CHAPTER 5-2

ECOPHYSIOLOGY OF DEVELOPMENT: SPORE GERMINATION

TABLE OF CONTENTS

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Figure 1. Maturing capsules of ***Oligotrichum hercynicum***. Photo by Michael Lüth, with permission.

Spore Maturation

Following meiosis, the spore must mature into the decorated unit that gets dispersed. The spore originally has only one plastid, but this number increases by fission (Mueller 1974). The typical spore wall in bryophytes is composed of three distinct layers: **intine**, **exine** and **perine** (Diego Knop Henriques, Bryonet 28 September 2011). The innermost is the **intine**, basically composed of fibrillar material, mainly pectin, and it plays a pivotal role in spore germination. The **exine** is a thin layer right outside the intine and has **sporopollenin** in its composition. Colpitts *et al*. (2011) demonstrated that spores of ***Physcomitrella patens*** (Figure 2) have the genetic information to produce sporopollenin in their spore walls, a gene that is expressed in the sporophyte generation. Sporopollenin is present in the intine of the spore and confers a great resistance to chemical and environmental factors, as it does in pollen. The **perine** is the outermost layer, also contains sporopollenin, and, in the majority of moss species, is the layer responsible for the spore ornamentation.

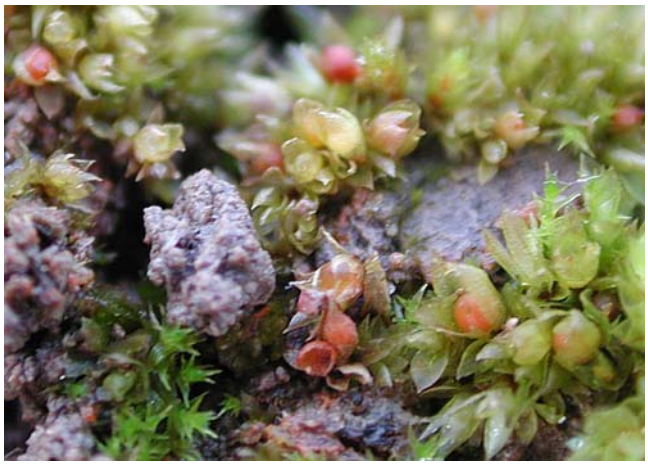

Figure 2. ***Physcomitrella patens*** with capsules. Photo by Michael Lüth, with permission.

Mueller (1974) described the formation of the spore wall in the moss ***Fissidens crispus*** (Figure 3). First the exine forms around the protoplast after meiosis. When the spore is fully enlarged, it is coated by the perine. Then the intine forms. Both the intine and exine originate from within the spore, but the perine comes from material within the capsule, but outside the spore. It is this deposited perine that forms the ornamentation on the spore wall.

Figure 3. ***Fissidens crispus*** capsule that has lost its spores. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Brown and Lemmon (1980) added to this wall description by using ultrastructural analysis of sporogenesis in the moss ***Ditrichum pallidum*** (Figure 4) to describe the internal events of the spore. They found that following meiosis, an extensive system of microtubules is present, underlying the entire distal spore surface where the exine deposition initiates. Following this, the lamellate exine thickens, extending to the proximal surface. The plastid and nucleus migrate to the proximal surface and an elaborate system of microtubules facilitates aperture development. Brown and Lemmon added a fourth layer to the description, a separating layer between the exine and intine. The developed aperture results from a modification of the proximal surface of the spore with a pore that contains fibrillar material surrounded by a thin ring (annulus).

Figure 4. ***Ditrichum pallidum*** with capsules. Photo by Bob Klips, with permission.

Maturation Seasons

Spore maturation tendencies differ with latitudes and climate. They also differ by families, at least in pleurocarpous mosses. Hedenäs (2007) summarized spore maturation seasons for a number of pleurocarpous families:

Winter:
- Brachytheciaceae
- Ctenidiaceae
- Heterocladioideae
- Hylocomiaceae

Summer:
- Plagiotheciaceae
- Amblystegiaceae
- Thuidiaceae

temperate
- Hypnaceae
- Rhytidiaceae

Does Dormancy Exist in Bryophytes?

Heinjo During, on Bryonet 4 March 2016, suggested that we know very little about dormancy in bryophytes. If it exists, it might require a trigger to initiate it. During suggested that low or fluctuating temperatures could be involved. I could also postulate that darkness within the capsule might initiate dormancy before the spores are dispersed. Once dormant, many studies suggest that light and moisture are needed for germination. But During points out that most papers suggest that dormancy of moss spores is rare or absent. Others argue that it may be less rare – lacking investigation.

The behavior of spores in ***Archidium alternifolium*** (Figure 5-Figure 6) suggest that it may experience some sort of dormancy (see Miles & Longton 1992). This species requires an unpredictable but long time to germinate. Could it be that, like some seeds, its spores are immature at the time of shedding and require certain conditions to complete maturation before germinating? This immaturity might be physiological without any morphological indication. Or might there be some inhibitor that must be washed away before it germinates, like some of the desert seeds?

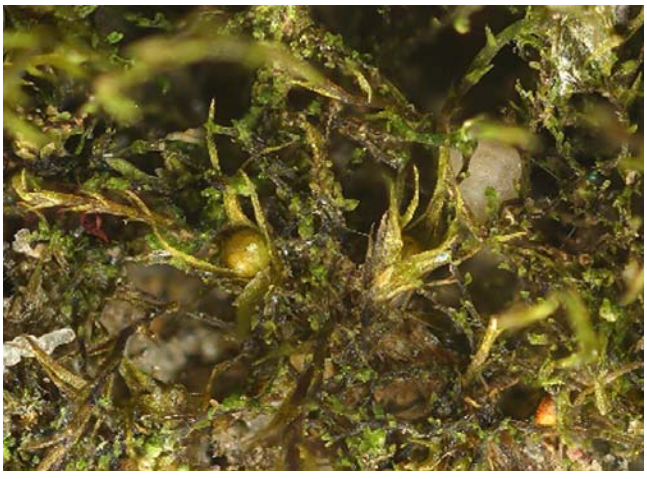

Figure 5. ***Archidium alternifolium*** with capsule. Michael Lüth, with permission.

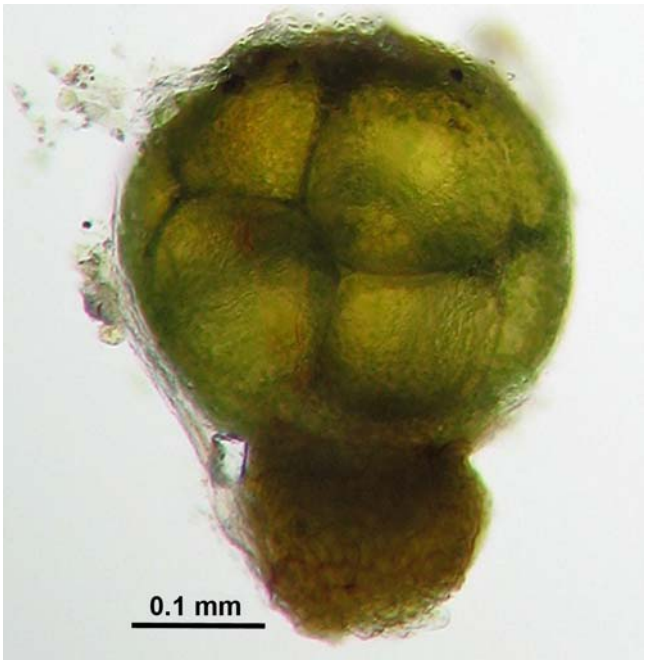

Figure 6. ***Archidium alternifolium*** capsule showing the large spores inside. Photo by Norbert Stapper, with permission.

Some indications of dormancy do exist. McLetchie (1999) examined dormancy/nondormancy cycles in the liverwort ***Sphaerocarpos texanus*** (Figure 39). He found the loss of dormancy increased as the length of time that spores were kept at the various incubation periods from 1-91 weeks. Furthermore, warmer temperatures aided in breaking dormancy. On the other hand, spores held at each of the three thermoperiods germinated best when transferred to 16/10°C and failed to germinate when transferred to the warmer combinations of 35/20 and 30/15°C (see below under Temperature). Thus, warmer temperatures both maintained dormancy and accelerated germination when that temperature dropped. Seasonal changes followed by low temperatures induced these spores to return to a secondary dormancy.

Hock *et al*. (2013) suggested that the spores of ***Phascum cuspidatum*** (Figure 7) in grassland exhibited dormancy. Watson (1983) suggested that chemical inhibition occurs among juvenile members of ***Polytrichum*** *s.l.* (Figure 8).

Figure 7. ***Phascum cuspidatum*** capsules. Photo by Michael Lüth, with permission.

Definition of Spore Germination

Successful germination is prerequisite to establishment in a new location, yet its consideration is lacking in nearly every ecological study. If we are to retain our rare and endangered species, we must understand the germination and establishment requirements that will permit them to become established in our conserved areas.

Bryophyte spores begin their life following meiosis in the capsule (Figure 1). There they wait and develop to maturity before dispersal. Generally, they do not germinate within the capsule.

There is no general agreement on the definition of spore germination. **Swelling** is the result of the uptake of water by the spore; **distension** occurs when the cell wall ruptures and the germ tube is formed. Some authors consider swollen spores as germinated (Bauer & Mohr 1959, Mogensen 1978a). But swelling of the spore is a passive process and therefore it does not fully satisfy a definition of germination. From the physiological standpoint, a spore has germinated when the spore wall has ruptured and when the germ tube has been formed, since these involve active processes. A more precise definition is given by Valanne (1966) who states that the "distension phase is the least ambiguous and most useful practical criterion for spore germination." In some species, among others ***Polytrichum commune*** (Figure 8), there is an intermediate phase between the swelling and the distension in which the germ tube is formed and the spore wall is stretched – the **protrusion phase** (Figure 22) (Karunen 1972).

Figure 8. ***Polytrichum commune*** with capsules. Photo by Kristian Peters, through Creative Commons.

Some species don't wait for environmental conditions become suitable. Rather, they germinate while still in the capsule (D'Rozario & Bera 2006). This is known for ***Marchantia palmata*** as well as a few other liverworts and some mosses. Two forms of germination occur among the bryophytes: **endosporic** and **exosporic**. **Endosporic** development is that development in which the spore cell divides within the cell wall, creating a multicellular structure before a protonemal thread emerges from the spore wall. In these cases, the spore wall stretches as the internal structure expands. This endosporic phase often coincides with **precocious germination**, that is,

development that occurs while the spore is still within the capsule (Nehira 1983). Such a developmental pattern occurs in ***Pellia epiphylla*** (Figure 9-Figure 10) and ***P. neesiana*** (Figure 11) (Bartholomew-Began 1996), distinguishing these taxa from other members of the **Metzgeriales** and from most bryophytes. Such a strategy would be an adaptive device for such taxa as ***Gymnostomum*** (Figure 12; pers. obs.) and others that live in dry habitats where a head start could permit them to reach sufficient size to survive before becoming dry. Nehira (1987) found that the endosporous habit was common among **epiphytic** (tree-dwelling) and **saxicolous** (rock-dwelling) liverworts and mosses. Other taxa, including the mosses ***Andreaea*** (Figure 13), ***Glyphomitrium*** (Figure 14), and ***Pylaisiella*** (Figure 15), and the liverworts ***Cavicularia*** (Figure 16), ***Radula*** (Figure 17), and ***Trichocoleopsis*** (Figure 18), may be endosporous, but do not become multicellular and stretched until after capsule dehiscence (Nehira 1983).

Figure 9. ***Pellia epiphylla***, a liverwort with endosporic development. Photo by David T. Holyoak, with permission.

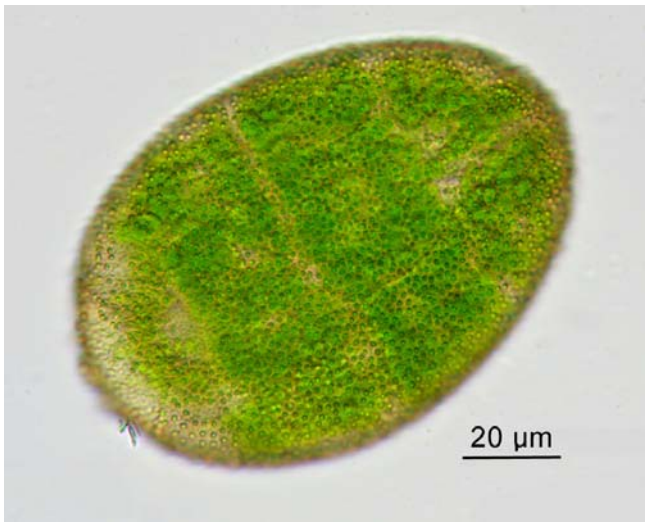

Figure 10. ***Pellia epiphylla*** spore showing endosporous development that occurs within the capsule. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 11. ***Pellia neesiana***, a species with endosporic development. Photo by Jan-Peter Frahm, with permission.

Figure 12. ***Gymnostomum aeruginosum*** with capsule. Photo by Michael Lüth, with permission.

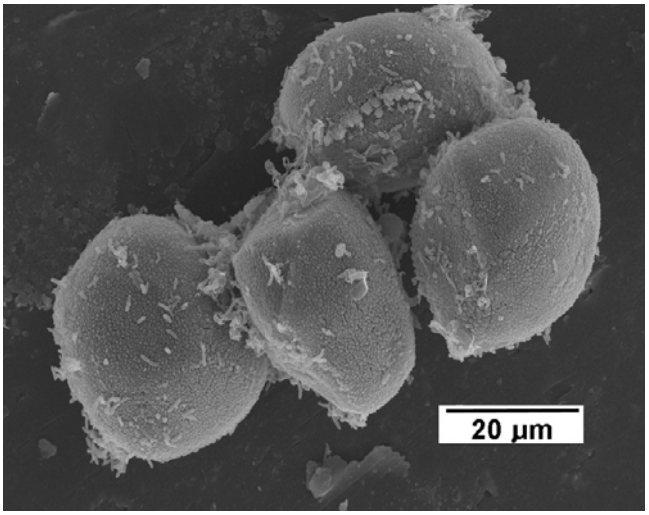

Figure 13. SEM of ***Andreaea rothii*** spores before germination. Photo courtesy of Karen Renzaglia.

Figure 14. ***Glyphomitrium davesii*** with capsules. Photo by Niklas Lönnell, with permission.

Figure 15. ***Pylaisiella polyantha*** with capsules. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 16. ***Cavicularia densa***. Photo by Harum Koh, through Creative Commons.

Exosporic development, occurring in most mosses and liverworts, has its first and only development outside the spore wall (Figure 22), a strategy more appropriate for wetter habitats than those used by species with endosporic development. Many sporeling types are known among the **Bryophyta** (Figure 12-Figure 15), **Marchantiophyta** (Figure 9-Figure 11, Figure 16-Figure 18), and **Anthocerotophyta** (Figure 19-Figure 20); (see Nehira 1983 for illustrations and a review). These are influenced not only genetically, but may also be modified environmentally (Alcalde *et al.* 1996). Even wavelength of light can affect germination patterns, as in ***Anthoceros miyabeanus***, where in red light it is exosporic, but in white light it is endosporic (Wada *et al*. 1984). Could such a difference in wavelength effect precocious development for those receiving mostly red light in the green capsule, but then stimulate exosporic development once the spore has left the capsule and become exposed to white light?

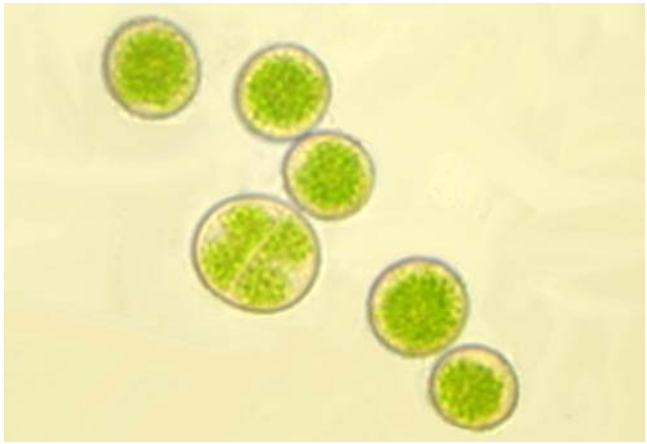

Figure 17. ***Radula recubans*** spores showing one with endosporic development. Photo by Adaíses Simone Maciel da Silva, with permission.

Figure 18. ***Trichocoleopsis sacculata***. Photo by Rui-Liang Zhu, with permission.

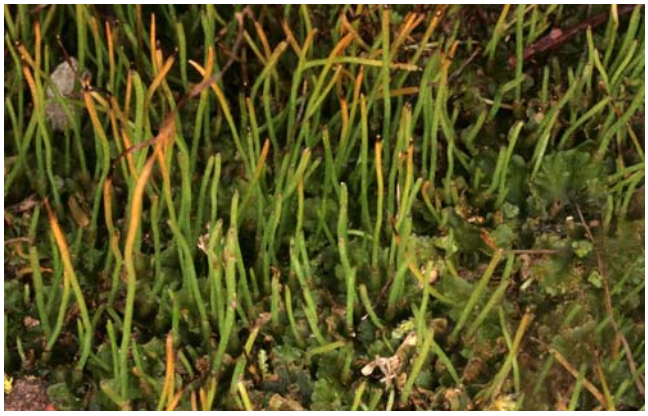

Figure 19. ***Anthoceros fusiformis*** with sporophytes. Photo by Jan-Peter Frahm, with permission.

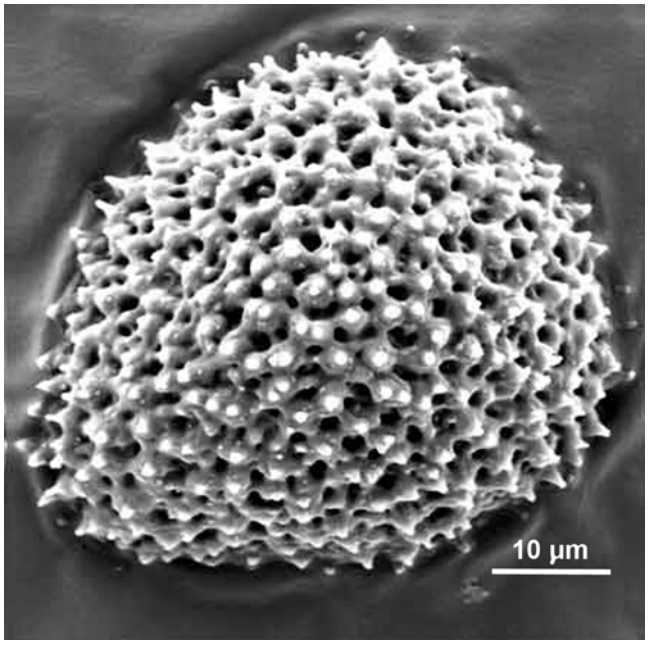

Figure 20. ***Anthoceros fusiformis*** spore distal view, SEM. Photo by Bill Doyle, with permission.

Figure 21. ***Macromitrium sulcatum*** with capsules. Photo by Manju Nair, through Creative Commons.

Figure 22. Exosporic germination of ***Fontinalis squamosa***. Photo by Janice Glime.

Germination Process

The germination process is one in which cell wall thicknesses change (Olesen & Mogensen 1978). For ***Polytrichum commune*** (Figure 8), ***Ceratodon purpureus*** (Figure 112), ***Funaria hygrometrica*** (Figure 23), and ***Macromitrium sulcatum*** (Figure 21), and probably most if not all species, this process involves a thickening of the intine in the region of the aperture, a decrease in the thickness of the exine there, presence of a lamellate structure next to the thin part of the exine, and accumulation of electron-dense material into the thin layer separating the intine and exine. In ***P. commune***, a knob-like structure forms in association with the thickened part of the intine. Water is absorbed through the aperture region, followed by swelling, rupture of the spore wall, protrusion, and recovery followed by spore distension. Spore swelling involves both symmetrical and asymmetrical swelling. The asymmetrical swelling results from swelling of the asymmetrical intine which protrudes beyond the exine and perine of the spore. The symmetrical swelling is not actually a part of the germination, but rather is the result of remoistening.

The swelling stage of spore germination requires water, whereas the distention phase requires light (Bhatla 1994). These requirements exhibit a certain amount of control over the timing of germination and help to prevent the needless loss of resources. These requirements are critical to the maintenance of spores in soil spore banks by preventing germination when the soil is wet but the spore is buried. Additional factors involved in germination are *p*H, calcium ions, and auxins (Bhatla 1994).

Water Needs

Based on studies conducted so far, all bryophytes require water for germination of the spore. The swelling phase of germination seems only to require the physical process of water absorption, resulting in rehydration (Bhatla 1994). Lack of sufficient water may in fact be the means that prevents germination of most spores within the capsule. On the other hand, mechanisms for rapid water uptake to seize upon germination opportunities could be important for some species.

Neidhart (1979) reports that spores of ***Funaria hygrometrica*** (Figure 23) withstand desiccation better in the capsule than when isolated. This seems reasonable since the capsule itself should prevent excessive drying on the interior. However, Neidhart used "young" spores and capsules but did not indicate whether the spores were swollen. Since one problem with desiccation is the leakage of nutrients through damaged membranes upon rewetting, it might be possible that spores in the capsule withstand desiccation better if the capsule can serve as a reservoir of nutrients after rewetting. Little evidence is available to tell us if the moss spores are able to draw upon nutritional sources of the moss as they continue their development in the capsule. Mogensen (1978a, 1981) has indicated that the columella serves as a reservoir of liquid, and that the smallest spores die first as that reservoir dries, permitting the larger spores to continue their growth. A similar series

of abortions of smaller spores occurs in ***Fontinalis squamosa*** (Figure 24; Glime & Knoop 1986). It would be interesting to examine this reservoir to determine if it in fact may be a source of sucrose or other nutritional substances as well.

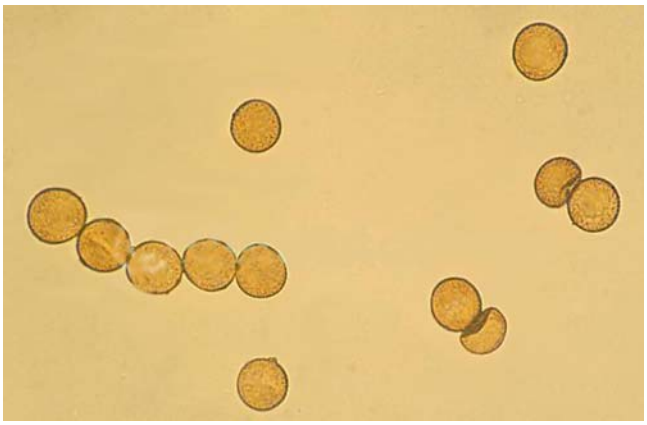

Figure 23. ***Funaria hygrometrica*** spores. Photo by Eugenia Ron and Tom Sobota, with permission.

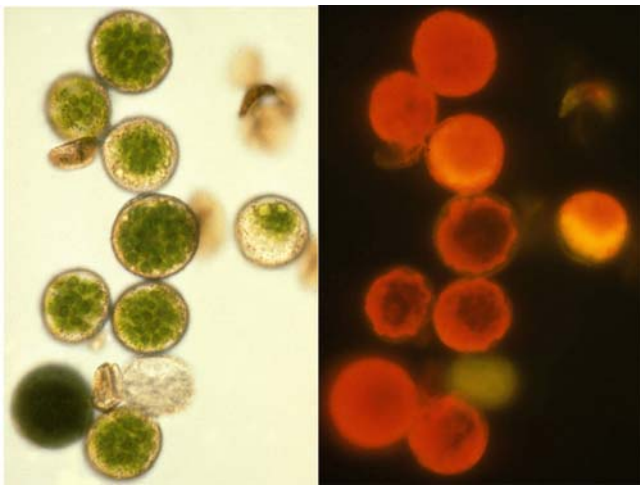

Figure 24. Comparison of chlorophyll in white light (**left**) and chlorophyll fluorescence in UV light (**right**) in large and small spores of ***Fontinalis squamosa***. Note dark gray areas in the photo on right are those areas lacking chlorophyll, and smaller spores tend to disappear in UV light. Photo by Janice Glime.

Once the spores leave the capsule, it is the structure of the spore itself that must serve to prevent desiccation. Bryophyte spores have an innermost layer called the **intine**, consisting of complex polysaccharides such as pectin and callose. The outer wall, the **exine**, is lamellated with plates believed to be **sporopollenin** (phenol-containing polymer known to impart high chemical resistance to exine of pollen), as in higher plants. In some species an opaque zone, termed the **separating layer**, may be seen between the intine and the exine.

Mosses have a further, poorly understood layer, the **perine**, which forms on the outside of the exine in some taxa. The **perine** often forms a pattern characteristic of the species. It is unusual in that it is formed by the sporophyte as an add-on to the outer wall of the spore (Mogensen 1983). It is made from old tissue of the columella and the mother spore wall (Crum 2001). Thus, such a layer is absent in liverworts, which lack a columella. Mogensen (1981) hypothesizes that the perine plays a role in avoiding germination during periods with too little water to survive, such as that provided by dew, and that it is of particular importance as a survival mechanism for the annual shuttle life strategy (living only one or few years in one location). However, we do not know how strongly the perine is bound(?) to the exine layer, or even how. It would be worthwhile to investigate SEM (scanning electron microscope) pictures of the perine of different moss species to see whether certain perine patterns are correlated with habitats liable to desiccation. Furthermore, it is possible that it plays an important role by providing capillary spaces that permit rapid uptake of water during precipitation events, or, as Mogensen suggests, its variation in thickness may provide "significant protection against desiccation of the spore."

Mogensen (1983) hypothesizes that the **exine**, or outer layer of the spore, serves to protect the spore from mechanical damage from the external environment. He bases this hypothesis on its loss of **tensibility** (strength when pulled end-to-end) at maturity, a phenomenon that seems to be common to all bryophytes. On the other hand, a thicker exine might also help to protect the spore from UV, permitting it to take advantage of those long-distance excursions by wind and updrafts.

The **intine** seems also to have a role in rapid uptake of water, through the aperture, facilitating distribution of water to all parts of the cell membrane (Mogensen 1983). The intine might also differ among species in its ability to facilitate this uptake and distribution. Since the thin part of the intine corresponds with the thick part of the exine and vice versa, perhaps water can move from one end of the cell to the other between the layers and thus need only to traverse the thin parts of each layer.

Energy Needs

The presence of water is a necessary prerequisite for the conversion of stored food reserves into glucose for the production of ATP. Any growth following swelling will necessarily require energy, so it is necessary to understand energy storage and requirements for conversion in order to interpret control over successful germination.

The requirement of light for spores to germinate permits them to remain where they have landed until conditions suitable for further development are present. Therefore, energy is not wasted by germination underground, under leaves, or under snow cover. However, even light-requiring moss spores can be induced to germinate by the addition of sucrose in dark conditions, indicating that the need for light is a need for energy. Sood (1975) found that 1.5% sucrose was optimum for germination, but that 4.8% was inhibitory for ***Pogonatum aloides*** (Figure 25), which does not germinate in the dark. Moss spores are green and chloroplast **grana** (stack of chlorophyll packets within the chloroplast where light reactions of photosynthesis take place) are already present before germination. Furthermore, when sufficient starch is present, the spores are able to make chloroplasts in the dark (Bhatla 1994). Therefore, the most obvious hypothesis to explain the need for light is that light causes photosynthesis, which produces glucose and the glucose is converted to sucrose that provides energy and contributes to swelling by causing osmosis.

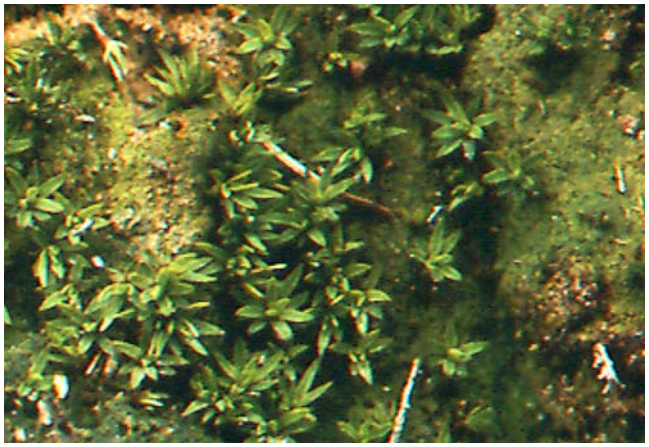

Figure 25. ***Pogonatum aloides*** with persistent protonemata. Photo by Janice Glime.

A second hypothesis is that stored carbohydrates break down into sucrose. We know that α-amylase, the enzyme that breaks starch down to glucose, increases its activity in short days and decreases it in long days in ***Marchantia polymorpha*** (Figure 26). Likewise, **GA_3** (a **gibberellin**) can mimic this photoperiod response (Maravolo 1980). We also know that gibberellin antagonists prevent starch disappearance (*i.e.* prevent metabolism to sugars). Gibberellins, therefore, seem to play a role in starch metabolism leading to germination. However, since gibberellins themselves are not light sensitive, we must look for a photoreceptor. Hahn and Miller (1966) demonstrated that increase in size of chloroplasts in ***Polytrichum commune*** (Figure 8) germinating spores was due to presence of starch. The reaction was red/far-red reversible (*i.e.*, interchanging these two light qualities would reverse the reaction), and only red and white light produced germination and chloroplast replication. Spores of the species would germinate only in light or in darkness + sucrose. The red/far-red reversibility is evidence of **phytochrome** activity, and the coupling of starch degradation with the multiplication of chloroplasts suggests that light is necessary for this starch to sugar conversion, thus supporting the second hypothesis.

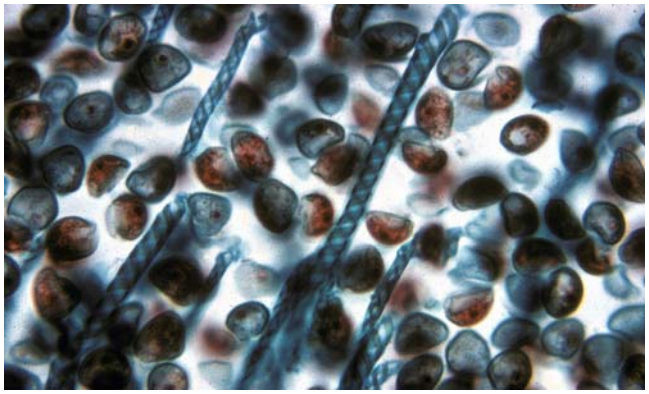

Figure 26. ***Marchantia polymorpha*** spores & elaters (stained) as they would appear in the capsule. Photo from Michigan State University teaching collection, with permission.

The response to short days is likewise consistent with phytochrome activity and would permit the spores to germinate in spring at the beginning of the growing season when most areas in the temperate zone have adequate rain and sufficiently cool temperatures for these C_3 mosses and liverworts to succeed. On the other hand, decreased germination in long days would prevent precocious germination of spring-produced spores that would most likely not succeed in the hotter, drier days of summer.

The decrease in stored carbohydrate (mostly as starch) over time may account for the inability of older spores, especially small ones, to germinate. It would be interesting to correlate stored carbohydrate with spore longevity. Our lack of field data on time of spore germination greatly inhibits our interpretation of the ecological implications of these physiological characteristics.

A third way to obtain sugars is breakdown of fatty acids through the glyoxylic acid cycle. This pathway has been described for germinating seeds, rich in fatty acids. Bryophytes also have fatty acids (Jamieson & Reid 1976; Suire & Asakawa 1981), and these are known in moss spores (Karunen & Liljenberg 1978). They play a role in spore germination of ***Polytrichum commune*** (Figure 8) (Karunen 1972), where, at the end of the protrusion phase, fatty acid degradation gives energy for development of chloroplasts.

It is clear that an energy source is necessary for many (probably all) spores. However, there is no rule that says the method must be the same for all, nor that only one of these could be in effect. Multiple sources of sugars and a variety of options would permit greater success in a wider variety of conditions. Perhaps having multiple possible sources of energy for spore germination is one factor that permits ubiquitous species of bryophytes to be ubiquitous. But what are the relative roles of photosynthesis, glyoxylate cycle, and breakdown of starch in production of sugars and energy during germination of the spore?

In very immature brown spores (lacking chlorophyll) we often see small lipid bodies. Chloroplasts are not yet formed and photosynthesis does not take place. It is reasonable that the first way to obtain sugars in such spores is through breakdown of lipids in the glyoxylate cycle, and lipid catabolism may occur prior to chloroplast formation.

In addition to gibberellins, IAA can have a stimulating effect on germination of spores in light but not in dark (Valanne 1966). How can we explain this? We know light has a stimulating effect on production of sugars. As a result of the change in osmotic potential of the cell, there is uptake of water. IAA makes the cell wall more elastic so that the spore can swell. In the dark there is no sugar production and exogenously supplied IAA has no effect. However, in the same experiment, Valanne noticed a decrease in percent of spores germinated when compared to control cultures with no growth substances. It might be possible that supplied IAA increased the IAA concentration above normal levels. High levels can induce the formation of IAA oxidase, resulting in the catabolism of IAA, and induce the production of ethylene, both of which could explain the lower percent germination of spores in IAA culture media compared with the control medium. This scenario would support hypothesis 1, that light is necessary because photosynthesis is necessary to provide sugars.

IAA probably has its main effect during the swelling of the spore. The inactivation of IAA by IAA oxidase is often correlated with an increase in GA content (Maravolo 1980). We know from tracheophyte studies that GA is sometimes formed in the day and used at night and that it

can cause the same response as a long day in long-day plants (Salisbury & Ross 1978). GA has a stimulating effect on α-amylase, and the resulting breakdown of starch provides material for cell wall formation. GA may thus play a role in the distension phase.

One might propose the following sequence: breakdown of lipid bodies prior to formation of chloroplasts; effect of IAA and photosynthetically derived sugars during the swelling phase; formation of gibberellic acid and breakdown of starch leading to the distension phase. This, however, is the reverse of the process known for tracheophytes. The position of lipid breakdown is the most tenuous, with Karunen's (1972) work showing degradation of fatty acids at the end of the protrusion phase, giving energy for chloroplast development.

It is clear that germination requires **energy**. Three potential pathways could provide that energy: 1) stimulation of **phytochrome** that initiates the **starch** to **sugar** conversion that precedes production of **chlorophyll**, possibly under control of **GA**; 2) conversion of **fatty acids** to sugars, providing energy for production of chlorophyll; 3) photosynthesis of green spores in the light. The requirement for light insures that spores will not germinate under soil or elsewhere where they will never get light. Small spores and older spores have poor germination success, most likely because of diminished energy stores. **IAA** provides the **elasticity** needed, sugar provides **energy** and the **osmotic potential** that brings in water, and **GA** stimulates the **α-amylase** production that precedes **distension**.

Light Needs

Most moss spores have chlorophyll at maturity, and that most likely helps to provide their energy as they germinate, through photosynthesis, as demonstrated in ***Funaria hygrometrica*** (Figure 23) (Krupa 1965).

Light is not required for swelling in most spores (Valanne 1966), but it is for germination. Even in species where swelling (germination) occurs in the dark, some individual spores require light. In ***Ceratodon purpureus*** (Figure 112), starch grains increase at the onset of darkness (Valanne 1971) but disappear from chloroplasts of those that swell in darkness, and the lipid bodies change shape (Valanne 1966). Since these changes do not occur in those species requiring light, it suggests that lack of germination may be due to the inability to mobilize food reserves. We have discussed the ability of **gibberellic acid** to mobilize starch in the presence of light, but what accounts for dark mobilization? Do spores differ in their content of **α-amylase**, with those rich in α-amylase waiting only for sufficient water to carry out their reactions? Is this mechanism purely a random distribution of materials at **sporogenesis** (spore production), or is it a genetically engineered device that conserves resources in some spores while permitting others to germinate early?

The clandestine ***Cryptothallus mirabilis*** (Figure 27), a liverwort that lives **within** a bed of ***Sphagnum***, lacks chlorophyll in the entire plant, including spores (Hill 1969) and has no requirement for light to germinate. It would be helpful to know if it has a ready supply of α-amylase.

Figure 27. ***Cryptothallus mirabilis*** with young sporophytes. Photo by Michael Lüth, with permission.

Although it seems that light intensity is the most important factor in germination of bryophyte spores, Kinugawa and Nakao (1965) found that photoperiod affected the termination of ***Bryum pseudotriquetrum*** (Figure 28). Most spores required more than a 5-hour photoperiod for germination, whereas more than about 12 hours seemed to make little difference, even though only about 75% of the spores were germinating (Figure 29).

Figure 28. ***Bryum pseudotriquetrum*** with capsules. Photo by Michael Lüth, with permission.

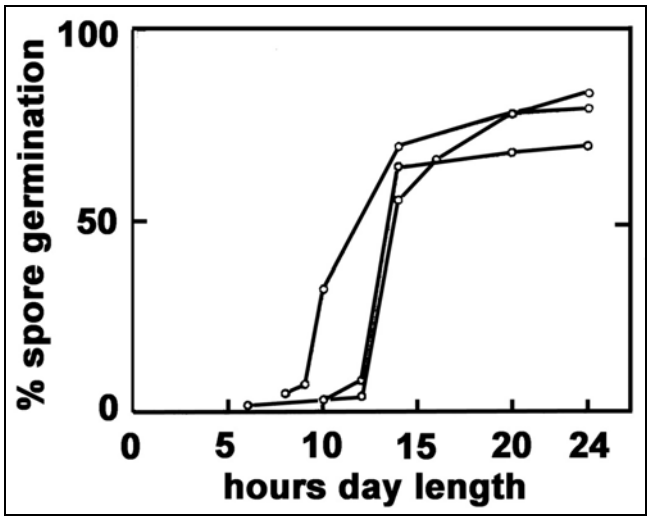

Figure 29. Effect of day length on germination of spores of ***Bryum pseudotriquetrum***. Redrawn from Kinugawa & Nakao 1965.

We know almost nothing about light intensities needed in the field for germination of spores. In the lab, we often add substances that could replace the need for photosynthetic activity. For example, ***Bartramidula bartramioides*** germinated best at 3500-4000 lux of continuous light in the lab, but the addition of a 1% sucrose solution may have had overriding effects to counter the low light and continuous illumination (Chopra & Rahbar 1982).

During (1979) assumes that lack of light and water in the capsule might restrain the germination of spores within the capsule, but it is questionable whether the capsule keeps all the light out. Spores can germinate under very low light intensities, *e.g.*: (1) Spores of ***Schistostega pennata*** (cave moss; Figure 30) germinate in the dark (Nehira 1967). (2) Geissler (1982) found that moss spores germinate under snow, thus under a greater far-red/red light ratio than sunlight (Winchester pers. comm.). (3) Spores of ***Dicranum scoparium*** (Figure 50) and ***Ceratodon purpureus*** (Figure 112) germinate at a light intensity of only 1 lux (Valanne 1966). (4) ***Cryptothallus mirabilis*** (Figure 27), which lives under a thick ***Sphagnum*** layer, is able to germinate in the dark, or under a very low light intensity. These examples show that low light intensity may not be a decisive factor to inhibit the germination of at least some kinds of spores within the capsule, or at least might not be the only factor involved.

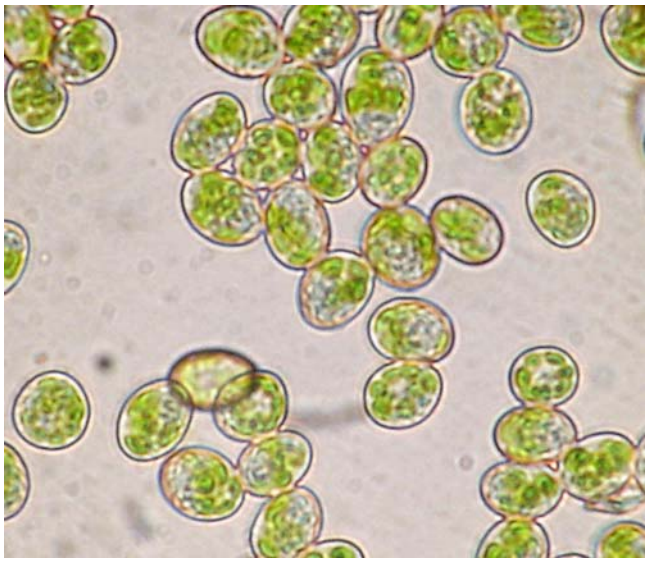

Figure 30. ***Schistostega pennata*** spores, a species where spores germinate in the dark and under snow. Photo by Misha Ignatov, with permission.

On the other hand, the wavelength of light inside the capsule may play a significant role. The capsule wall changes its color during maturation and the color of the capsule at the time of spore maturation could have an effect on the germination of spores. We know that spores of different species germinate under different wavelengths (Valanne 1966). For example, ***Funaria hygrometrica*** spores (Figure 23) will germinate at long wavelengths (580-700 nm) at low light intensities, but require high intensities at short wavelengths (362-500 nm) (Krupa 1967). Since short wavelengths are likely to be filtered out most easily, this could be an effective inhibitory mechanism. Valanne (1966) reported that far red and blue light inhibit swelling of ***F. hygrometrica*** spores and that blue-green light reduces distention in ***Funaria*** and ***Ceratodon*** (Figure 112) to lower than that in the dark. On the other hand, she found that ***Ceratodon*** and ***Dicranum*** (Figure 50) are indifferent to far red light. Bauer and Mohr (1959) showed that the ratio of red to far-red light is important in the germination of ***Funaria***.

In a later study on ***Ceratodon purpureus*** (Figure 112), Valanne (1971) found that illumination with blue light resulted in sporelings with chloroplasts that were richer in starch, had denser stroma, and had more mitochondria than those in red light. The sporelings in red light made more effective use of reserve lipids.

Bauer and Mohr (1959) found that the initiation of germination in ***Funaria*** (Figure 23) depends on phytochrome. The involvement of phytochrome could explain why Krupa (1967) found retarded germination in green light, but after 18 hours at 680 nm (optimum wavelength), followed by 24 hours at 544 nm (green), germination was greater than when 680 nm illumination was followed by dark. In working with ***Octoblepharum albidum*** (Figure 31) spores, Egunyomi (1979) also found that wavelength was important in the onset of germination. Red, cyan, green, mimcro-7, and orange light resulted in germination, but blue, mercury green, deep yellow, and deep red inhibited it. White light resulted in germination and reversed the effects of the inhibitory light, except for the inhibition by mercury green. It might be worthwhile to follow the germination capabilities of the spores of different species during ripening of the capsule, and to relate the spore maturation stages with the changes of color of the capsule. Such color changes in the capsule might be important in preparing the spores for germination at their maturity while preventing it if they are dispersed while still immature.

Figure 31. The epiphytic moss ***Octoblepharum albidum*** with capsules. Photo by Janice Glime.

Not only does the capsule wall change color during the maturation process, but ambient light will change considerably between early spring and summer. As the snow melts and the trees still lack leaves, white light is able to reach the ground. But in a few weeks to months, depending on the latitude, canopy leaves filter out the red light and transmit light high in green and far-red (Bjorkman 1981). These light quality changes could likewise serve as signals to spore germination, and, in combination with

capsule color, could be effective inhibitors for mature spores still inside the capsule.

In some species, such as ***Mnium hornum*** (Figure 32), instead of depending directly on its environment, the spore has an endogenous development cycle that results in the immediate germination of the spore (Newton 1972a, b). Nevertheless, although the germination is independent of both light and temperature, light is still important in maintaining the internal clock; a slight delay of the development caused by short days helps to maintain an annual rhythm in spore maturation (*i.e.*, it resets the clock) and subsequent germination. It is possible that temperature plays a similar role.

Figure 32. ***Mnium hornum*** with capsules. Photo by Jan-Peter Frahm, with permission.

Whereas most bryophytes require light to germinate, a few that live in very low light do not and others require as little as 1 lux. In culture, sugar can substitute for the presence of light and its presence may explain the germination of some species in the dark in nature. Furthermore, the presence of α-amylase could permit spores to convert stored starch to sugar for germination without light. The wave length of light seems to be important for some mosses and could safeguard spores against germinating in the wrong habitat. There are insufficient studies on requirements for spore germination to draw any generalizations about light requirements and habitat, but we can hypothesize that most sun-loving species are more likely to require red light than those that grow in the forest and other low-light habitats. Nevertheless, as mentioned above, ***Ceratodon purpureus***, often found in high light situations, can germinate at only 1 lux. Clearly something more than light intensity and photosynthesis is involved.

Environmental Control over Germination

The three requirements already named – water, energy, and light – obviously will exercise primary control over the germination of spores. However, specific requirements of individual species will further narrow the window of germination. These controls can include *p*H, nutrients, temperature, photoperiod, and exogenous substances, all interacting with internal substances that respond to these environmental cues.

Delay until the right weather (temperature, moisture) occurs is easily perpetuated genetically, but what selects for genes to prevent germination on the wrong substrate? Unless the spore can be re-dispersed, there is no selective advantage that would favor inhibition of germination. Yet there are species where the nature of the substrate does control germination and further development. For example, calcium enhances germination success in the **calciphile** (calcium loving) ***Orthotrichum cupulatum*** (Figure 33), but germination of ***Dicranella cerviculata*** (Figure 34) is depressed by calcium (Vaarama & Tarén 1963). In ***Stereophyllum radiculosum*** (Figure 35), control cultures and those at 22 ppm Ca produced one protonema per spore, whereas those at 50-150 ppm each produced two (Olarinmoye *et al*. 1981). When the leafy liverwort ***Cheilolejeunea clypeata*** was grown on a Ca-free medium, the spores became distended, but the protonema failed to develop during the next five months of culture, whereas in the normal medium young plants had developed (Geldreich 1948).

Are these alternatives in protonemal production adaptive, suggesting that more calcium should be able to support more gametophores? A species loses nothing by germinating in an unsuitable habitat, as opposed to no germination at all. Yet it seems that many spores hang on tenaciously to life for years, awaiting the right set of conditions for germination. And sometimes those needed changes may actually occur.

Figure 33. ***Orthotrichum cupulatum*** capsule that has expelled its spores. Photo by Vita Plasek, with permission.

Figure 34. ***Dicranella cerviculata*** with capsules. Photo by David T. Holyoak, with permission.

Figure 35. ***Stereophyllum radiculosum***. Photo by Scott Zona, with permission.

*p*H

Apinis (1939) contended that most moss spores are almost indifferent to *p*H range. The spores germinate in a wide *p*H range, the protonema range is more restricted, and the *p*H range of the leafy plant in culture corresponds closely to its range in nature. Philippi (1969), on the other hand, found that species from acid or raw humus reacted uniformly, preferring acid, whereas species from wood had a strong divergence of *p*H range. Armentano and Caponetti (1972) felt that *p*H may be the factor that limits the habitat for ***Funaria hygrometrica*** (Figure 23) and ***Tetraplodon mnioides*** (Figure 36), both of which germinate better at a basic *p*H. Vishvakarma and Kaul (1988) found that in culture two liverworts, ***Plagiochasma appendiculatum*** (Figure 37) and ***Reboulia hemisphaerica*** (Figure 38), had an optimum *p*H for germination and thallus growth of 6.0.

Figure 36. Capsules of the dung moss ***Tetraplodon mnioides***. Photo by Zen Iwatsuki, with permission.

But how does *p*H affect spore germination? Does each species have a spore wall requiring a characteristic *p*H, such as that found on tree bark? What is the effect of *p*H on the cation exchange between spores and the substrate? A change in the *p*H can affect enzymatic activities, but it can also affect the solubility and release of certain ions in the substrate and cause, indirectly, a toxic effect. Could it be that *p*H is simply an indicator of needed ions that are associated with the higher or lower *p*H? Vishvakarma and coworkers (1987) found that calcium enhanced spore germination in ***Plagiochasma*** (Figure 37) and magnesium did likewise in ***Reboulia*** (Figure 38); both of these ions are generally associated with high *p*H. Furthermore, as we have seen above, calcium is involved in germination of some species, and its transport may be affected by *p*H.

Figure 37. ***Plagiochasma appendiculatum***. Photo by Michael Lüth, with permission.

Figure 38. ***Reboulia hemisphaerica*** with archegoniophores. Photo by Gideon Pisanty, through Creative Commons.

Nutrients

Although only water and light are generally considered necessary for germination, Arnaudow (1925) was unable to get spores of ***Dicranum scoparium*** (Figure 50) to germinate in water for four weeks, but when particles of earth were added to the water, they germinated in two days.

The cosmopolitan ***Funaria hygrometrica*** (Figure 23) seems to have some precise requirement that is elusive. Its

germination occurs over a wide range of temperature, light intensity, and chemical conditions. According to Hoffman (1966), the soils where it grows have no consistently high or low nutrients and *p*H is neither high nor low. Yet, Hoffman's efforts to grow the moss on soils with various nutrient conditions failed, but soil from burned areas supported growth. In experiments with heated soils, Hoffman found that it grows well on C horizon soils (inorganic parent rock material) heated to 200-300^{O}C, but grows poorly or not at all if the soil has been heated to over 300^{O}C. However, if N and P are added to soils heated to 600^{O}C, it grows well. This suggests that loss of N and P at high temperatures account for its inability to grow. On the other hand, Southorn (1977) relates the presence of ***Funaria hygrometrica*** to the change of source of N and P in the soil. He found that ammonia-N inhibits germination, and that replacement of ***Funaria hygrometrica*** by other bryophytes was correlated with a decrease of phosphate-P. The decrease in abundance may also partly be a result of changing nutrient concentration due to leaching by rain water. Yet Chevallier (1975) demonstrated the requirement of manganese as a **micronutrient** (those required in small quantities) for germination. The restriction of ***F. hygrometrica*** to relatively open areas is consistent with its requirement for light for germination.

But what do other bryophytes require? Most bryophytes have been grown from tissue cultures (see Sargent 1988) using one of several standard media. No comprehensive study in the lab or the field has provided any information on the nutrient requirements, if any, for germination success. Most likely the requirements are few, if any, until after germination and the protonema requires them for growth.

Temperature

One might conjecture that temperature could control when and where species germinate and thus limit distribution. For example, Longton and Greene (1969) found that germination rate steadily increased within a temperature range between 5º and 20ºC in ***Pleurozium schreberi*** (Figure 98), a normal temperature range for spring and autumn. One advantage to this ability to germinate over a wide range of temperatures, with an optimum adjusted to the climate, is that it would permit multiple chances to take advantage of changeable weather in a given season without forfeiting an entire year's crop of spores due to an inopportune germination time. Certainly such strategies exist, as in this ***Pleurozium*** example.

In ***Sphaerocarpos texanus*** (Figure 39), as discussed above, loss of spore dormancy increases as length of time at a suitable temperature increases (McLetchie 1999). Spores kept at 35/20ºC lost dormancy faster than those at 30/15ºC or 25/15ºC. However, the best germination occurred when these spores were subsequently placed at 16/10ºC (typical temperate spring or fall temperatures) and it failed at 35/20ºC and 30/15ºC (late spring and summer temperatures).

At first, McLetchie and Johnson (1997) found that the size of the ***Sphaerocarpos texanus*** (Figure 39) spore tetrad affected the male:female ratio; spores were normally dispersed in tetrads of 2 males and 2 females. However, if the tetrad was less than 90 µm, the sex ratio was female biased. Then McLetchie (2001) found that spores of ***Sphaerocarpos texanus*** behave like eggs of alligators, wherein gender is determined by temperature of the eggs! In this dioicous liverwort the sex ratio is affected by the temperature at which the spore loses its dormancy! At 25/15°C, the population became female biased, whereas at higher temperatures (35/20, 30/15°C) it was not, suggesting a differential survivorship at the spore stage.

The development of physiological races for germination temperature optima in different localities is probably a widespread phenomenon. Dietert (1977) tested ***Funaria hygrometrica*** (Figure 23) and ***Weissia controversa*** spores (see Figure 40) and found optimum temperatures that differed among populations of one species. Populations from colder habitats showed lower germination optima than populations from warmer habitats, thus suggesting that survival of the sporeling did not require the greater temperature. At first, this seems intuitively to be backwards. This temperature relationship is the reverse of McNaughton's (1966) results for *Typha* (cattail) seeds, where a higher temperature requirement for germination of northern seeds protected the seedlings from late freezing that was not a problem for southern populations. On the other hand, this system of cold-adapted species germinating at a lower temperature than those from warm areas provides a longer growing season for individuals in colder climates than would be possible if they had a higher temperature optimum. Since bryophytes are less susceptible to damage by cold and its accompanying desiccation than most tracheophytes, germination early in the season may not be a problem.

Figure 39. The thallose liverwort ***Sphaerocarpos texanus***. Photo by Paul Davison, University of North Alabama, with permission.

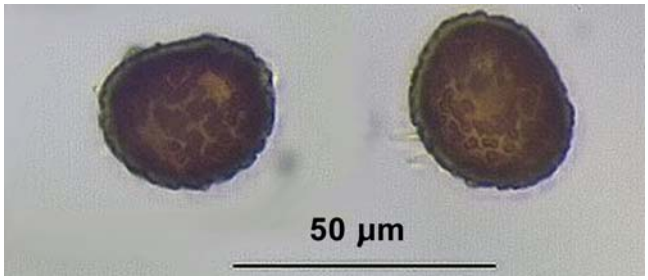

Figure 40. ***Weissia longifolia*** spores, a species that differs among populations in optimum germination temperatures. Photo by Kristian Peters, with permission.

The lack of need for warmer temperatures for sporeling survival is supported by Dietert's later work (1980) that showed optimum germination temperatures for

Funaria hygrometrica (Figure 23) of 30°C, protonema growth at 25°C, and a requirement for cooler temperatures for gametangial formation. In this case, requirements seem to agree with McNaughton's (1966) conclusions that a high germination temperature is necessary to protect the organism from late freezing conditions, but once germination has occurred, sufficiently warm temperatures are assured so there is no selection pressure for the higher temperature optimum. In other words, there is a strong selection pressure against those individuals that germinate at lower temperatures and then experience sub-zero temperatures, but once the temperature has reached 30°C, it is not likely to be sub-zero again, thus permitting those individuals to survive; there is apparently no selection pressure for high or low temperature for development in this case, unless this positions the moss to germinate in the fall and develop over winter.

One problem for spores that germinate and must overwinter as protonemata is desiccation. Frost and ice crystals are hygroscopic and draw the water from the delicate filaments. But if water is available, at least some species can overwinter safely, as can be seen for ***Dicranella heteromalla*** that live through winter in acid mine water (Figure 41).

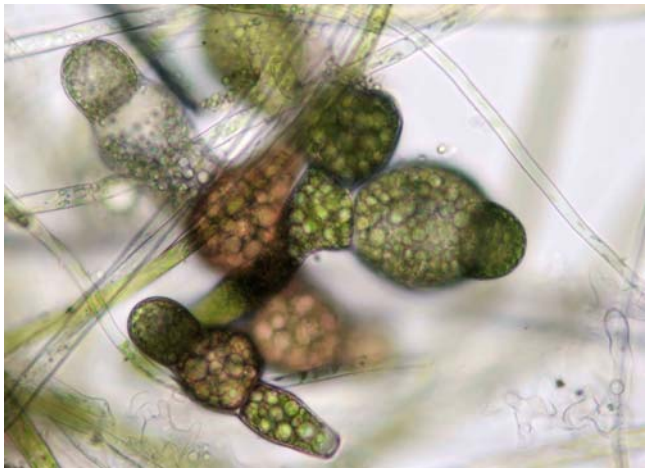

Figure 41. ***Dicranella heteromalla*** protonemata that survived winter in an acid mine lake. Photo by Jan Fott, with permission.

Chopra and Sood (1973) experimented with vernalization and temperature in the thallose liverwort ***Riccia crystallina*** (Figure 42). After 3-4 months only a few spores germinated, and those were in only 4-5% of the cultures. However, a cold treatment of 8-15°C not only increased the percentage of germination but also shortened the dormancy period to 15 days.

In summary, a requirement for a minimum temperature would prevent early germination and the increasing number of spores germinating as the temperature rises protects the population against loss of all germlings at one time in a bad weather event. Populations from colder climates may adapt by having a lower requirement for germination, thus providing them with a longer growing season. In some species, the temperature at which the spores lose their dormancy affects the gender and thus the sex ratio of the colony.

Figure 42. ***Riccia crystallina***, a species that requires a cold period to germinate. Photo by David T. Holyoak, with permission.

Vernalization

We must distinguish between the ability of spores to withstand low temperatures and the necessity for chilling (**vernalization**) for germination. For example, Van Zanten (1976, 1978a, b) froze spores in order to study **freezing tolerance** to demonstrate the possible long-range dispersal of mosses. Some spores kept their ability to germinate after 36 months of freezing.

But withstanding freezing is quite different from the need for cold temperatures for germination. Geissler (1982) illustrated the possible necessity for freezing in some taxa, mentioning that some bryophytes have a hibernation period of two winters, most likely requiring cold, but perhaps merely exhibiting immature spores, as found in seeds of some flowering plants. In ***Orthotrichum anomalum*** (Figure 45) and ***Leucodon sciuroides*** (Figure 43), freezing is favorable for the germination of the spores (During 1979), although it may not be a necessity, whereas ***Splachnum vasculosum*** (Figure 44) does require freezing (-5°C) (During 1979). However, survivorship is greater if spores are frozen in the capsule than if they are fully hydrated (in distilled water). It is likely that water activates the spores before freezing is accomplished and then freezes them in an active rather than a dormant state.

Figure 43. ***Leucodon sciuroides*** with capsules. Photo by Michael Lüth, with permission.

Figure 44. ***Splachnum vasculosum*** with capsules. Photo by Dick Haaksma, with permission.

Membrane damage can occur during freezing of an active cell, causing leakage of necessary nutrients, and a recently activated cell is more likely to have used up the energy resources for repair of membrane damage caused by desiccation (Bewley 1979). Furthermore, leaching of nutrients from a cell with a damaged membrane would be greater in distilled water than in almost any natural medium. This short period of hydration before freezing could leave insufficient nutrients and energy for repair when the cell is reactivated after freezing, and energy could, therefore, be insufficient for normal germination processes.

The achlorophyllous ***Cryptothallus mirabilis*** (Figure 27) actually germinates sooner if exposed to temperatures of -18ºC (Benson-Evans & Hughes 1960 in Schuster 1966). This is perhaps not surprising since it grows among ***Sphagnum*** species, thus being more frequent in northern habitats.

Cold, but not freezing, temperatures could be important for some species to facilitate the conversion of starch to sucrose. Glier and Caruso (1974) found that the activity of starch degradative enzymes of cold-requiring plants increased after a long exposure at 4^{o}C. It is thus possible that cold-requiring bryophyte species use this exposure to metabolize their starch. Species that do freeze and survive could also be cold-requiring, passing through the cold, but above-freezing, temperatures as the temperature warms in spring.

Such aquatic species as ***Fontinalis*** (Figure 22, Figure 24) might require other inhibitory mechanisms to block conversion and subsequent germination in winter since they will seldom experience temperatures below 1^{o}C in the water, or perhaps they are adapted to winter germination, which would coincide with capsule maturation and dispersal.

Germination Inhibitors

Under favorable conditions, most dispersed spores germinate fairly rapidly. Spores of ***Campylopus*** (Figure 46), ***Microdus*** (Figure 47), and ***Hymenostylium*** (Figure 48) germinate in 2, 3, and 4 days respectively (Mehta 1988). ***Funaria hygrometrica*** (Figure 23) spores germinate in 3-5 days. Although some spores have specific temperature requirements, most spores germinate when shed, provided they have suitable light and water, suggesting that they lack dormancy in the form of germination inhibitors and must depend on the sporophyte to permit major dispersal only at a suitable time. Van Zanten (1976, 1978a, b) has demonstrated long-term survivorship for spores of a number of species, suggesting that dryness effectively maintains dormancy. Others survive burial in soil, where darkness maintains dormancy.

Figure 45. ***Orthotrichum anomalum*** with capsules and surrounded by snow, evidence of its benefit for spore germination. Photo by Michael Lüth, with permission.

Figure 46. ***Campylopus flexuosus*** with capsules. Photo by Dick Haaksma, with permission.

Figure 47. ***Microdus brasiliensis***, in a genus with rapid spore germination in the presence of water. Photo by Jan-Peter Frahm, with permission.

Figure 48. ***Hymenostylium recurvirostrum*** with capsules, a genus with rapid spore germination. Photo by Paul Wilson, with permission.

Nevertheless, some spores are shed under what would seem to be suitable germination conditions. What makes them wait? Why don't spores simply germinate on leaves of their parents where most of them land? Certainly avoidance of such a tactic is desirable because they would deprive the parent plant of light, but what is it that prevents such an occurrence? It seems that at least some leafy mosses [*e.g.* ***Syntrichia*** (Figure 49) & ***Dicranum*** (Figure 50)] can provide a diffusible substance, not yet named or characterized, that inhibits the germination of the spores (Mishler & Newton 1988). Such inhibition has been known for a long time in ***Marchantia polymorpha*** (Figure 26), where the gemmae remain dormant on the parent, but begin growing immediately when dispersed from that parent onto a suitable substrate. In fact, it appears that mature plants may inhibit successful germination of both spores and asexual structures in at least some mosses (Newton & Mishler 1996).

Figure 49. ***Syntrichia ruralis*** with capsules & water drops. This genus inhibits germination of its own spores. Photo by Peggy Edwards, with permission.

For desert mosses, brief periods of moisture could cause germination, but subsequent drying would be lethal. Therefore, it would be beneficial for spores to have an inhibitor that prevented germination until sufficient water was present. In some desert seeds, an inhibitor is leached out of the seed by rain water (Fitter & Hay 1981). When rain continues, the concentration of the inhibitor in the seeds decreases below a critical level and germination occurs. When rain stops before this critical level is reached, the inhibitor is resynthesized and germination is postponed until a later rain period.

The same scenario might apply to mosses. We know that mosses can contain high concentrations of phenolic compounds (often serving as inhibitors), especially in some of the capsules that house the spores. These compounds, known to prevent germination in seeds, are likely mechanisms for preventing germination of spores within the capsule. This mechanism may also be important for inhibiting germination of spores that fall onto humic substrata or older moss parts where phenolic compounds are present. Some of the compounds could travel with the spores as they disperse, perhaps inhibiting some individuals more than others, and thus spreading the water requirements and period of dormancy over a wider range that might take advantage of unpredictable conditions.

ABA and ethylene are both known inhibitors of seed germination and could serve as well to inhibit bryophyte germination, with lunularic acid as a possible inhibitor in liverworts. Ethylene could be an effective inhibitor of spores buried in soil, building up in the small spaces there, but is a spore large enough to produce sufficient quantities on a predictable scale to inhibit germination? We don't know if this ever occurs, or even if these substances are present in bryophyte spores. These ideas are conjecture since experimental studies on the effects of either internal or external inhibitors on moss spores are lacking.

Hormonal Regulation

Like phenolic compounds, hormones may intervene in germination of spores. Shukla and Kaul (1991) found that low concentrations of five kinds of auxins, ascorbic acid, benzoic acid, and gibberellic acid all stimulated germination in the liverwort ***Plagiochasma appendiculatum*** (Figure 37), but at concentrations greater than 5 ppm, growth was inhibited. High concentrations could accumulate within the capsule, diminishing after operculum dehiscence. Could hormones from decomposing leaf litter possibly inhibit spore germination? Or could it be that newly dispersed spores have high concentrations that get leached from them by water?

Experiments by Arnaudow (1925) suggest that the gametophyte could exercise control over the germination of spores within the capsule. When embryos of ***Dicranum scoparium*** (Figure 50) were transplanted to ***Atrichum undulatum*** (Figure 53) archegonia (and that was without the help of a computer to guide his hands!), normal development ensued, producing larger capsules than in controls, but remarkably many ***D. scoparium*** spores germinated in the capsules of transplanted sporophytes, producing 3-4-celled protonemata.

Such a phenomenon of germination within the capsule is rare in mosses, occurring for example in ***Dicnemon*** (Figure 51) and ***Eucamptodon*** (Figure 52) (Goebel 1930). Arnaudow found no germinated spores in ***Dicranum scoparium*** (Figure 50) or ***Atrichum undulatum*** (Figure 53) controls, and suggested that nutrition could account for the difference. Could it be absence of an appropriate inhibitor? Or possibly a hormonal stimulant (Table 1)?

Figure 50. ***Dicranum scoparium***, a moss used by Arnaudow (1925) for embryo transplant studies. Photo by Michael Lüth, with permission.

Figure 51. ***Dicnemon calycinum*** with capsules. This is a genus in which spores germinate within the capsule. Photo by Zen Iwatsuki, with permission.

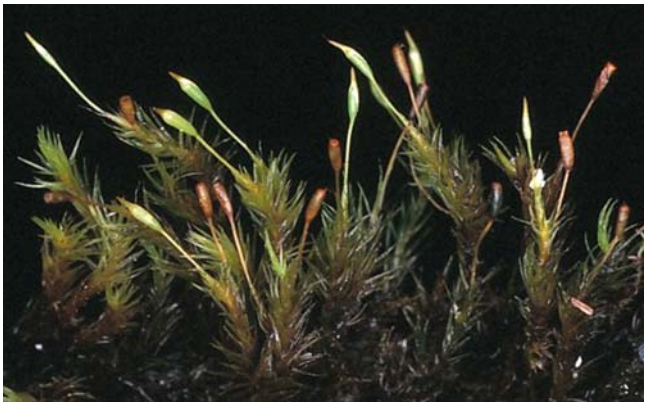

Figure 52. ***Eucamptodon perichaetiale*** with capsules. In this genus, spores germinate within the capsule. Photo by Jan-Peter Frahm, with permission.

There is reason to suppose that gibberellin might be involved; GA stimulates breakdown of starch and causes subsequent swelling of spores. If this is true, under natural conditions one should expect swollen spores in ***Atrichum undulatum*** (Figure 53) and not in ***Dicranum scoparium*** (Figure 50) capsules. Nehira (1963) found ripe spores of ***Dicranum caesium*** (Figure 54) to be 20 µm in diameter and greenish brown. On germination the spores stretched slightly. This suggests that spores of at least this ***Dicranum*** species do not swell in the capsule. On the other hand, Longton and Miles (1982) found 66-81% of ***Atrichum*** spores to be green and round, dependent on habitat. An obvious experiment to test our hypothesis for explaining Arnaudow's observations would be to supply fruiting ***D. scoparium*** plants with GA. Germination of spores within the capsule will support the hypothesis.

Figure 53. ***Atrichum undulatum***, a species of moss used by Arnaudow (1925) for embryo transplant studies. Photo by Michael Lüth, with permission.

Figure 54. ***Dicranum caesium***, a species for which spores do no swell in the capsule. Photo from Digital Museum, University of Hiroshima, with permission.

Table 1. Theorized hormonal control of spore dormancy in ***Dicranum scoparium***, based on experiments of Arnaudow (1925), where embryos of ***D. scoparium*** were transplanted into the archegonium of ***Atrichum undulatum***, causing ***D. scoparium*** spores to germinate within the resulting transplanted capsule. (Z and X are hypothesized substances.)

	Control		**Experimental**
gametophyte	*D. scoparium*	*A. undulatum*	*A undulatum*
	-	Z	X
sporophyte	*D. scoparium*	*A. undulatum*	*D. scoparium*
	-	Z	-
germination	no	no	yes

Suppose, then, that the sporophyte of ***Atrichum*** (Figure 53) might produce abscisic acid, which reduces the

effect of GA (Goodwin & Mercer 1983). In this respect, Oppenheimer (1922) and Buch (1920) mention formation of chemical substances that emanate from the capsule wall and inhibit germination. Such an inhibitor, **lunularic acid**, is known to inhibit germination of gemmae in the liverwort ***Lunularia cruciata*** (Figure 55) while they are retained by the parent thallus (Schwabe 1976). In mosses, where lunularic acid is unknown, abscisic acid could have a similar role (Pryce 1972). This hypothesis is further supported by the fact that operculum dehiscence is usually correlated with spore maturation in mosses (Hancock & Brassard 1974), and abscisic acid could promote this dehiscence, a role similar to that of autumn leaf dehiscence. On the other hand, if abscisic acid does not cause dehiscence of cells, we may find that drying of the capsule is the major factor in determining time of dehiscence, and that the ring of weak cells that facilitates this is under enzymic control or perhaps ethylene control at an earlier stage of development.

Figure 55. ***Lunularia cruciata*** with gemmae in cups and on the thallus. The thallus inhibits their germination. Photo by Martin Hutten, with permission.

In any event, it appears that we should also look closely at the gametophyte as a potential controlling generation for spore dormancy. Hughes (1954) found that control of sporangium production in ***Pogonatum aloides*** (Figure 25) and ***Polytrichum piliferum*** (Figure 56) is photoperiodic, sensed by the gametophyte, and communicated to the sporophyte. Another explanation then is that in transplanted ***Dicranum scoparium*** (Figure 50) sporophytes, communication for spore dormancy was not sent at the proper time by its ***Atrichum undulatum*** (Figure 53) gametophyte.

Another hormonal effect may intervene in dispersal of the entire capsule in such desert mosses as ***Goniomitrium*** (Figure 57) and ***Bryobartramia*** (Scott 1982). Both mosses have a short seta, a **cleistocarpous** (lacking regular mechanism for opening such as operculum or lines of dehiscence), globose capsule, and a calyptra that covers the capsule completely until dispersal (Scott & Stone 1976). Ethylene produced by the sporophyte could accumulate and cause release of capsules. Ethylene inhibits cell elongation, perhaps accounting for the short setae. The autocatalytic ability of ethylene, if captured in enclosed space under the calyptra, may cause **abscission** (breaking away) and **senescence** (aging). In higher plants abscission is the result of synthesis and secretion of a wall-degrading enzyme. Ethylene also softens the cell wall (Salisbury & Ross 1978), and its presence increases production of abscisic acid (ABA).

Figure 56. ***Polytrichum piliferum*** with capsules. Spores in this species respond to photoperiod to germinate. Photo by Michael Lüth, with permission.

Figure 57. ***Goniomitrium enerve*** with capsules. In this genus, the entire capsule disperses. Photo by David Tng, with permission.

Few species experience the germination of spores within the capsule. This inhibition could be caused by insufficient light or by the presence of an inhibitor. Such an inhibitor could be produced by either the gametophyte or sporophyte. We know that high concentrations of auxins, GA, and other hormones can inhibit germination, and the sealed capsule could accumulate such substances to inhibitory levels. Ethylene remains an unexplored possibility in this inhibition and may also play a role in the abscission of the capsule to release the operculum.

The role of hormones in germination of bryophyte spores is poorly understood. It appears that the gibberellins, growth hormones, are involved in at least some cases (Anterola *et al*. 2009). By inhibiting the production of gibberellins in ***Physcomitrella patens*** (Figure 2), Anterola and coworkers demonstrated a reduction in spore germination rate.

Inter- and Intraspecific Interactions

Exogenous inhibitors are those substances produced by other organisms that inhibit spore germination. Some species get downright nasty in their competition. For example, species of the lichen *Cladonia* can produce chemical inhibitors that prevent or reduce moss spore germination (Lawrey 1977). For ***Funaria hygrometrica*** (Figure 23), ***Weissia controversa*** (Figure 58-Figure 59), ***Plagiomnium cuspidatum*** (Figure 60), and ***Physcomitrium pyriforme*** (Figure 111), inhibition by ***Cladonia subcariosa*** (Figure 61), ***C. cristatella*** (Figure 62), and ***Cladonia squamosa*** (Figure 63) in acetone extract was complete, whereas germination was 90% or greater in acetone controls in all except ***Physcomitrium pyriforme***. The ubiquitous pollution-tolerant ***Pohlia nutans*** (Figure 64) exhibited only 34% germination in controls, but maintained from 0.8 to 5.6% germination in the three lichen extracts. The least affected species was ***Amblystegium serpens*** (Figure 64), with 91% germination in controls, and 15-71% germination with lichen extracts. However, such concentrations of lichen extracts may never exist in nature where adhesion onto soil **colloids** (substances having particles that remain dispersed in solution) may render them ineffective, or they may not leave the lichen in sufficient quantity to have any effect (unless bryophytes leach the acids out with acetone!). On the other hand, dead or damaged thalli could indeed leach out lichen acids. Such inhibition can account for some of the moss to lichen successional patterns observed in nature.

Figure 58. ***Weissia controversa*** with capsules. Photo by J. C. Schou, with permission.

Figure 59. ***Weissia controversa*** peristome with spore. Spore germination in this species is inhibited by several species of the lichen ***Cladonia***. Photo by Dick Haaksma, with permission.

Figure 60. ***Plagiomnium cuspidatum*** with capsules. Spore germination in this species is inhibited by several species of the lichen ***Cladonia***. Photo by Bob Klips, with permission.

Figure 61. ***Cladonia subcariosa***, a species that can inhibit germination of some moss spores. Photo through Creative Commons.

Figure 62. *Cladonia cristatella* (British soldier lichen), a species that can inhibit germination of some moss spores. Photo by Janice Glime.

Figure 63. ***Cladonia squamosa***, a lichen species that inhibits germination of spores of some moss species. Photo by Paul Cannon, through Creative Commons.

Figure 64. ***Pohlia nutans*** with capsules, a species in which spore germination is reduced in the presence of lichen extracts. Photo by J. C. Schou from Biopix, with permission.

Figure 65. ***Amblystegium serpens*** with capsules, a species in which spore germination is slightly reduced in the presence of lichen extracts. Photo by Dragiša Savić, with permission.

Gardner and Mueller (1981) found that the effectiveness of lichen acids in inhibiting germination of ***Funaria hygrometrica*** (Figure 66-Figure 67) spores was dependent upon *p*H. At *p*H 7, none of eight lichen acids tested had any effect on germination at concentrations of 2.7×10^{-5} M, but at lower and higher *p*H levels many became increasingly more toxic, whereas others resulted in better germination at *p*H values other than 7. These differences could account for the success or failure of bryophyte species in soils of various *p*H levels where lichens are also growing.

Based on the ease of growing ***Funaria hygrometrica*** (Figure 66-Figure 67) in the laboratory (Bopp 1980), one would expect to find germlings of this species everywhere in nature. Yet this simply is not the case. Longton (pers. comm.) has found he could not grow ***Funaria*** on soil in nature where he had collected it, yet he could grow it there on a Petri plate. If one considers the fact that ***Funaria*** remains only 1-2 years in burned areas (Hoffman 1966), and seldom remains longer than that where it invades other disturbed areas, it appears that the moss must suffer from either self-inhibition, **allelopathy** (influence of plant metabolites on other plants – *i.e.*, chemical warfare), or competition. In fact, Klein (1967) showed that ***F. hygrometrica*** protonemata release Factor H (probably a cytokinin) to the substrate and that it greatly reduces protonemal differentiation. Furthermore, old cultures of ***Funaria*** exhibit senility after about one year (Bopp & Knoop, pers. comm.), suggesting that a diffusable substance might accumulate in the substrate.

To test this theory of inhibition by older protonemata, I (Glime unpubl.) grew spores of ***Funaria hygrometrica*** (Figure 66-Figure 67) on agar that had been previously treated with 1-cm plugs of agar containing old protonemata, plugs with mature plants, and fresh agar. In all treatments, germination occurred within 48 hours, and spores even germinated on some of the plugs. Buds appeared within 10 days, with abundant buds on plates with protonemata, young plants, or mature gametophores. Furthermore, new buds were induced on the protonemata of mature plants. We must therefore conclude that either ***Funaria*** is not inhibited by any chemical that is diffused from existing plants into the agar or that the older cultures were too old and the inhibitor had broken down or become too dilute. These results do not, however, preclude the possibility of an accumulation of products as the plant grows, or the production of a gas (ethylene?) that inhibits encroaching plants.

Figure 66. ***Funaria hygrometrica*** mature plants with capsules. Photo by Michael Lüth, with permission.

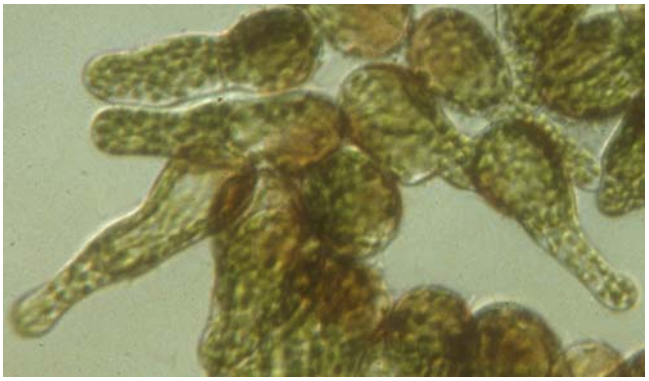

Figure 67. ***Funaria hygrometrica*** germinating spores. Photo by Yenhung Li, with permission.

Since ***Funaria*** (Figure 66-Figure 67) grows in a wide range of habitats, allelopathy seemed unlikely, though not impossible. The toxic source should be a widespread one such as that of humic acid decomposition (Hoffman 1966). Humic acids could lower the *p*H, and Armentano and Caponetti (1972) have shown that a lower *p*H retards its germination. It is significant that ***Funaria*** seldom occurs among other vegetation. Bopp (pers. comm.) has suggested that its growth after fires might be possible because of the ability of charcoal to absorb an inhibitor, although it might relate to nutrient availability as discussed above.

Therefore Raeymaekers and Glime (unpubl.) chose to experiment with humic acid effects on germination, using humic concentrations from 0 to 10%. Mean percent of germinated spores two days after inoculation decreased as the concentration of humic acid increased (Figure 68). At high humic acid concentrations (5% and 10%) the protonemata grew upward (away from the agar) and clustered together with other protonemata. Some protonemata in those concentrations formed swollen cells similar to those found by Sood (1975) in ***Pogonatum aloides*** (Figure 25). Buds were observed 8 days after inoculation in control plants, and 10 days after inoculation on protonemata of the 0.5% and 1% humic acid treatments. No buds were formed after 14 days on protonemata of the 5% and 10% humic acid treatments; however, after three weeks buds were present in 5% and 10% treatments, but in lower quantities than in the other humic acid treatments and the control.

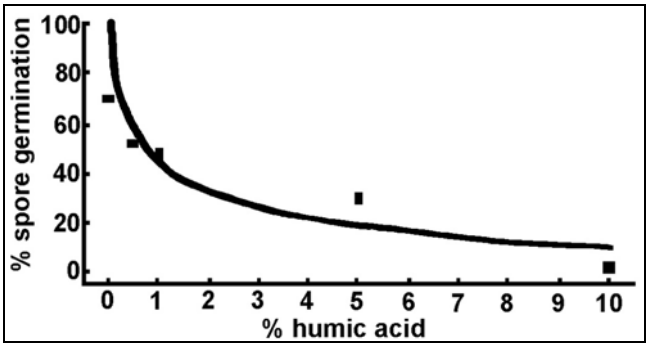

Figure 68. Effects of humic acid concentration on mean percent spore germination in ***Funaria hygrometrica***. From Raeymaekers, unpublished data.

We can conclude that spore germination and bud formation are retarded at high concentrations of humic acids. A concentration of 5% humic acid is not unusual in nature and occurs, at least in the **A horizon** (dark-colored soil layer with organic content and minerals intermixed), of spruce (*Picea*) forest soils (Remezov & Progrebnyak 1965). Fire can remove the humic acids by burning off the organic matter and returning the soil to the mineral layer, or **C horizon**. This may be a factor in permitting such bryophytes as ***Funaria hygrometrica*** (Figure 66-Figure 67) and ***Marchantia polymorpha*** (Figure 26) to colonize rapidly. But how quickly can new growth on a burned area return the lethally high concentrations of humic acids?

Could humic acids alone account for the disappearance of ***Funaria*** (Figure 66-Figure 67) from the areas where it has been a pioneer? It appears that leaf litter may offer more of a deterrent than simply blocking light. That litter is a major source of humic acids. In my moss garden, I discovered that when I left the leaf litter on the mosses all winter, they did poorly the next growing season, even though I removed the litter within days after snowmelt. Even the hardy *Fissidens* that had been doing well for several years showed signs of stress. But this unreplicated anecdotal record hardly is conclusive evidence.

In addition to endogenous inhibitors, spore germination may be affected by its surroundings. The lichen *Cladonia* can be a strong inhibitor, as can humic acids. Such signals would prevent spores from germinating in habitats that would otherwise be unsuitable, on one hand by competition for space from lichens, and on the other by competition for light with trees that drop leaves that release humic acids. A species can even stimulate bud production of its own colony, as in ***Funaria hygrometrica***, by releasing substances that stimulate protonemata to produce buds.

Interspecific Competition

Competition can be a problem of limited physical space, nutrients, or shading (light competition). For a tiny moss, physical space is available between larger plants that invade, and such spaces are usually still available long after ***Funaria*** (Figure 66-Figure 67) has disappeared. Because most nutrients are absorbed through the leaves in **ectohydric** mosses (those conducting water outside the plant) like ***Funaria***, nutrient competition can occur when a canopy intercepts and absorbs or diverts rainwater nutrients before they reach the moss. Since mosses such as ***Funaria hygrometrica*** absorb little or no nutrients from the **rhizosphere**, early invading roots present little nutrient threat.

Light quality alone could account for the restriction of ***Funaria*** (Figure 66-Figure 67) to exposed, barren habitats because the predominant wavelength transmitted through vegetation is green. However, this simple explanation cannot be applied to the distension phase of ***Funaria*** germination, wherein maximum distension occurs in yellow-green and far-red light, with the fewest protonemal cells in blue-green and red light (Valanne 1966). With such a seeming contradiction, I decided to culture ***Funaria*** spores under *Taraxacum* (dandelion) leaves to determine if in fact germination was less successful than in the open.

Few spores germinated on agar under *Taraxacum*, and protonema development was very slow. After 14 days all

control cultures at 29.5 μE m^{-2} s^{-1} (2000 lux) had buds, but those cultures under *Taraxacum* leaves at 9.4 μE m^{-2} s^{-1} (700 lux) failed to produce buds during the next four days, except for a few at the edge of the plate where white light entered. By 23 days, one experimental plate had young plants that were strongly bent toward the light at the edge of the plate. All gametophores under the *Taraxacum* were **etiolated** (abnormally elongated stems, usually in response to low light). While this demonstrates the possible role of other plants in inhibiting germination, it does not indicate whether the difference was caused by light quality or light intensity. As already discussed, the change in ratio of red to far-red light may have been the inhibitory factor (Bauer & Mohr 1959)

External Growth Promoters

It is interesting that bryophytes respond positively to application of herbicides (Balcerkiewicz 1985). On paths sprayed with herbicides, ***Funaria hygrometrica*** (Figure 66-Figure 67), together with ***Marchantia polymorpha*** (Figure 26), stayed a long time and was only slowly replaced by ***Marchantia***, which is a perennial (Raeymaekers pers. obs., Bowers *et al*. 1982). This suggests that herbicides might provide some growth-promoting substance. On the other hand, it might simply be absence of competitors and whatever they do to alter the environment.

Fungi are common growth promoters because of their production of gibberellic acid, which invades their environment. Experiments on ***Dicranum scoparium*** (Figure 50), ***D. undulatum*** (Figure 69), ***Dicranoweisia crispula*** (Figure 70), and ***Pogonatum urnigerum*** (Figure 71), using 0.01% GA, showed that GA can promote both spore germination and protonema growth (Vaarama & Tarén 1959). But most of these experiments with gibberellic acid failed to cause any increase in germination of bryophyte spores, *e.g.* in ***Tetraphis pellucida*** (Figure 72-Figure 73), ***Racomitrium fasciculare*** (Figure 74), and ***Polytrichum strictum*** (Figure 75). Gemmrich (1976) tried to induce germination of ***Marchantia polymorpha*** (Figure 26) in the dark by using GA, but was unsuccessful. However, Vaarama and Tarén discovered that spores stored dry at room temperature lost their viability, but that GA stimulated them to germinate.

Figure 69. ***Dicranum undulatum*** with capsules, a species for which GA promotes both germination and spore growth. Photo by Jan-Peter Frahm, with permission.

Figure 70. ***Dicranoweisia crispula*** with capsules, a species for which GA promotes both germination and spore growth. Photo by Hermann Schachner, through Creative Commons.

Figure 71. ***Pogonatum urnigerum*** capsules, a species in which spore germination is promoted by GA. Photo by Kristian Peters, with permission.

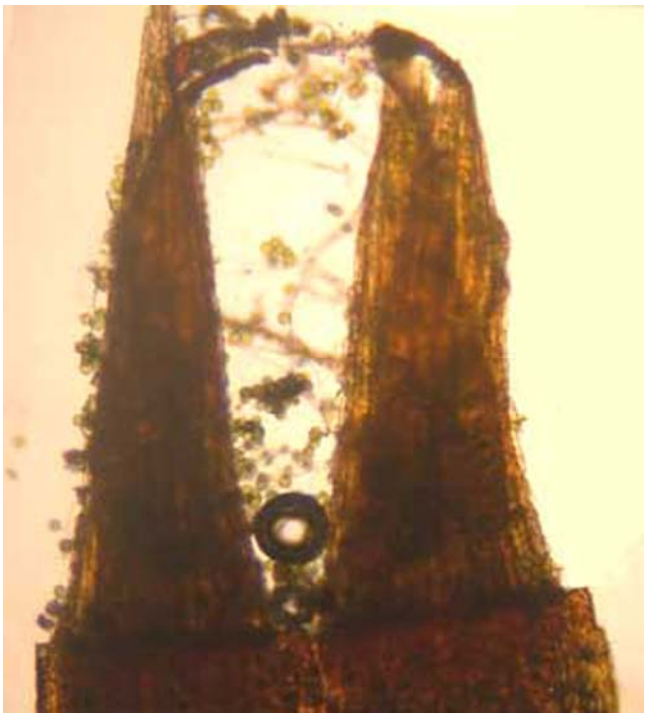

Figure 72. ***Tetraphis pellucida*** teeth and spores. Photo from Botany website, UBC, with permission

Figure 73. ***Tetraphis pellucida*** growing on stumps where wood-decaying fungi can provide GA. Photo by Janice Glime.

Figure 74. ***Racomitrium fasciculare*** with capsules, a species in which GA does not induce germination. Photo by Michael Lüth, with permission.

Figure 75. ***Polytrichum strictum*** with capsules, a species in which GA does not induce germination. Photo by Michael Lüth, with permission.

Merwin (2003) reports that in orchards post-emergence herbicides favor bryophytes. Several studies have noted that long-term use of these herbicides promote the growth of ***Bryum argenteum*** (Figure 76) and ***Marchantia polymorpha*** (Figure 26) under the trees. These actually provide an advantage to the orchard growers because they stabilize the soil, resist trampling, and do not compete with the trees for nutrients. In this case, spores may not be involved because of vegetative propagules, but they cannot be ruled out.

Perhaps the most ecologically relevant evidence in those experiments is that several fungi (*Aspergillus flavus*, *Penicillium martensii*, *Mucor racemosus*, *Fusarium scirpi*, *Rhodotorula mucilaginosa*) promoted germination and growth even more than GA! Fungi isolated from the rotting wood where ***Tetraphis pellucida*** (Figure 73) was growing also stimulated the germination of the spores (Figure 72), but that does not account for its ability to grow on rock faces. It may be interaction with fungal GA that accounts for the production of gametophores of ***Fontinalis squamosa*** (Figure 77, Figure 93) in contaminated laboratory cultures when none of the sterile cultures reached that stage, suggesting that ***F. squamosa*** protonemata might be most likely to succeed on damp rocks that have a fungal mat on them (Glime & Knoop 1986). Vaarama & Tarén obtained similar stimulatory results with fungi and ***Pogonatum urnigerum*** (Figure 71), a soil moss. However, they failed to obtain germination of spores from the rock-dwelling ***Racomitrium fasciculare*** (Figure 74) when culturing it with the mold *Aspergillus flavus*. Although results have varied widely in the GA treatments, one certainly cannot ignore the potential influence of fungi in the development of at least some bryophytes.

Figure 76. ***Bryum argenteum*** with capsules, a species in which herbicides promote growth. Photo by Jan-Peter Frahm, with permission.

Figure 77. ***Fontinalis squamosa*** protonema. Photo by Janice Glime.

Additional evidence for fungal intervention in bryophyte development occurs in ***Funaria hygrometrica*** (Figure 66-Figure 67). Hahn and Bopp (1972) concluded that the addition of fungi hastened bud formation in this species and considered this to be a symbiotic interaction.

Inorganic substances also have an effect on germination and may account for the presence or absence

of species on newly disturbed soil. Gemmrich (1976) found that while gibberellic acid did not induce dark germination of ***Marchantia polymorpha*** (Figure 26), various forms of Fe and Ca did, as well as KNO_3 and $MgSO_4$, with optimum germination on $Ca(NO_3)$.

Pollutants

We seldom consider germination when considering the effects of environmental contaminants. Yet, reductions in numbers of bryophytes from many substrates may indeed be the result of failure to germinate. For example, Francis and Petersen (1989) recommend that spore germination is a good bioassay technique for determining the toxicity of heavy metals. But much work remains to determine the effects of the many contaminants on the many species of bryophytes.

Numerous possibilities of inhibition exist with the presence of pollutants. These can include greater dryness, UV exposure, and a myriad of chemicals. Field studies on effects of such pollutants on spores are lacking. However, laboratory studies can suggest potential problems. One early study on pollutant effects on spores is that by Lewis (1973) on suspended solids from coal. She found that increasing concentrations of coal particles resulted in decreasing germination of spores of ***Platyhypnidium riparioides*** (Figure 78) suspended in Bold's (nutrient culture) medium (Figure 85).

Figure 78. ***Platyhypnidium riparioides*** with capsules, a species in which suspended coal particles caused decreased germination. Photo by Hermann Schachner, through Creative Commons.

Spore Size

Greater spore size may offer an advantage at germination by providing a reservoir of energy that permits long-term storage (see Chapter 3-1, Polyploidy and Spore Size). The trade-off, one would presume, is that large spores do not disperse far, so we should expect taxa with extremely large spores, such as ***Archidium*** (Figure 5-Figure 6) (50-130 µm), to have a small distribution. Surprisingly, ***Archidium*** is relatively widespread in southeastern North America, Eurasia, and New Caledonia (Schofield 1985), and because it is so often overlooked due to its small size, it is likely that it is even more widespread and frequent than that reported. Its large spores seem to permit it to be successful on disturbed soils, but its means of arrival remains a mystery.

Convey and Smith (1993) considered that short-lived species in the Antarctic typically had large spores that could help them in local colonization, whereas small spores characterized more widespread species In assessing the spore sizes of Michigan mosses, as published in Crum (1973), I found that the perennial, pleurocarpous mosses all had relatively small spores, the largest being 24 µm. Acrocarpous mosses, on the other hand, ranged up to 68µm with roughly 40% of the species larger than 24 µm. ***Buxbaumia aphylla*** (Figure 79), a species with one of the largest capsules, has the smallest spores of 6.5-8 µm, perhaps accounting for its ability to colonize disturbed sites. The largest Michigan spores, being multicellular and measuring 60-100 µm, occur on ***Drummondia prorepens*** (Figure 80), an epiphyte. ***Sphagnum*** shows no correlation of spore size with plant size, ranging from a minimum of 17 µm in ***S. squarrosum*** (Figure 82) and ***S. warnstorfii*** (Figure 83) to a maximum of 42 µm in ***S. cuspidatum*** (Figure 84).

Figure 79. ***Buxbaumia aphylla*** capsules with the smallest spores, exposed in upper capsule. Photo by Janice Glime.

Figure 80. ***Drummondia prorepens*** on wood, the species with the largest spores in Michigan. Photo by Dale Vitt, with permission.

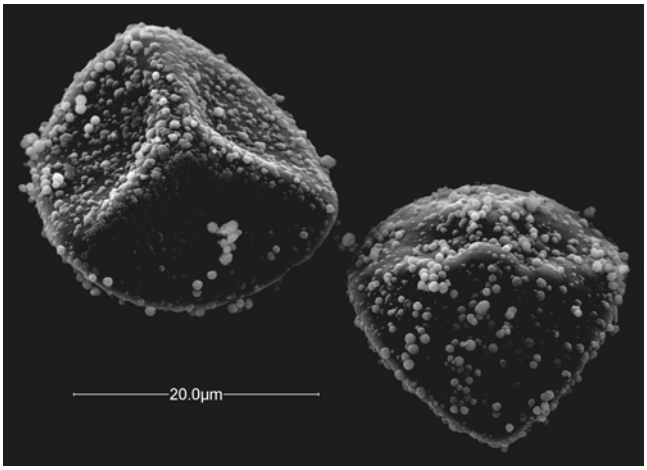

Figure 81. ***Sphagnum*** spore SEM. Photo by Whitaker & Edwards 2010, with permission.

Figure 82. ***Sphagnum squarrosum*** with capsules, a ***Sphagnum*** species among those with the smallest spores. Photo by Michael Lüth, with permission.

Figure 83. ***Sphagnum warnstorfii***, a ***Sphagnum*** species among those with the smallest spores. Photo by Michael Lüth, with permission.

McLetchie and Johnson (1997) found an interesting effect of spore size in the liverwort ***Sphaerocarpos texanus*** (Figure 86). As discussed earlier, this liverwort disperses its spores in tetrads with two male and two female spores, ensuring close neighbors of the opposite sex. However, when the spore size is abnormally small (<90µm), the sex ratio is biased toward females.

Figure 84. ***Sphagnum cuspidatum*** with capsules, a ***Sphagnum*** species with the largest spores. Photo by Bobby Hattaway (DiscoverLife), through Creative Commons.

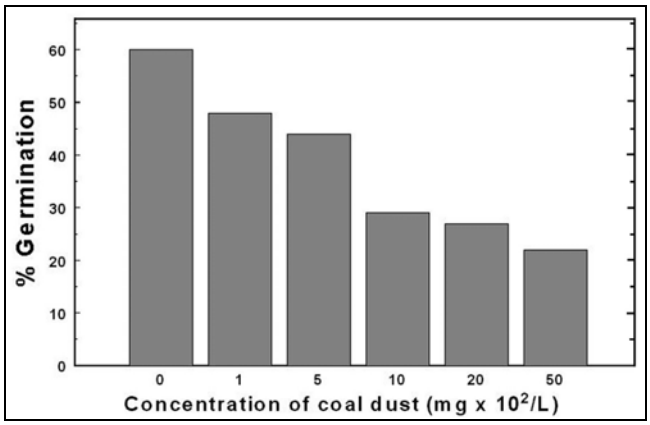

Figure 85. Inhibition of germination of ***Platyhypnidium riparioides*** spores resulting from suspended coal particles. Redrawn from Lewis (1973).

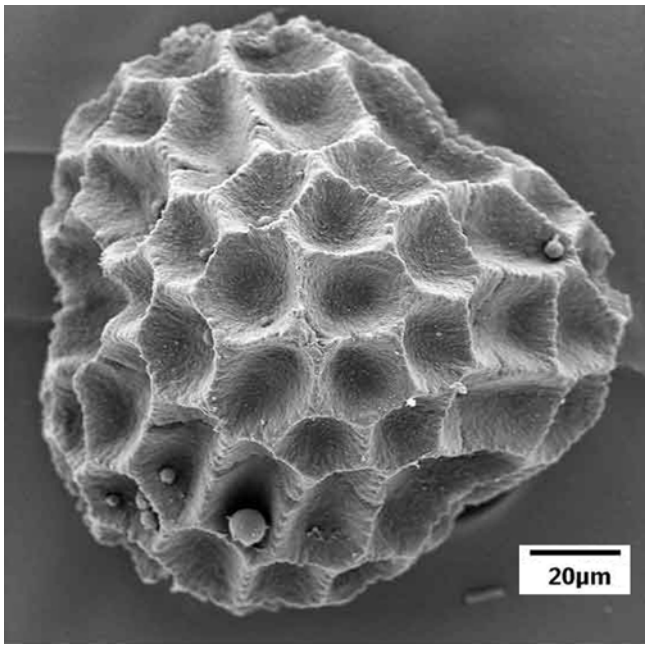

Figure 86. SEM of ***Sphaerocarpos texanus*** distal spore wall. Photo by William T. Doyle, with permission.

Anisospory and False Anisospory

Most mosses and liverworts have only one size of spore, *i.e.*, they have **isospory**. Few have **anisospory**, or two different spore sizes determined genetically. However, **false anisospory** (non-genetic size differences) exists in several genera. Mogensen (1978b) used acetocarmine stain to demonstrate that false anisospory in ***Fissidens dubius*** (Figure 87), ***Macromitrium incurvum***, and ***Rhizomnium magnifolium*** (Figure 88) was due to death of spores; only live ones stain red. In this case, some spores may abort at some point during development, rendering them smaller than those spores that have continued to develop. These arrested spores seem unable to germinate. However, in other cases, there appears to be arrested development of some spores, perhaps due to crowding, that permits other spores to continue their development in the limited space inside the capsule. These aborted spores may or may not be able to germinate, apparently depending on their ensuing conditions. This relationship is much like that of baby birds. The larger (often older) babies get all the food, sometimes leaving the smaller ones to starve, rendering them small or dead. It does not appear that any particular spore has a genetic predisposition to develop or to abort, so the two sizes diverge randomly and there can be multiple sizes due to more than one event of arrested or aborted development.

Figure 87. ***Fissidens dubius*** with capsules, a species in which some spores abort, creating large and small spores. Photo through public domain.

Figure 88. ***Rhizomnium magnifolium***, a species in which some spores abort, creating large and small spores. Photo by Michael Lüth, with permission.

Most reported cases of anisospory seem to be in mosses, not liverworts. However, Pant and Singh (1989) reported the possibility in the liverworts ***Targionia*** (Figure 89-Figure 90) and ***Cyathodium*** (Figure 91). They found a few cases of abnormally shaped spores of unequal size in several species of these two genera. It is more likely, however, that these were again cases of false anisospory due to spore abortion.

Figure 89. ***Targionia hypophylla*** with capsule in the black marsupium. Photo by Des Callaghan, with permission.

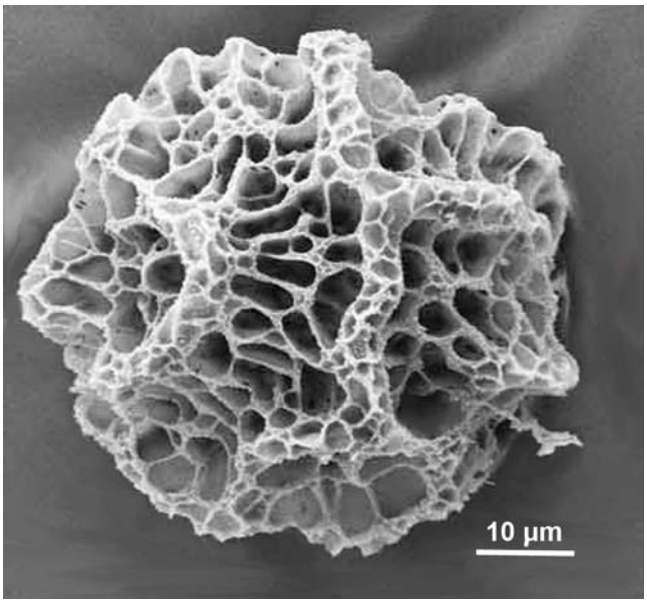

Figure 90. ***Targionia hypophylla*** distal spore wall SEM. This genus sometimes has unequal spore sizes. Photo by William T. Doyle, with permission.

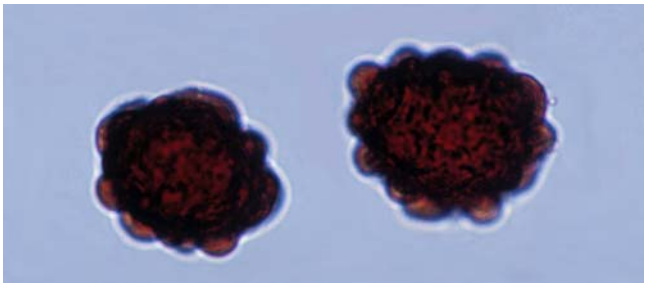

Figure 91. Spores of ***Cyathodium***, where spores are sometimes of unequal size. Photo courtesy of Noris Salazar Allen.

Fontinalis (Figure 93) has false anisospory. At the completion of sporogenesis, tetrads frequently have 1, 2, or occasionally 3 collapsed spores (Figure 92; Glime & Knoop 1986). At any subsequent stage of development of the capsule, one can find two sizes of spores in the same capsule (Figure 93). In early stages, these can both be brown, and only the larger spore becomes swollen and green when cultured on nutrient agar. At later stages, both large and small spores can be green. Large green spores become distended after five days of culturing, whereas small green ones do not. It appears that the smaller ones never germinate, but they do swell in response to the culture medium. These might have insufficient food reserves to succeed.

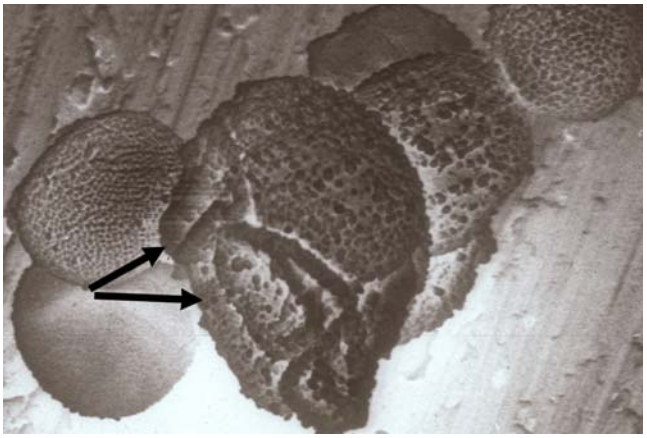

Figure 92. SEM of spore tetrad of ***Fontinalis squamosa*** showing one normal and at least two aborted spores (**arrows**) in the middle tetrad. The remaining visible spore is larger than nearby spores. Photo by Janice Glime.

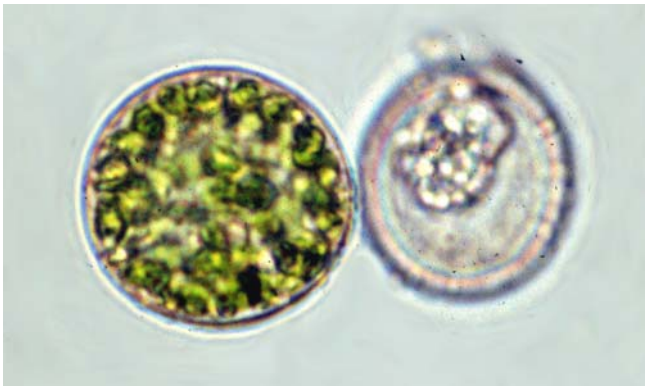

Figure 93. Normal (**left**) and aborted (**right**) spores of ***Fontinalis squamosa***. Photo by Janice Glime.

In the moss ***Bryowijkia ambigua*** (Figure 94), DeLuna (1990) found that anisospory is really a case of aborted small spores and large, multicellular spores. He found that in a tetrad, two spores abort and two remain viable, dividing to become multicellular.

These examples demonstrate the inequality of the spores, but do not provide any genetic clues. The randomness with which collapsed spores of ***Fontinalis*** (Figure 92-Figure 93) occur in the tetrads precludes sex linkage. Furthermore, in a conversation with Gert Mogensen, I was convinced that I could not rule out the continual death of spores, so that there is always a mixture of healthy spores and smaller dead ones. This explanation is further supported by the absence or reduction of chlorophyll fluorescence in the smaller spores, even when they are still green (Figure 24).

Figure 94. ***Bryowijkia ambigua***, a species that has anisospory due to aborted smaller spores. Photo by Li Zhang, with permission.

By using acetocarmine to stain the nucleus, Mogensen (1978a) has demonstrated false anisospory from spore mortality in ***Cinclidium*** (Figure 95). In that genus, mortality predominates in the stationary spore stage, with little occurring later, contrasting with ***Fontinalis squamosa*** (Figure 24, Figure 92), where it occurs at all phases. If Mogensen's model applies, a physiological failure can result from a pair of lethal genes on separate chromosomes, one of which is a sex chromosome. If this results in failure of a developmental process, then we should expect death of the spores to occur at one developmental stage, as Mogensen observed in ***Cinclidium***. Valanne (1966) observed that some spores fail to use their reserves in the dark, even with added GA, IAA, or kinetin, and therefore require light to provide the energy to move to the next life cycle stage, suggesting a potential mechanism for failure.

Figure 95. ***Cinclidium arcticum*** with capsules, a genus in which death of the spores occurs at one developmental stage. Photo by Michael Lüth, with permission.

In ***Fontinalis***, lethality does not seem to be so simple, as it apparently can occur at several spore stages rather than

one. I observed about equal numbers of two spore types in the capsules of ***Fontinalis squamosa*** (Figure 92-Figure 93) at three different stages in spore development. If the early smaller spores were dead, then a new set of dead ones appeared when spores were larger. Without obtaining counts of spores at each of these stages, we cannot eliminate the possibility that two sets of truly anisosporous spores were developing in consort, with no spore death, and that perhaps the two sizes had different germination requirements.

If we assume that spores are dying, one advantage for post-meiotic death would be to reduce competition for resources such as moisture, space, and sugar reserves within the capsule. Furthermore, 50% retarded development could provide a dispersal advantage. Small spores, if still viable, would be adapted for long-distance dispersal, larger ones for germinating close to home. This strategy of functional heterospory is known for the epiphytic moss ***Leptodontium viticulosoides*** (Figure 96) in the Andes (Kürschner & Parolly 1998).

Figure 96. ***Leptodontium viticulosoides***, a species with functional heterospory due to delayed development of some spores. Photo by Claudio Delgadillo Moya, with permission.

If the theory of retarded development is correct, why is non-sex-linked (false) **anisospory** unique to bryophytes? In other groups of plants, **heterospory** is associated with sex, with the female being larger. In bryophytes this is usually not the case, with large females and dwarf males resulting from anisospory known only in ***Macromitrium*** (Figure 97; Ramsay 1979). In flowering plants, retarded development of the seed can permit some seeds to germinate in the fall and others to wait until spring, as in *Melampyrum*. But in that case, it is a result of seed production over an extended period of time, causing different degrees of maturity at fall germination time.

In other groups of plants, heterospory usually occurs in those organisms having endosporous development of the female gametophyte. There is an advantage for the female to be large and provide food for the developing embryo, and it is also an advantage for the male to be small for dispersal (e.g. *Marsilea*, *Selaginella*, seed plants). These are strong selection pressures that would favor sex-linked heterospory in endosporous organisms. In mosses, endosporic development does not occur, although a few taxa do increase to about 4 cells before germination. Hence, this sex-linked advantage is lacking. On the contrary, there is an advantage for heterospory to occur within both sexes to provide for both long distance dispersal of some of the smaller spores and immediate fitness of large spores, in both sexes. Van Zanten and Pocs (1981) feel that green spores are adapted for immediate fitness and short dispersal only, and non-green spores are adapted for long range dispersal. However in ***Macromitrium*** (Figure 97), where dwarf males must sit on females (Ramsay 1979), one might argue for an advantage to short distance dispersal of the annual male so that the perennial female has a supply of sperm each year.

Figure 97. ***Macromitrium*** sp., a genus with true anisospory. Photo by Janice Glime.

However, ***Fontinalis*** (Figure 92-Figure 93) does not have dwarf males. Is it possible that long-range dispersal might occur in the immature brown spores, with germination being a slow process in a suitable habitat, and immaturity delaying germination, allowing an even greater chance for distance dispersal? Certainly their small size would permit them to have wind dispersal, and their roughened surface might serve as protection in the atmosphere.

This leaves us with a developmental question. What determines that non-sex-linked spores in a capsule will be of two sizes? Genetic differences can exist to program different developmental rates. Environmental differences within the capsule could alter the rate of development. If a genetic difference exists, it must separate at meiosis. In this case, we would predict equal numbers of large and small (or fast and slow) spores in all capsules only if the controlling gene is on a sex chromosome. In fact, however, we see varying percentages: 0-14% abortion in *Pleurozium schreberi* (Longton & Greene 1979); 49-61% physiological anisospory in ***Ceratodon purpureus*** (Figure 112) (Valanne 1966); 11-50% in ***Cinclidium*** (Figure 95) (Mogensen 1978a). If the trait is genetic, either it is absent in both gametes, present in only one, or present in both. Following meiosis, three combinations could occur: all small, half small - half large, all large. This pattern is not evident, but Mogensen (1981) has suggested this may be due to the counting technique. On the other hand, if the trait is coupled with differential viability, some capsules of the species should exist with only one kind of spore. This is not the case for ***Fontinalis squamosa*** (Figure 92-Figure 93); however, differential viability might not be 100% effective. If we can demonstrate that both types of spores

germinate, we have proved that Mogensen's explanation for ***Cinclidium*** does not apply to this case.

Whereas Mogensen used acetocarmine, a vital stain, to demonstrate viable DNA in ***Cinclidium*** (Figure 95), we used germination to demonstrate that at least some small spores in ***Fontinalis*** (Figure 92-Figure 93) could germinate. We have not tested both species by the same method, and we do not have evidence that viable DNA in the spore means it is capable of germination. If the spore lacks sufficient stored energy, it still is unlikely to be able to germinate and reach the distention or protonema stage in nature.

The second developmental possibility, internal environmental differences, could result from unequal nutrition or moisture within the capsule. This can easily account for differences in percentages between capsules, as different plants and different positions within the capsule could have different abilities to provide energy. In fact, differentiation could be related to the position of the cells at the time of meiosis. This is supported with the suggestion that the columella serves as a water reservoir, and it could also serve as a nutrient source.

Longton and Greene (1979) found a bisporic composition of spores in ***Pleurozium schreberi*** (Figure 98), similar to the ***Fontinalis squamosa*** (Figure 92-Figure 93) condition. Spores were of two types: green and papillose, or small, brown, and hyaline. Viability of large, green spores was 90-100%, whereas total spore abortion was commonly 0-40%. No "aborted" spores germinated. The observations on ***Fontinalis squamosa*** can likewise be compared with those of Paolillo and Kass (1973) for ***Polytrichum*** (Figure 8). In the two species they studied, they could obtain no germination from "immature" spores. Perhaps they did not wait long enough, or the conditions in the culture did not permit ripening of the ***Polytrichum*** spores, but the spores may have been dead. Some immature spores germinated on agar with sucrose, indicating the importance of nutrition and confirming that not all the small spores were dead, but rather that they lacked sufficient energy.

Fischer (1911) found that non-green fern spores took 4-210 days to germinate. ***Fontinalis squamosa*** (Figure 24) required only five days for ripe spores to germinate in culture, but 18 days for unripened spores, and during that same period spores in capsules at 10ºC in the dark also ripened (Glime & Knoop 1986). This observation on ***F. squamosa*** (Figure 92-Figure 93) suggests that light is not necessary for maturation of spores in the capsule, and that food reserves of the sporophyte or gametophyte suffice for ripening. Those spores cultured in the dark on agar, on the other hand, did not become green and swollen during this time. This indicates these spores are dependent on having either light or a parent plant to provide energy during ripening.

Based on these responses, it appears that maturation of ***F. squamosa*** (Figure 24) spores is dependent on a sugar source. The obvious experiment is to culture immature spores in the dark on agar with sucrose or glucose. However, Paolillo and Kass (1973) used a 2% sucrose solution with ***Polytrichum*** spp. (Figure 8), but spores that lacked fluorescence (suggesting no active chlorophyll) did not germinate in 14 days of culture at 11,800 lux, 28ºC. Possibly the light was too high for maturation, or the temperature too high, but one would expect at least a small percentage to germinate. Spores kept in the capsule for seven days did germinate. This suggests that the mechanism in ***Polytrichum*** (Figure 8) might require more than sugar, or that development outside the capsule was much slower than in the capsule.

Figure 98. Branches of moss ***Pleurozium schreberi*** showing the red stem that distinguishes it. Photo by Michael Lüth, with permission.

Three spore size conditions exist among bryophytes. **Isospory** is the typical condition in which all spores are the same size. **Anisospory** exists in only a few taxa in which there are genetically determined size differences among spores. In some species of ***Macromitrium*** the small spore develops into a **dwarf male**. The remaining species with two spore sizes appear to be cases of **false anisospory** in which some spores abort or mature more slowly, most likely with different causes in different species, some resulting from spore death and some developing more slowly from insufficient nutrition or water. Either of these conditions could be caused environmentally or genetically. If small spores are simply less developed but viable, the two sizes could provide the bryophyte with a bet-hedging strategy in which large spores are ready to germinate and most likely fall close to their parents. Small spores, on the other hand, could require more time for maturity, perhaps outside the capsule, and would be small enough to travel greater distances.

Tradeoffs

As already mentioned, having large spores insures a greater success at germination, but decreases the range of dispersal. Large spores also result in a smaller number of spores, both between species and within a species. But another tradeoff exists that may be more costly. A smaller number or absence of asexual propagules coincides with having large spores in Great Britain (Söderström & During 2005). This may be especially important for many annual shuttle species whose life cycle is too short to accomplish production of both.

Wiklund and Rydin (2004) suggested that spores may have a tradeoff between moisture and suitable *p*H. They interpreted the interaction between *p*H and moisture to indicate that spores can germinate at suboptimal *p*H when abundant water is available, and vice versa. The wood-inhabiting ***Buxbaumia viridis*** (Figure 99) germinated

better than did the epiphytic ***Neckera pennata*** (Figure 100-Figure 101) at low *p*H. ***Neckera pennata***, on the other hand, had earlier spore germination in conditions of low water potential and spores survived longer in a dry state. The researchers considered this represented a trade-off between the ability to colonize substrates with low moisture-holding capacity and low *p*H, favoring ***Buxbaumia viridis***, vs the positive effect that high *p*H has on germination by permitting it to exploit short, moist periods, favoring ***Neckera pennata***.

Figure 99. ***Buxbaumia viridis*** on a log that has lost most of its bark. Photo by Michael Lüth, with permission.

Figure 100. ***Neckera pennata*** showing its tree bark habitat. Photo by Janice Glime.

Figure 101. ***Neckera pennata*** showing capsules. Photo by Michael Lüth, with permission.

Germination Success

Most of what we know about success of germination is based on laboratory results. Field success is likely to be much lower due to decay, herbivory, and inappropriate location. In a study by Hassel and Söderström (1999), it would appear that most spores might be successful if the appropriate conditions are found. They grew spores from ***Pogonatum dentatum*** (Figure 102) on Petri plates and had 96.6% germination after 21 days. However, when they sowed the spores from a half, one, and two capsules in 10x10 cm plots on a newly built forest road in Sweden, only 11, 10, and 12 shoots per block developed, respectively, after one year. However, more appeared the second year, resulting in 17, 20, and 22 shoots. These late appearances could have come from protonemata already established the first year rather than from new germinations. In any case, the success rate from the estimated 712,000 spores per capsule is quite low!

Figure 102. ***Pogonatum dentatum*** with capsules, a species in which not all spores germinate the first year. Photo by Matt Goff <www.sitkanature.org>, with permission.

Germination Time

Germination times vary with type of propagule, size, age, and available water. And light seems to be required for most spores to germinate, although some germinate in the low light of the capsule. ***Aloina*** (Figure 103-Figure 104) and ***Bryum*** (Figure 28, Figure 76) spores germinate in 7-10 days (Llo Stark, pers. comm. 3 February 2015). On the other hand, propagula can germinate in 2-4 days in ***Bryum*** and ***Syntrichia*** (Figure 49). Germination of ***Pogonatum dentatum*** (Figure 102) spores occurred after 21 days (Hassel & Söderström 1999). Bhatla (1994) states that ***Funaria hygrometrica*** (Figure 66-Figure 67) spores germinate in 48 hours, a time period known for a number of mosses, but Krupa (1964) found that some (1%) germinate in as little as 15 hours in continuous light. The epiphytic ***Lindbergia brachyptera*** (Figure 105) spores germinate in 3 days, with 95% germination in 8 days (Zhao *et al*. 2004). ***Brachythecium velutinum*** germinated in 13-39 days from fresh material (Herguido & Ron 1990).

Figure 103. ***Aloina aloides*** capsules, where some spores germinate in the low light within the capsule. Photo by Jan-Peter Frahm, with permission.

Figure 104. ***Aloina aloides*** peristome & spores that sometimes germinate within the capsule. Photo by Kristian Peters, with permission.

Figure 105. ***Lindbergia brachyptera*** with capsules, a species whose spores germinate in 3 days. Photo by Martin Hutten, with permission.

Maciel da Silva *et al*. (2010) found that nutrients affect the time required for germination in ***Bryum argenteum*** (Figure 76). In distilled water, the spores required three days to germinate, whereas when nutrients were added they germinated in two days. Following germination, nutrients were needed for protonema growth to occur.

Heald (1898; Meyer 1948) established the need for light for germination in ***Funaria hygrometrica*** (Figure 66-Figure 67), ***Brachythecium rutabulum*** (Figure 106), ***Bryum algovicum*** (Figure 107-Figure 108), and ***Plagiomnium cuspidatum*** (Figure 109). These species all germinated in three days in the light, but had not germinated after one month in darkness.

Figure 106. ***Brachythecium rutabulum*** with capsules. Photo by J. C. Schou, with permission.

Figure 107. ***Bryum algovicum*** with capsules. Photo by David T. Holyoak, with permission.

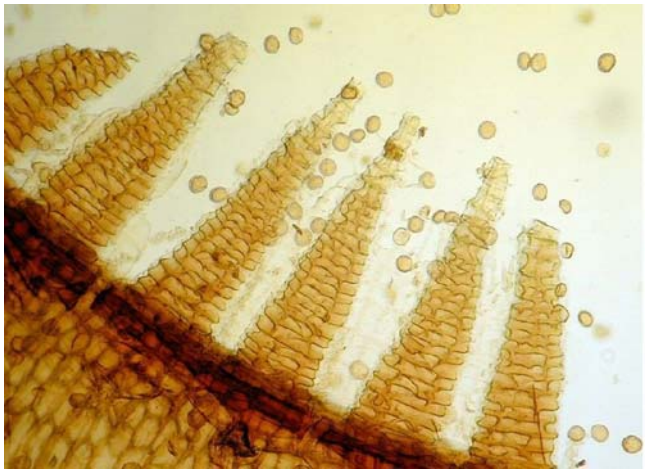

Figure 108. ***Bryum algovicum*** peristome and spores. These spores germinate in the light in three days. Photo by Michael Lüth, with permission.

Figure 109. ***Plagiomnium cuspidatum*** with capsules. Spores of this species germinate in the light in three days. Photo by Bob Klips, with permission.

In summary, germination time depends on the species and the germination conditions.

Spore Resiliency and Longevity

The most remarkable physiological observations I have made are on the capabilities of the spores themselves. I have observed ***Funaria hygrometrica*** (Figure 66-Figure 67) growing from spores that remained in a plate that had been autoclaved at 120ºC, +1 G for 20 minutes. A similar resiliency is demonstrated by the retention of the green color of ***Fontinalis squamosa*** (Figure 92-Figure 93) spores after critical point drying for SEM observation. Becquerel (1932) even reported that moss spores could survive to near absolute zero when dry in a vacuum tube.

Spores of widespread taxa such as ***Funaria hygrometrica*** (Figure 66-Figure 67) are able to survive for more than a year under more natural conditions. During (1986) has found ***Funaria*** gametophytes growing from soil samples that had been stored for two years before sowing them in the greenhouse. However, those sown in the field did not germinate. Kessler (1914) reported germination after four years and Lesage (1918) reported germination after seven years. However, Janzen (1909) was unsuccessful at germinating them after eight and twenty years.

Meyer (1941) collected spores of ***Physcomitrium pyriforme*** (as ***P. turbinatum***; Figure 111) from seven herbaria and attempted to germinate them. Only those collected in the current and previous year germinated. In the same study, spores of ***Funaria hygrometrica*** (Figure 23) germinated for the most recent eight years.

More strikingly, Malta (1921) germinated spores of ***Grimmia pulvinata*** (Figure 110) from specimens that had resided in a herbarium for 70 years, but then he retracted this claim (Malta 1922) when he was unable to repeat the success, assuming that the specimen had been contaminated with fresh spores. In his study of 200 species (Malta 1922), those with the greatest longevity were ***Funaria hygrometrica*** (13 years; Figure 66-Figure 67) and ***Ceratodon purpureus*** (16 years; Figure 112). Mogensen (1983) reports that spores can survive from only an hour to decades. But do we have any clear evidence that bryophyte spores are viable for lengthy periods similar to those of lotus seeds, reputedly of 1000 years? Although Schimper (1848) reported spore viability for fifty years, Wettstein (1925) felt this claim required re-examination. The experience of Malta (1922) supports this caution. When we examine bryophyte specimens, it is not unusual to be looking at another herbarium specimen to verify a new collection. While we are careful not to mix the specimens, spores can easily escape and join the nearby open packets. Such contamination could lead to a misrepresentation of the viability. And herbarium conditions do not represent those found in nature. Quite to the contrary, the dry conditions of the spores may permit them to go into a suspended animation state (Lipman 1936) in which respiration is all but stopped.

Figure 110. ***Grimmia pulvinata*** with capsules. Note the ungerminated spores on the outsides of some capsules. Photo by Michael Lüth, with permission.

Figure 111. ***Physcomitrium pyriforme*** with capsules, a moss that seems to have short-lived spores. Photo by Li Zhang, with permission.

Figure 112. ***Ceratodon purpureus***, with its typically prolific capsules. Photo by Michael Lüth, with permission.

Van Zanten (1976, 1978a, b) has demonstrated the long viability periods of various spores, but even more remarkable is the resiliency of the spores to adverse conditions. Van Zanten (1978a, b) found that even though spores of many species could survive 2-7 months of desiccation, these species did not occur on neighboring land masses that could easily be reached in that time. In his experiments UV radiation was definitely deleterious. Perhaps long exposures to high light intensities and longer day lengths at low temperatures in the atmosphere could result in spore death during dispersal.

Even the aquatic habitat can serve as a sporebank, although we do not have many indications of the longevity. ***Riella americana*** spores (Figure 113) from dried mud germinated after 13 years of storage (Studhalter (1931). In a Delaware River freshwater tidal wetland, Leck and Simpson (1987) found that the greatest densities of spores occurred in the upper 2 cm, and that ***Bryum*** (Figure 28, Figure 76) species were the most common bryophytes, perhaps due to prolific capsule production. Spores of mosses (and ferns) from these muds were much slower to germinate than seeds.

Figure 113. ***Riella americana*** showing spores and decaying thallus. Photo by Jan-Peter Frahm, with permission.

In fact, in flood plains of the Murray River valley of Australia, borders of cypress swamps in Florida, and low areas of southern Illinois, and most likely numerous other places, taxa such as ***Riccia*** (Figure 114-Figure 115) typically appear and survive in these periodically disturbed habitats. Spore longevity in this genus, such as that of ***Riccia albovestita*** reported by Perold (1990) to germinate from six-year-old spores, could favor rapid colonization on such disturbed sites.

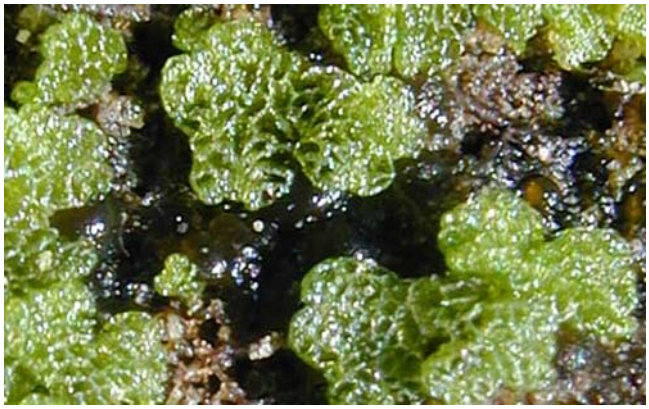

Figure 114. ***Riccia cavernosa*** on mud. Photo by Michael Lüth, with permission.

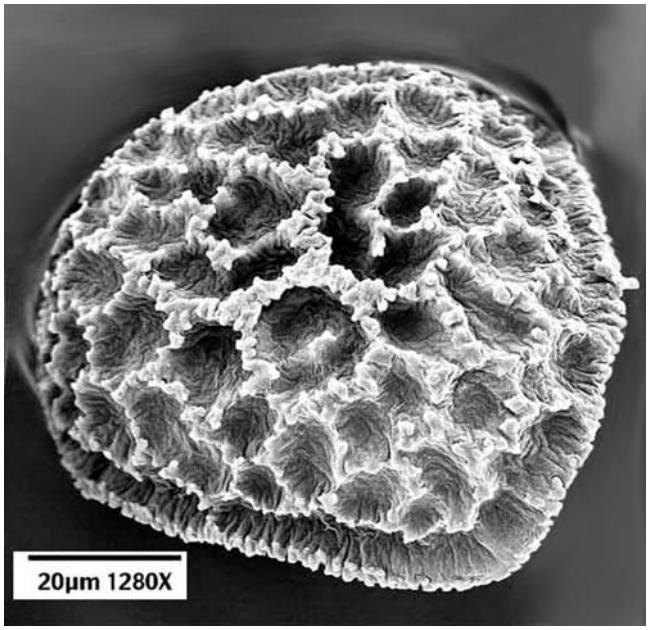

Figure 115. SEM of ***Riccia cavernosa*** spore SEM. Photo by William T. Doyle, with permission.

Even in wet peatlands, ***Sphagnum*** spores persist and germinate after several years. Sundberg and Rydin (2000) found that while viability decreased, spores buried at various depths in peat still germinated after three years. Oddly, the light-colored spores of ***Sphagnum balticum*** (Figure 116) and ***S. tenellum*** **(**Figure 117**)** maintained a higher viability than did the dark-colored spores of ***S. fuscum*** (Figure 118) and ***S. lindbergii*** (Figure 119). Surprisingly, spores that were under wet aerobic conditions survived better than did spores under wet anaerobic conditions, which died in 2-3 years. Another anomaly is that the small spores from small capsules of ***S. balticum*** and ***S. tenellum*** survived better than did the spores from medium and large capsules of the same species. Refrigerated spores maintained 13-15% viability for 13 years. Based on experiments, they estimated that ***Sphagnum*** spores can maintain a half-life in sporebanks for 1-20 years. Sundberg and Rydin attributed the widespread occurrence of ***Sphagnum*** in northern climates to the long viability of their spores in sporebanks and the ability for the spores to germinate whenever favorable conditions become available.

Figure 116. ***Sphagnum balticum*** with capsules. Photo by Michael Lüth, with permission.

Figure 117. ***Sphagnum tenellum*** with capsules. Photo by Dick Haaksma, with permission.

Figure 118. ***Sphagnum fuscum*** with capsules, a species with dark-colored spores. Photo by Dale Vitt, with permission.

But wet habitats are not favorable locations for all spores. Dalen and Söderström (1999) found that survival of spores from five species of mosses was much better when dry than in water. The highest survival rate was for ***Schistidium rivulare*** (Figure 120), perhaps accounting for its common occurrence on emergent rocks in streams. Success was lower in ***Ceratodon purpureus*** (Figure 112), ***Dicranoweisia crispula*** (Figure 70), ***Oligotrichum hercynicum*** (Figure 121), and ***Racomitrium aciculare*** (Figure 122). Nevertheless, survival of some spores for up to six months meant that submerged spore banks and water transport cannot be ruled out. See further discussion of spore longevity in the chapter on dispersal.

Figure 119. ***Sphagnum lindbergii*** with capsules. Photo by Michael Lüth, with permission.

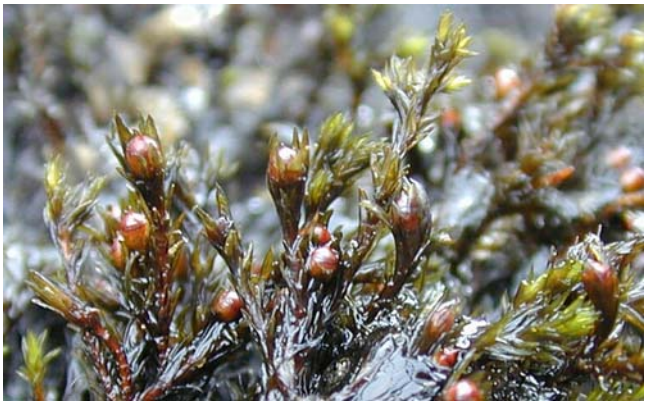

Figure 120. ***Schistidium rivulare*** growing on a wet, emergent rock. Photo by Michael Lüth, with permission.

Figure 121. ***Oligotrichum hercynicum*** with capsules. Photo by Michael Lüth, with permission.

Figure 122. ***Racomitrium aciculare*** with capsules. Photo by Michael Lüth, with permission.

Bryophyte spores are known to survive dormant in mud for up to 13 years, but reports of up to 23 years exist for herbarium specimens. Herbarium specimens can become contaminated with more recent spores; field spores are subject to damage by UV, earthworms, decay, and loss of energy, whereas herbarium specimens are protected from all those factors. Nevertheless, some dormant spores from the sporebank permit bryophytes to colonize newly disturbed sites.

Adaptations to Moisture Extremes

Most spores are adapted to travelling in a dry atmosphere that permits them to be wafted vertically considerable distances. Although spores could be dispersed on damp, cool, cloudy days, they can become clumped and heavy under these conditions, preventing long-distance dispersal. But when it is time to germinate, spores need water. The thickness of the exine layer of the spore may be an adaptation to desiccation. More water needs to be present for distension of the spores when the exine layer is thicker, and this requirement might be a protection against precocious germination.

Certainly the problems of germination of desert mosses differ considerably from those of aquatic mosses. On the one hand, the spore must delay germination until sufficient water is present to permit not only germination but subsequent development of the protonema. On the other hand, spores that are constantly surrounded by water must time their germination with a season during which they can get established and grow, *i.e.*, not too hot, not imbedded in snow or ice, and not subjected to torrential water flow that carries them off to some less suitable place.

Dry Habitats

Although some protonemata may have the ability to withstand desiccation, this ability is more likely to occur in a mature protonema than in one just emerging from the spore, when cell walls are still thin and pliable to permit elongation. Therefore, it appears that timing of spore germination is critical.

Desert bryophytes can be, compared to non-desert bryophytes, very fertile, at least in Australia. Their spore production there is high and asexual production low (Scott 1982). (See Mishler and Oliver, 1991, for contrary evidence in ***Syntrichia ruralis*** (Figure 49) in North American deserts). This high rate of fertility, together with their life strategy (**annual shuttle species**), is an adaptation to the **xeric** (dry) environment. Salt-tolerant, or **halophytic**, species share the same characters with desert bryophytes and are often very productive, *e.g.* ***Schistidium maritimum*** (Figure 123), ***Hennediella heimii*** (Figure 124), ***Ulota phyllantha*** (Figure 125). Some species form polymorphic spores, so that not all spores germinate at once and a false start with too little water will not use up all the spores (Scott 1982), a phenomenon discussed above for some non-desert taxa.

Figure 123. ***Schistidium maritimum*** with capsules. Photo by David T. Holyoak, with permission.

Figure 124. ***Hennediella heimii*** with capsules. Photo by David T. Holyoak, with permission.

Figure 125. ***Ulota phyllantha*** with capsules. Photo by Michael Lüth, with permission.

An interesting adaptation to desiccation is formation of **multicellular spores**. Parihar (1970) gives a complete list of species with multicellular spores. In hepatics these are mainly thallose liverworts and in mosses the species belong to closely related families: Dicnemonaceae, Calymperaceae, and Pottiaceae, all from relatively dry habitats. Mogensen (1981) interprets multicellular spores as an adaptation to desiccation and, at least in mosses, we see that the species that show this characteristic are relatively **xerophytic** (adapted to dry habitats).

Multicellular spores are possible when the **glyoxysomes** [organelle in plant or microorganism cell, containing catalase, where acetate and fatty acids can be used as sole carbon source (glyoxylate cycle)] are not blocked and material for the cell wall can be provided (Neidhart 1979; Mogensen 1981). This is possible through the **glyoxylate cycle** that provides sugars as a source for the carbon skeletons and energy for the synthesis of new cell walls. In unicellular spores the glyoxysomes are blocked prior to germination (Neidhart 1979). This seems to parallel the seeds that are adapted to dry habitats and are rich in fatty acids, using the glyoxylate cycle to germinate.

The environmental signals that cause spores to divide and that prevent germination are not known. From higher plants we know that chilling (5ºC for 6 hours) lowers the **isocitratase** activity. Isocitratase is an enzyme of the glyoxylate cycle and its activity is depressed by an exogenous source of succinic acid (Noggle & Fites 1964). Succinate is a product in the biochemical pathway from fatty acids to carbohydrates. Perhaps the low temperature causes an accumulation of succinate, thus halting germination. A careful study of timing of multicellular development in moss spores and temperature might be an interesting approach to finding mechanisms of control of germination.

Precocious Germination

Precocious germination, like a precocious child, reaches a developmental stager earlier than usual. In the case of germination, the spores germinate within the capsule. This is not a general occurrence among bryophytes.

In ***Brachymenium leptophyllum*** (Figure 127) in South Arabia, spores germinate within the capsule (Kürschner 2004). In this habitat, it permits new plants to establish rapidly near the mother plant, decreasing their risk of extinction in long-range dispersal.

Dendroceros (Figure 126) is a tropical hornwort that differs from other hornworts by growing on tree bark and leaves (Schuette & Renzaglia 2010). It produces green multicellular spores which begin as unicellular **tetrads** (groups of four) following meiosis. These spores expand to 60-75 µ in diameter. These fill the available space around them, resulting in many different shapes and sizes of spores within the capsule. When the spore divides, the resulting cells develop a single large, star-shaped chloroplast with a **pyrenoid** (organelle that facilitates starch formation by concentrating CO_2) in each cell. Individual cells become smaller during this division process. Cell content increases, particularly the protein storage bodies in vacuoles. As in ***Brachymenium leptophyllum*** (Figure 127), this multicellular condition appears to be an adaptation to drying. ***Dendroceros*** is the only desiccation-tolerant hornwort and this same adaptation is also present in a number of other epiphytes among the mosses and leafy liverworts (*e.g.* **Porellaceae,** Figure 128).

Figure 126. ***Dendroceros crispus*** with sporophytes. Photo by Jan-Peter Frahm, with permission.

Figure 127. ***Brachymenium*** cf. ***leptophyllum*** with capsules. Spores in this species germinate within the capsule. Photo by Li Zhang, with permission.

Figure 128. ***Porella cordaeana*** with capsules, in a family with some a desiccation-tolerant species. Photo by Ken-Ichi Ueda through Creative Commons.

Desert mosses have several adaptations within their spores to increase their chances of success. Those in the Mojave Desert contrast sharply with those in Australian deserts, with the latter producing prolific sporophytes. Among these, one strategy is to have a delayed germination in which not all spores germinate at one time, thus providing **multiple chances** to have sufficient water following germination. There seems to be a good correlation between those spores that succeed in xeric conditions and the **absence of an inhibitor** of the **glyoxysomes**. When glyoxysomes are free to operate, they are able to provide a **carbon source** for building **cell walls** through the breakdown of **fatty acids**. Others succeed by having **precocious** germination.

Aquatic

In submerged aquatic mosses such as ***Fontinalis*** (Figure 131), the opposite problem exists. Special adaptations must be present to prevent germination within a continuously wet capsule. One can suppose that the dark-colored capsule might have a high concentration of phenolic compounds that could serve as inhibitors (Figure 129). On the other hand, just by being in a dark-colored capsule, spores may fail to germinate due to lack of light. Furthermore, the glossy, thick capsule wall might effectively prevent water from entering the capsule. However, spores can become swollen and green within the capsule (Glime, pers. obs.; Figure 130). Since these swollen green spores fail to show distension, an inhibitory factor might be implicated. On the other hand, as already discussed, light is most likely necessary for distension, and the level inside the capsule may be too low.

Figure 129. Dark mature capsule of ***Fontinalis squamosa***. Photo by Janice Glime.

Figure 130. Longitudinal section through nearly mature capsule of ***Fontinalis squamosa*** showing green spores and dark capsule wall. Photo by Janice Glime.

Elssmann (1923-1925) has made the interesting observation that at least several species of aquatic bryophytes fail to have operculum dehiscence: ***Platyhypnidium riparioides*** (Figure 78), ***Fissidens fontanus***, (Figure 132), and ***Fontinalis antipyretica*** (Figure 133), as I have in ***F. novae-angliae*** **(**Figure 134**)** and ***F. dalecarlica*** (Figure 131). In most mosses, the annulus forms a circle of cells delineating the separation between operculum and capsule. These cells are often mucilaginous. According to Elssmann, there are small "rifts" in the cuticle due to stresses as the capsule dries, and these provide entry regions where moisture can reach the mucilaginous cells of the annulus. This of course causes the annulus cells to swell and can henceforth separate the operculum from its capsule. For such a process to occur, the capsule must experience drying to create the rifts and permit entry of moisture that swells the annulus. Dihm (in Elssmann 1923-1925) also believed the annulus was important in this context, and indicated "that the ring attains a lower degree of development and mechanical effectiveness in mosses growing on moist earth." Elssmann points out that Loeske likewise referred to a "retrogressive" annulus in ***Fontinalis*** (Figure 129-Figure 135) and ***Fissidens fontanus***. Elssmann sectioned the capsule and determined that annulus cells of ***Fontinalis antipyretica*** were very small and seemed to have no mucilage at all (or perhaps in a very dilute form). In ***Fontinalis***, it appears that abrasion may be a more important factor in exposing the inside of the capsule, and hence the spores.

Figure 131. ***Fontinalis dalecarlica*** capsules, a species which often fails to dehisce its operculum. Photo by Janice Glime.

Once the spores are liberated into the aquatic environment, they face the problem of germinating at the right time. Unless they are under ice and snow, we can assume they have both water and light. Some amphibious mosses appear to solve this problem by producing their capsules only when they are above water. But this requires "planning" – coordinated timing of capsule maturation and spore dispersal. What do they use as signals?

Figure 132. ***Fissidens fontanus***, a species in which capsules do not open. Photo by Michael Lüth, with permission.

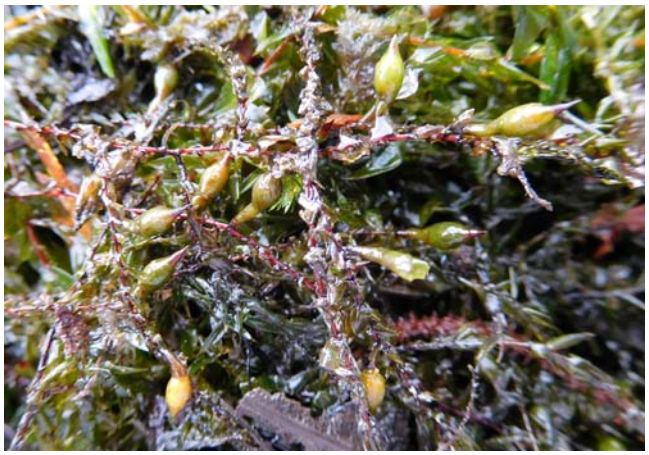

Figure 133. ***Fontinalis antipyretica*** with capsules, a species with very small annulus cells that do not dehisce. Photo courtesy of Rienk-Jan Bijlsma (per Joop Kortselius).

Temperature differences in streams and lakes are moderate compared to those on land, and therefore we might hypothesize that temperature has little influence on time of germination. But in ***Fontinalis squamosa*** (Figure 135), temperature does seem to play a role. At any given time, there are usually two sizes of spores within these capsules: small brown ones, presumably less mature, and larger green ones. It took 18 days before any of the brown ***F. squamosa*** (Figure 24) spores germinated, with many more germinating at 20ºC than at 14ºC (Glime & Knoop 1986).

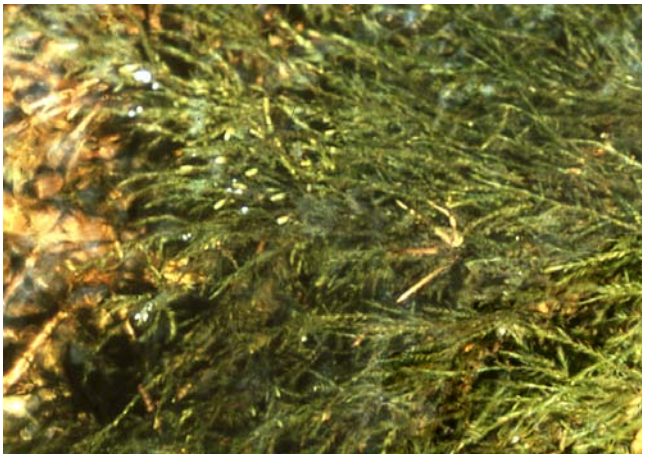

Figure 134. ***Fontinalis novae-angliae*** with capsules, a species that seems to fail in operculum dehiscence. Photo by Janice Glime.

Glime and Knoop (1986) reasoned that ***Fontinalis squamosa*** (Figure 24) is able to take advantage of a long period of spore dispersal, but with different behaviors on the part of the two spore sizes. Since capsules seem to depend on abrasion for dispersal of spores, this is likely to be a somewhat unpredictable event, most likely occurring among the capsules over an extended period of time. Since the greatest abrasion will occur with spring runoff, the cold temperature of the water during runoff could prevent germination, or at least protonema formation, and once warmer temperatures arrived in the spring, the moss could be assured of having continued warm water and no ice to block the light. Once the ice is gone, the temperatures warm rapidly, providing conditions more favorable to the protonemata. But it would seem that germination at 20^{o}C would in most cases be detrimental to ***Fontinalis*** because prolonged exposure of the gametophore to that temperature causes growth to cease in most of its species (Fornwall & Glime 1982, Glime 1982, 1987a, b), and danger of desiccation is imminent due to low stream and lake water levels. Perhaps this higher temperature permits the protonema to become well established over a sizeable area before it produces its temperature-sensitive gametophores, hence permitting development of numerous gametophores that afford each other protection from the drag effect of running water by "safety in numbers."

Figure 135. The brook moss, ***Fontinalis squamosa***. Photo by Michael Lüth, with permission.

Summary

Spores are protected by an inner intine, outer exine, and plates most likely of **sporopollenin**. **Perine** may be deposited by the sporophyte from disintegrating **columella** tissue and the sporocyte wall. Germination of spores begins with **swelling** that results from water intake, followed by **distension** that requires light, resulting in **rupture** of the cell wall and formation of the **germ tube**.

Germination and production of the germ tube require energy that may either be stored in the spore or result from immediate photosynthesis. Various hormones may be involved either in promoting germination or maintaining dormancy, both in the capsule and after dispersal. Evidence of the role of temperature, *p*H, and nutrients, especially in field conditions, is scant. However, some spores require **vernalization** (chilling).

Capsule characteristics may contribute to within capsule **dormancy** through such interventions as light blockage, altered wavelength, lack of water, and dormancy hormones.

Other species, such as the lichen *Cladonia*, may inhibit germination of some species, whereas hormones from some fungi might promote it. Humic acid from litter breakdown may also inhibit germination and contribute to the scarcity of bryophytes on the deciduous forest floor.

Some bryophytes have two sizes of spores, but with the exception of ***Macromitrium***, these appear to be a case of **false anisospory** resulting from one or more abortion events during spore development within the capsule.

Although germination success in the lab is generally high, success of the same species in the field is extremely low. Spore survival, on the other hand, can be extensive, lasting for up to 20 years in some, and probably longer.

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