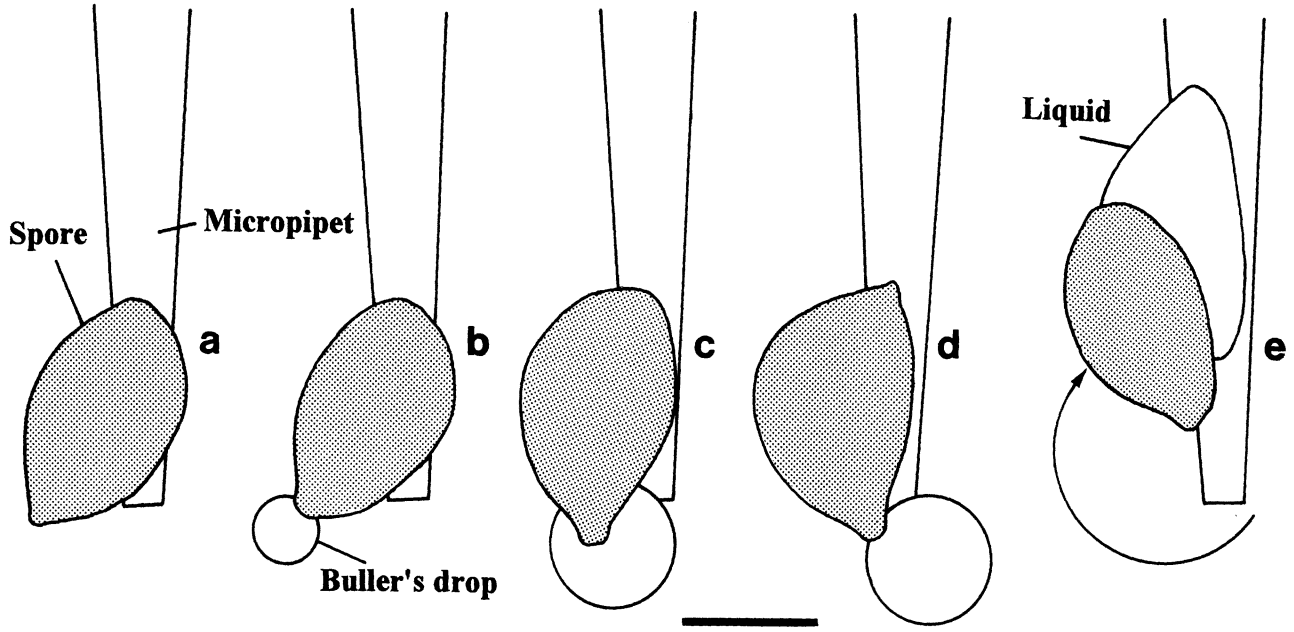

FIG. 5. Behavior of detached ballistospore of *Itersonilia perplexans* on tip of micropipet. a. Ballistospore adheres to tip of micropipet in natural orientation with hilar appendix pointing downward; b. Buller's drop initiated; c, d. grows to maximum size causing slight shift in the position of the spore; e. Buller's drop contacts surface of micropipet and ballistospore wall pulling spore up on pipet. This experiment demonstrates that Buller's drop is a mass of liquid not a gas bubble. The changes in position of the spore are not directly comparable with the natural behavior of the ballistospore attached to a sterigma, but do demonstrate the cohesion between spore and drop. Scale bar = 10 μm. Figure redrawn from Webster et al. (1984).

then found that drop expansion could be slowed by transferring fruit-body slices to tap water agar supplemented with mannitol. This was a clever experiment in which the reduction in water potential within the agar medium caused by the addition of mannitol would also decrease the water availability (or relative humidity) in the gaseous phase just above the agar surface. As the ballistospores formed over medium supplemented with more and more mannitol, the rate of drop expansion decreased (FIG. 6). Clearly, the relative humidity of the air surrounding the spore affected the development of the drop, entirely consistent with the idea that condensation of water from the surrounding air results in the expansion of Buller's drop (Webster et al., 1984a, b).

Webster et al. (1984b) were also the first to suggest that drop expansion might be driven by the extrusion of osmotically active ions or molecules from the *punctum lacrymans*. In response to the appearance of a hygroscopic nucleus at the *punctum lacrymans*, water molecules would condense from the atmosphere causing the rapid expansion of Buller's drop on the hilar appendix. Goates and Hoffman (1986) reached exactly the same conclusions independently during a study of ballistospores of the smut *Tilletia foetida*. Two testable predictions arise from this idea. First, Buller's drop should contain osmotically active solutes, and second, the measured concentration of osmolytes should support the measured rate of drop expansion according to an accurate mathematical model. Webster's group tested both ideas to solve the mechanism of ballistospore discharge.

4.2. TEST 1: DOES BULLER'S DROP CONTAIN OSMOLYTES?

The presence of osmolytes in Buller's drop was first demonstrated by Goates and Hoffmann (1986) who found that drops dried on the outer surface of a glass micropipet tip rehydrated when positioned close to an agar surface (where relative humidity is highest). In *Itersonilia* the maximum volume of a Buller's drop is only about 0.5 pL (drop with radius of 5 μm). Nevertheless, Webster et al. (1995) were able to analyze the solute content of drops using microfluorimetry and gas-liquid chromatography. Microfluorimetric analyses were possible by collecting up to four drops at a time with a micropipet. After collection, these minuscule liquid samples were ejected from the micropipet under mineral oil (paraffin) to minimize water loss, and the oil was held in an aluminum ring on a microscope slide. To measure hexose sugars, samples were treated with a cocktail of hexokinase, phosphoglucose isomerase, and glucose-6-phosphate dehydrogenase (method described in detail by Tomos et al., 1994). Hexokinase and phosphoglucose isomerase catalyze the oxidation of glucose and fruc-

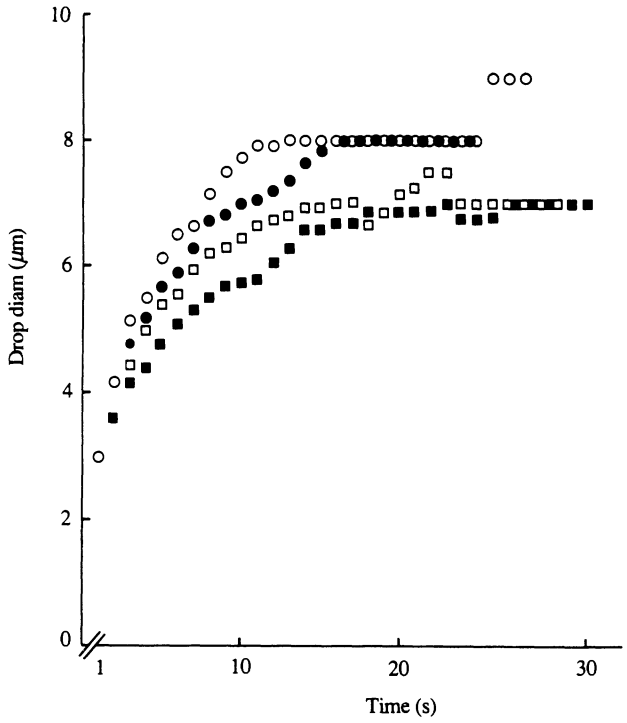

FIG. 6. Effect of relative humidity (controlled by mannitol addition) on rate of expansion of Buller's drop in *Auricularia auricula-judae*. Diam of drop measured at 1 s intervals from time-lapse photographic images. Plots show drop diam on unsupplemented tap water agar (○), tap water agar supplemented with 1% mannitol (●), 2% manitol (□), and 3% mannitol (■). Rate of expansion decreases with addition of increasing concentrations of mannitol that lower the relative humidity above the agar surface. From Webster et al. (1989).

tose to form glucose 6-phosphate, and glucose-6-phosphate dehydrogenase then catalyzes the formation of 6-phosphogluconolactone. The formation of 6-phosphogluconolactone is coupled to the reduction of NADP to NADPH, and because NADPH is autofluorescent, the level of this nucleotide can be measured with a microscope fluorimeter. The mannitol content was measured by adding mannitol dehydrogenase to the reaction mixture, to form fructose, and the mannitol levels were calculated from the consequent increase in NADPH fluorescence resulting from fructose oxidation. Measurements showed that the samples contained femtomole amounts of mannitol and hexoses (29 ± 7 fmol/spore mannitol, 21 ± 9 fmol/spore hexoses, for a total of 50 fmol/spore). The mannitol and hexose content of washings from discharged spores was also determined by microfluorimetry. By combining fluorimetric measurements from expanding drops and spore washings Webster et al. (1995) calculated that the mannitol concentration in Buller's drop would range from 21 to 58 mM, and the hexose content from 14 to 42 mM. Since the volume of Buller's drop increases in the seconds preceding spore discharge, the concentration of mannitol and hexoses will obviously decrease unless the growing drop continues to be supplied with solutes from the spore. Gas-liquid chromatography confirmed the presence of mannitol in washings taken from deposits of ballistospores from 19 basidiomycete species discharged onto glass, and also indicated the presence of glucose in four species.

4.3. TEST 2: RELATIONSHIP BETWEEN COMPOSITION AND EXPANSION OF BULLER'S DROP

Webster et al. (1989) made the first attempt to model drop expansion to test the hypothesis of drop growth by condensation of water vapor. They began with the assumption that Buller's drop contained dissolved solutes (note that this model was published six years before the fluorimetric analyses described in the previous section were reported), reasoning that such solutes would lower the vapor pressure at the drop surface, providing a driving force for mass transfer of water molecules toward the drop. Turner and Webster (1991) improved the model by incorporating the effects of the temperature increase from the latent heat of condensation, and the surface tension of the drop. The effect of the latent heat of condensation is particularly important because any increase in the temperature of Buller's drop will reduce the driving force for mass transfer of water to the drop surface, reducing the rate of drop expansion. Based on this refined treatment, Turner and Webster (1995) later calculated that 56 fmol of an ideal solute would drive the rate of expansion characteristic of *Itersonilia*. The agreement between this figure and the solute content of 50 fmol/spore (mannitol plus hexoses) measured by microfluorimetry is amazing!

4.4. A FLY IN THE OINTMENT, AND POSSIBLE RECONCILIATION

We owe much of our understanding of ballistospore development and structure to the superb electron microscopic studies by David McLaughlin at the University of Minnesota (McLaughlin, 1977; McLaughlin and Beckett, 1987; McLaughlin et al., 1985; Yoon and McLaughlin, 1984, 1986). Buller's drops attached to hilar appendices of *Coprinus cinereus* and *Boletus rubinellus* are preserved in both chemically-fixed and frozen-hydrated preparations of ballistospores (FIGS. 7, 8; McLaughlin et al., 1985). Chemical fixation only preserves the youngest drops, whereas fully-enlarged drops are preserved in frozen-hydrated specimens.

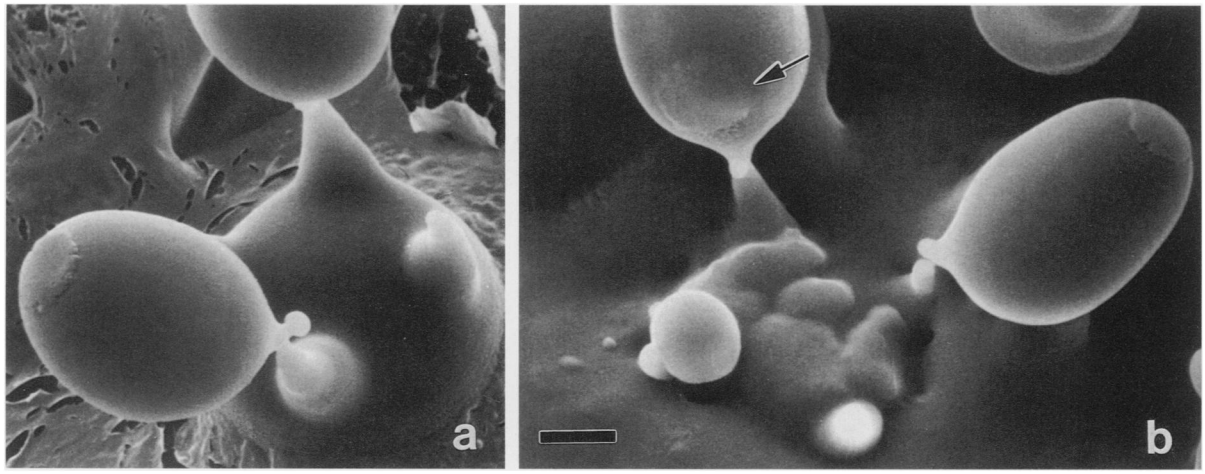

FIG. 7. Scanning electron micrographs of partially frozen-hydrated basidia of *Coprinus cinereus* showing Buller's drops (compare with FIG. 2). a. Small drop projecting from hilar appendix. b. Two basidiospores with small Buller's drops. Fluid on spore surface (arrow) above hilar appendix is preserved on one spore. The roughly spherical structure shown at lower left may be a larger Buller's drop dislodged from an adjacent hilar appendix. Scale bar = 2 μm. From McLaughlin et al. (1985).

But the fact that even the smallest drops are preserved by chemical fixation is very surprising given the evidence presented in previous sections of the liquid nature of the structure, because surface liquid will be dispersed when specimens are immersed in fixatives. An explanation may be found in transmission electron micrographs which show that Buller's drops of *Boletus rubinellus* are delimited by a pellicle or membrane early in their development (FIGS. 8, 9; McLaughlin et al., 1985; Yoon and McLaughlin, 1986). This observation shows that Webster's description of drop structure and development is incomplete, but the disagreement is not as significant as it might at first seem.

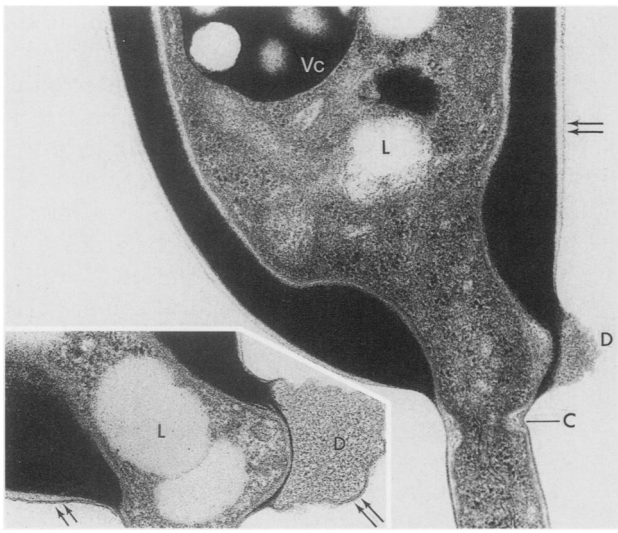

FIG. 8. Transmission electron micrograph of chemically-fixed basidiospores of *Boletus rubinellus*. Main subject shows drop initiated at the hilar appendix; spore fixed in glutaraldehyde followed by osmium tetroxide, × 30 000. Inset shows different spore with drop surrounded by a pellicle preserved by semisimultaneous fixation in glutaraldehyde and osmium tetroxide, × 37 620. Arrows indicate pellicle surrounding spore and drop. C, constriction at tip of sterigma; D, Buller's drop; L, lipid inclusion; Vc, vacuole. From Yoon and McLaughlin (1986).

McLaughlin's electron micrographs show that the structure surrounding young Buller's drops in *B. rubinellus* is identical to the pellicle that surrounds the whole spore. McLaughlin et al. (1985) speculated that the pellicle might be derived from a layer of material that surrounds the entire hymenium at earlier stages of development, which is consistent with the following interpretation. In recent years we have learned that a wide variety of fungal surfaces exposed to air are coated with cysteine-rich polypeptides called hydrophobins (Wessels, 1996; Kershaw and Talbot, 1998). Hydrophobins are secreted proteins that play significant roles in sporulation, fruit body development, and pathogenesis. So it is possible that a raft of these proteins is carried from the sterigmal or spore surface around the expanding Buller's drop. Electron micrographs show that fully-expanded Buller's drops in *B. rubinellus* are no longer enveloped by a pellicle (McLaughlin et al., 1985; Yoon and McLaughlin, 1986) suggesting that the liquid of the expanding drop is exposed some time before discharge. However, these observations do complicate our picture of drop initiation because the presence of a hydrophobic pellicle would surely inhibit drop growth by condensation of water from the surround-

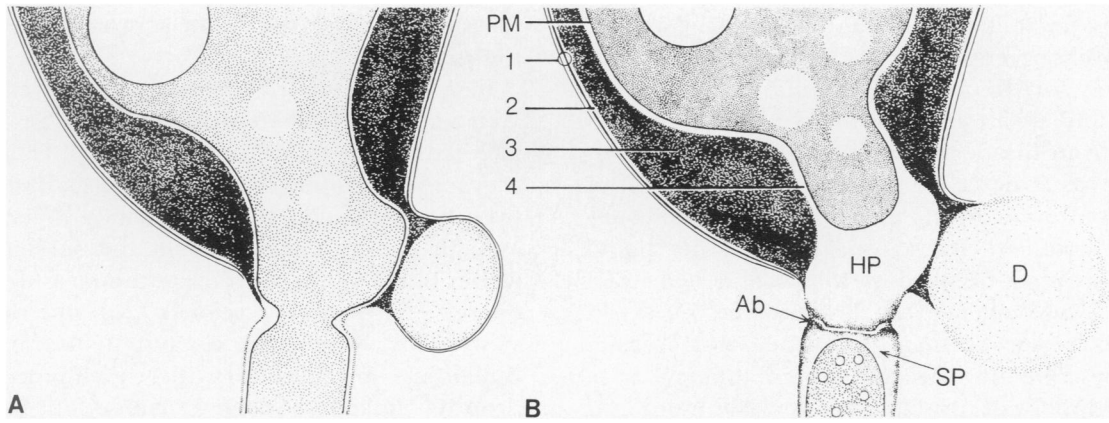

FIG. 9. Diagram of basal region of basidiospore of *Boletus rubinellus* (based on electron micrographs), illustrating the process of drop expansion. A. Drop surrounded by pellicle. B. Expanded drop no longer delimited by pellicle. Ab, abscission layer; D, Buller's drop; HP, hilar plug, and SP, sterigmal plug (plug of wall material); PM, plasma membrane. Numerals 1 through 4 indicate discrete wall layers identified in electron micrographs. From Yoon and McLaughlin (1986).

ing air. It is possible that in the seconds before the pellicle is broken that the water that initiates drop expansion is derived from fluid within the interstices of the spore wall rather than the surounding air. Interestingly, fluid droplets on the surface of sclerotia are also surrounded by a pellicle (Colotelo et al., 1971).

It should be noted that while many of the most important experiments on ballistospore discharge have been performed on *Itersonilia,* we have a much clearer picture of the fine-structure of the thick-walled spores of *Boletus.* Therefore, we do not know whether or not the Buller's drop in *Itersonilia* is surrounded by a pellicle, but in the absence of critical information we hypothesize that the fundamental characteristics of ballistospore discharge are the same in all basidiomycetes.

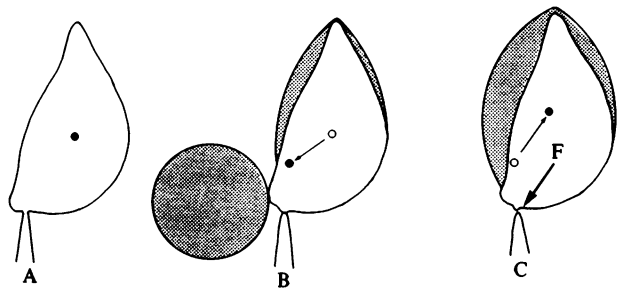

FIG. 10. Webster's mechanism of ballistospore discharge. A. Ballistospore is attached to the tip of the sterigma. The center of mass of the spore is indicated as a closed circle. B. Buller's drop develops at the hilar appendix and liquid accumulates over the larger spore surface (stippled areas). Center of mass is displaced toward hilar appendix. C. Buller's drop fuses with the liquid on the adaxial surface of the spore. The center of mass of the spore is rapidly shifted away from the hilar appendix, and the spore and liquid gain kinetic energy and momentum in the same direction as the moving center of mass, exerting a force (F) on the sterigma. From Webster et al. (1988).

5. WEBSTER'S SOLUTION TO THE DISCHARGE MECHANISM

Webster and collegues first proposed their model of ballistospore discharge in 1984 (Webster et al., 1984a), but a further decade of experiments and calculations (reviewed in Sections 4.1–4.3) was necessary before the power of the theory became apparent. The mechanism hinges on the redistribution of mass when Buller's drop contacts and coalesces with an additional shallow meniscus of liquid on the adjacent surface of the spore (FIG. 10). As Buller's drop expands, the center of mass of the spore moves from the center of the spore toward the hilar appendix and the tip of the sterigma. When coalescence occurs, the center of mass is suddenly shifted away from the sterigma, back toward the physical center of the spore, and the spore and surrounding fluid are projected away from the sterigma along the axis of the changing center of mass.

To analyze the theory in more detail it is necessary to introduce some physical principles to explain why the two volumes of liquid coalesce and how this process imparts momentum (= mass × velocity) to the spore. As the surface area of the growing Buller's drop increases, the surface free energy within the drop resulting from surface tension also rises. When Buller's drop reaches a critical size and makes contact with the liquid on the adjacent spore surface, coalescence is induced by the thermodynamic tendency to minimize surface free energy. Simple calculations show that the dispersion of the liquid in Buller's drop over the larger spore surface achieves

the necessary reduction in the total surface area of liquid (Webster et al., 1984a, 1988). It follows that the energy for the motion of liquid over the spore surface, and the resulting redistribution of mass, is derived from the decrease in surface free energy at the time of coalescence. According to calculations made by Webster et al. (1984a), drop coalescence must be complete within one μs, with the liquid spreading over a distance of 10 μm at a velocity of 8.8 m s^{-1} (coalescence can be observed when discharge fails; see Section 3). Turner and Webster (1991) describe the spore and drop snapping together at the instant of fusion, "like an elastic band". As Buller's drop disperses over the spore surface it loses surface energy and gains kinetic energy and momentum, and we have already seen that the momentum of the liquid drives the redistribution of mass that propels the ballistospore. This view of the mechanism led to the term "surface tension catapult" (Turner and Webster, 1995), because the surface tension of Buller's drop and the adjacent liquid on the spore surface is the source of the free energy for discharge. Some of the surface free energy must also be lost in the form of heat (how much defines the efficiency of the mechanism). But irrespective of heat loss, only a small proportion of the total surface energy present in Buller's drop and the film of liquid on the adjacent spore surface is actually converted into kinetic energy, because the discharged spore is bathed in this liquid after discharge and it is still under tension.

Accumulation of liquid on the spore surface is often visible as a change in the outline of the ballistospore, coincident with the expansion of Buller's drop (FIG. 2). Although sometimes referred to as the adaxial drop, it is probably the most visible part of a liquid film that covers the entire spore surface. Unlike Buller's drop, it is very difficult to measure the volume and rate of expansion of this liquid, but it does appear to expand in the seconds preceding spore discharge. Accumulation of this liquid must also be driven by the presence of mannitol and hexoses since washings from discharged spores (that are soaked in the fused masses of liquid from Buller's and adaxial drops) contain exactly the same solutes as the picoliter samples of Buller's drops (Webster et al., 1995). This raises the possibility that excretion of solutes is not limited to the *punctum lacrymans,* but is spread over a much larger part of the spore surface. Alternatively, solutes may originate at the *punctum lacrymans* and diffuse over the larger spore surface. These details do not affect the essence of the discharge mechanism which can be summarized in the following way: (i) fusion of two liquid drops causes the rapid displacement of the center of mass of the spore; (ii) this exerts a force that is opposed by the sterigma; (iii) the spore is projected away from the sterigma.

In a very limited sense, A. H. R. Buller scooped Webster by more than 70 yr by suggesting that surface tension in Buller's drop could provide the energy for spore discharge (Buller, 1922). Based on this hypothesis, Ingold (1939) calculated that there was enough energy available from the surface tension within Buller's drop to provide the measured kinetic energy (= ½mass × $velocity^2$) of the discharged spore. This is fascinating because neither Ingold nor Buller had any idea how the development of the drop was linked to spore discharge, but simply reasoned from its appearance and disappearance that it must play some essential role.

6. ROLE OF THE STERIGMA

Newton's second law of motion tells us that the force projecting the ballistospore from its sterigma is equal to the product of the mass of the spore (with its associated surface liquid) and its acceleration. According to Webster's model, force comes from the rapid redistribution of mass. In this case, force is exerted against the sterigma so that the spore-drop complex is catapulted away from the sterigma. Consider an analogy. When the charge in the cartridge of a rifle bullet is detonated, the bullet accelerates because the force of the explosion is opposed by both the closed chamber of the rifle and by the person holding the firearm. Therefore, the bullet accelerates out of the barrel, and the person firing the weapon experiences a recoil from the rifle. In ballistospore discharge, any recoil occurs within the sterigma. The sterigma must be capable of withstanding a force of about 10^{-16} N (the force driving discharge), and Turner and Webster (1991) calculate that the sterigma might flex by about 2 μm when subjected to this force. This calculation is based on estimates of the Young's modulus (ratio of stress to strain) of the sterigma wall. However, the turgor pressure within the sterigma should also be considered. After spore discharge, sterigmata remain erect (FIGS. 2–4), but like other types of aerial hyphae they collapse when they dry out (if, for example, a Petri dish is left open in the laboratory). Therefore, it is the pressurized cell sap within the sterigma that is primarily responsible for its form and function; the cell wall alone cannot maintain the erection. Although the pressure within the sterigma has not been measured, it is reasonable to suggest (with reference to turgor pressures measured from hyphae), that its pressure will be of the order of a few tenths of one MPa (Money, 1994). Internal pressures between 0.1 and 0.4 MPa (1 to 4 bars) will generate forces of about 10^{-8} to 10^{-7} N at the tip of the

sterigma where contact is made with the hilum (contact area treated as a circle with radius of 0.25 μm). So it is unlikely that the minuscule force exerted upon the sterigma at the time of spore discharge causes any significant flexing; these considerations negate the type of springboard model developed by Gregory (1979) that depends on a highly flexible sterigma.

7. HOW FAR AND HOW FAST ARE THE SPORES PROPELLED?

Again we return to Buller (1909) who calculated the horizontal discharge velocity (u_0) of spores of *Amanita vaginata* using the following relationship for the motion of a sphere in a viscous medium:

$$u_0 = gl/u_1$$

where

u_0 = horizontal discharge velocity (m s^{-1}),

g = acceleration due to gravity = 9.8 m s^{-2},

l = horizontal distance of discharge

= 2×10^{-4} m, and

u_1 = terminal velocity of sedimentation

= 5×10^{-3} m s^{-1}.

The horizontal distance of discharge and the terminal velocity of spores during free fall were measured from pieces of agaric fruit bodies using a horizontal microscope. The equation predicted that the spores are discharged over a distance of 0.2 mm at an average velocity of 0.39 m s^{-1}, before falling vertically under gravitational acceleration.

Turner and Webster (1991) applied a different equation to estimate ballistospore velocity in *Itersonilia*:

$$u_0 = 6\pi\mu al/m$$

where

u_0 = discharge velocity (m s^{-1}),

μ = viscosity of air = 1.8×10^{-5} N m^{-2} s at 20 C,

a = particle radius = 5×10^{-6} m.

l = horizontal distance of discharge = about 1×10^{-3} m (a much greater range than *Amanita*; Webster et al., 1984),

and

m = mass of the spore plus Buller's adaxial drops = 1.5×10^{-12} kg,

This equation provides an estimate of ballistospore velocity of 1.1 m s^{-1} for *Itersonilia,* somewhat faster than calculated by Buller for *Amanita.* At this speed, the ballistospore will cover a distance of 1 mm (100 times its own length) in 1 ms, and discharge could be captured only with a camera with a shutter speed faster than 50 μs (or with a cinecamera running at 20 000 frames s^{-1}; calculation based on a camera field of view with a diameter of 50 μm). Ballistospore velocities are not particularly fast when we compare them to rifle bullets (muzzle speeds of 3000 m s^{-1}; Vogel, 1988), or rocket-powered vehicles like the Space Shuttle (7800 m s^{-1} during first 8.5 min of flight). However, ballistospores accelerate away from sterigmata at astonishing rates: velocities of one m s^{-1} or more are reached from a standing start in about one μs, and in that process the spore is subjected to thousands of *g*. Astronauts, and indeed any other human beings, cannot withstand accelerations above a few *g,* but this is due to the fact that the stress produced within the body by high accelerations are enormous compared with those experienced by microscopic spores. It is also interesting to note that the spore utilizes only 1% of its total mass (in the form of mannitol and hexoses excreted from the *punctum lacrymans*) to achieve discharge (Turner and Webster, 1995), while the Space Shuttle consumes 50% of its own weight in solid fuel during the first 2 min of flight.

8. EVOLUTION OF THE DISCHARGE MECHANISM

The evolutionary origins of a mechanism of this apparent sophistication is problematic: from what precursor or intermediate form of the mechanism could the discharge process have evolved? Ingold (1990a, b) argued that it is difficult to imagine anything but the complete system conferring any selective advantage to the organism. Corner (1991) disagreed with this view, but the validity of his opinion is diminished by the fact that Corner remained convinced that the turgor pressure within the basidium was responsible for ballistospore discharge, an idea which I hope this review has dispelled. The fossil record is of little use in resolving this question, since spores of the oldest fossil of a gilled mushroom, the 90–94 million-yr-old *Archaeomarasmius* exquisitely preserved in amber, possess hilar appendices identical to those of living fungi, suggesting that they were functional ballistospores (Hibbett et al., 1995, 1997). But there are some observations that may be useful in generating hypotheses about the evolution of the discharge mechanism. At least one aspect of the process seems to be very widespread among fungi, namely drop formation on the surfaces of fungal cells. The surfaces of cultured mycelia are often bejewelled with fluid drops, and the sporangiophores of *Pilobolus* (FIG. 11)

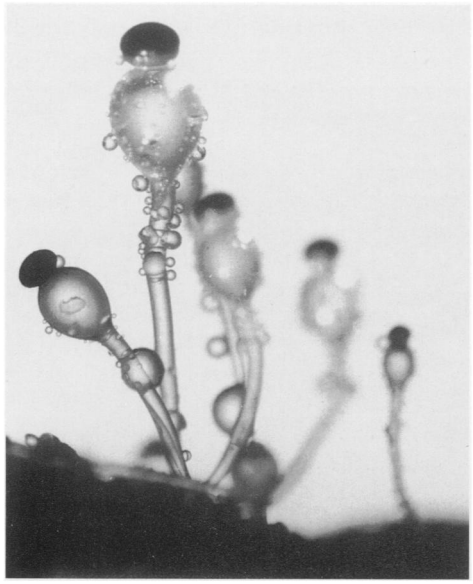

FIG. 11. Fluid droplets on the surface of *Pilobolus* sporangiophores growing on rabbit dung. Photograph courtesy of John Webster.

and *Phycomyces* are decorated in a similar fashion. In many cases, these drops have been shown to contain solutes and enzymes, but the source of the water has not been determined experimentally. In *Phycomyces,* Cosgrove et al. (1987) suggest that water is exuded from the cytoplasm through the plasma membrane, an interpretation shared by Jennings (1991) for aerial hyphae of *Serpula lacrymans.* But Jennings based his explanation on assumptions about water potential gradients between the drops and the surrounding air, and in the absence of real data it is equally likely that drop expansion is driven by condensation rather than extrusion in these fungi. Therefore, it is possible that the formation of Buller's drop may be a specialized case of this widespread fungal phenomenon, and we can imagine (fantasize in the early hours of the morning) steps by which the evolution of the discharge mechanism might have occurred. Incidently, Ingold has been thinking along similar lines, and discusses this in a fascinating essay (Ingold, 1996). Localization of solute leakage or transport to a particular region of the surface of a spore would be one of several refinements necessary for ballistospore discharge by the surface-tension catapult, but the evolution of such a complex mechanism is not as unlikely as one might at first think.

9. THE DISCHARGE MECHANISM AND BASIDIOCARP FORM AND FUNCTION

It was established in Section 4 that Buller's drop grows by the condensation of water vapor. This component of the mechanism is consistent with both laboratory and field observations that ballistospore discharge is stimulated by high relative humidity (see review by Kramer, 1982; Webster et al., 1989). Most studies suggest that evidence of circadian rhythms in ballistospore release from fruit bodies are actually due to underlying changes in humidity rather than any constitutive clock (Kramer, 1982); for example, the diurnal pattern of spore release reported for *Pleurotus ostreatus* is abolished by maintaining constant temperature and humidity (McCraken, 1972). These observations make perfect sense in the light of Webster's theory of ballistospore discharge, and the theory also supports the idea that protection from rain is one reason for the convergence of basidiocarp form on the familiar umbrella- and bracket-shapes (Ingold, 1971). Webster's hypothesis predicts that for successful discharge the mature ballistospore must be surrounded with air saturated with water vapor, but not washed with rain, because liquid water will disperse the solutes secreted from the *punctum lacrymans.* Ingold (1992) has also discussed the importance of the relationship between the spacing between mushroom gills (and the diameter of tubes) and the distance of ballistospore discharge.

The signal role of the condensation of water vapor raises other interesting questions about fruit body form and function. Falck (1904) presented evidence that the brackets of *Polyporus squamosus* generate heat (up to 9.6 C above ambient temperature), and proposed that the resulting thermal gradient between the fruit body and surrounding air produces convection currents. Falck suggested that convection currents would help disperse the spores from the fruit body. Such a mechanism for enhancing dispersal becomes particularly attractive when we consider the minuscule diameter of the tubes of fungi like *Ganoderma applanatum,* and the close packing of gills in *Coprinus atramentarius* (Ingold, 1971). However, Falck's observation of heating is surprizing, because at constant humidity, any heating will inhibit condensation of water onto the *punctum lacrymans* and disrupt discharge (this is an implicit prediction of the equations of Turner and Webster, 1991). The phenomenon of fruit-body heating, along with many other aspects of fruit body function, are ripe for reevaluation using modern instrumentation.

On a final speculative note, the presence of fluid drops enriched with polyols and sugars could provide a useful lure for insects, and insects feeding on Buller's drops could act as vectors for spore dispersal. While many field mycologists must have noted the intense activity of mosquitos and other flies around basidiomycete fruit bodies, there has been little research on these interactions. It is interesting to con-

sider the idea that a mechanism of spore dispersal by insects might evolve (or have already evolved) from the process of ballistospore discharge described in this review.

10. EPILOGUE

Observations and experiments by many mycologists during the last century of research led to the present explanation of the mechanism of ballistospore discharge, but the contributions by the group at the University of Exeter should not be understated. Their work represents a superb combination of talents and knowledge, supported by a clarity of experimental design that has left few questions unanswered. Further experiments to determine the location of osmolytes within the spore, the mechanism by which osmolytes are transferred to the surface of the *punctum,* and analysis of pellicle composition and function would enrich our appreciation of this mechanism. But this is one of those rare cases in experimental mycology in which we can claim to have solved a complex problem. Therefore, this article celebrates a masterful achievement.

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