CHAPTER 5-1
ECOPHYSIOLOGY OF DEVELOPMENT: HORMONES

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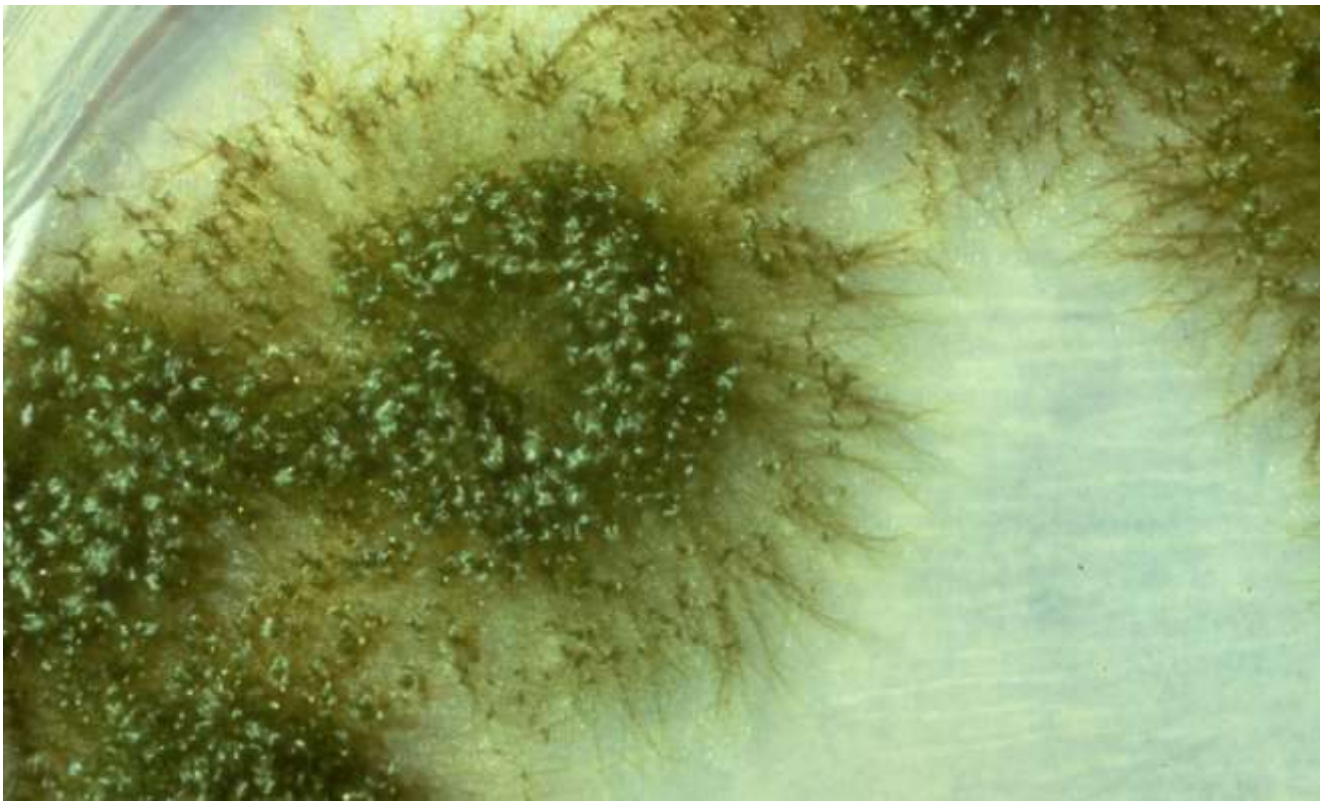

Figure 1. ***Funaria hygrometrica*** demonstrating the doughnut-shaped growth typical of cultures. Photo by Janice Glime.

Introduction

Although the field of development usually attracts scientists with very different interests from those of the ecologist, the two fields nevertheless have important overlaps that define the niche of the organism. It is the development and life cycle that permit the organism to time its life so that it can survive, from having water to grow, to dispersing its sperm and spores, to being dormant when the going is rough. Thus, it is appropriate for the ecologist to have some rudimentary understanding of the environmental controls on the physiological aspects of development and to understand the sorts of responses that might occur.

Bryophytes are limited in their occupancy of the world by a lack of lignin. This compound, providing strength and structure for the mighty sequoia, permits tracheophytes to attain heights unimaginable for the unlignified bryophyte. Height for most mosses standing alone is but a few centimeters, achieving greater heights when supported by their neighbors, the power of the clone! Yet some mosses, like ***Dawsonia*** (Figure 2), achieve heights exceeding 2 dm, with enough strength to maintain it alone.

Figure 2. ***Dawsonia superba***, the tallest stand-alone moss. Photo by Janice Glime.

In some cases, lignin-like compounds may add strength to the cellulose walls of the cells. But perhaps a new discovery may help in understanding how bryophytes maintain their strength. **Extensins**, previously known from tracheophytes, have just been found in mosses for the first time, in what else – ***Physcomitrella patens*** (Figure 3; Schipper *et al*. 2002). These glycoproteins, rich with hydroxyproline, comprise about 5-10% of the dry weight of most primary cell walls and serve to strengthen the walls (Taiz & Zieger 1991). Taiz and Zieger (1991) claim that tracheophyte fibers with a tensile strength similar to that of steel wire may gain their strength from the combination of both lignin and extensin. The importance of extensin to bryophyte strength remains to be demonstrated.

Figure 3. ***Physcomitrella patens*** in its natural habitat. Photo by Michael Lüth, with permission.

Bryophytes, with a very thin cuticle, if any, and leaves only one cell thick, easily lose water. Yet, there are about 15,000 species, more than any other group of plants besides flowering plants. How is it that they are able to survive in such harsh environments where they might completely dry out for months at a time? How do they live in places that **never** get any rainfall?

Then there is the problem of sexual reproduction, of transferring gametes from a male organ to a female organ when the male gamete, the sperm, requires water in order to swim! It seems that one of the best solutions was to produce gametes only when water was available, but that requires developing the gametangia well in advance of the fertilization event in order to be ready on time. Something has to trigger the plants to stop using all their energy for growth and put some of it into making gametangia. A method of receiving and responding to environmental signals was necessary.

Finally, these plants needed ways to get to new homes when theirs were being destroyed, whether by erosion, fire, or other unpredictable events. They needed reproductive structures that could travel in a medium of air and survive without water for a long period of time. Hence, they needed spores that did not swim and these needed a thick cover to prevent total desiccation.

All of these events had to be carefully controlled, timed to take advantage of seasons when water was available for fertilization and when dry air was available for spore dispersal. These "primitive" bryophytes have been successful at organizing their morphology, their biochemistry, and their life cycles in a way best suited to their individual environments.

For these organisms to complete their life cycles, a coordinated set of developmental stages and environmental signals must exist. If this coordination is lacking, the plant may find itself in a life cycle stage that has requirements the environment is unable to supply. Unlike animals, the plant cannot move to a new habitat when the going gets rough. When the spore lands and germinates, a bryophyte must be able to develop its protonema, produce a leafy gametophore, develop archegonia and antheridia, achieve fertilization, develop a sporophyte with a capsule, and disperse its spores without changing its location.

As we have studied the taxonomy of bryophytes during the last two centuries, numerous examples of life cycle adaptations have become apparent through our descriptions of the genera and species that grow in a variety of habitats. It is obvious that many strategies exist, from the **neotenous** (having juvenile traits retained in adults) habit of ***Buxbaumia*** (Figure 4) to produce sporophytes without developing an upright gametophyte, to the highly developed gametophyte of ***Fissidens obscurus***, where sporophytes are generally unknown. Some mosses readily form gametophores on nutrient-poor soil, such as the pioneer ***Funaria hygrometrica*** (Figure 1), whereas others such as ***Pylaisiella*** (Figure 25) seem to benefit from products of associated organisms (Spiess *et al*. 1971, 1972). Some rely predominantly on spores for dispersal, whereas others depend on abundant gemmae. Control of these life cycle differences depends on a complex evolutionary interaction with the environment to select the strategy that bests adapts the bryophyte to its particular set of circumstances.

Figure 4. ***Buxbaumia aphylla***, demonstrating the neotenous development of reproductive structures and ultimately a sporophyte without the development of a leafy gametophyte. Photo by Janice Glime.

While our understanding of development has been progressing since the early descriptive work of Goebel (1930) and Lorch (1931), so has our understanding of moss ecology. During (1979) began to bridge the fields of development and ecology by his presentation of life cycle strategies. He has suggested that the ability to occupy a habitat is dependent upon life span, type of reproduction, time required for maturity, spore size, spore longevity, and growth form. Based on the review presented by Bopp (1981) and knowledge of the importance of growth hormones in regulating development in higher plants, it is

possible now for us to consider the role of hormones during all stages of the life cycle. Reviews on developmental physiology by Bopp (1981), on biochemical constituents by Suire and Asakawa (1981), and recently the review on control of development by Christianson (2000a) begin to make it possible to evaluate environmental signals as they relate to known physiological responses that determine development.

Developmental Adjustments

Like some of the insects that can adjust their life cycle mid course, changing their developmental rates, at least some bryophytes likewise adjust their developmental periods based on seasonal and temperature effects. For example, ***Fontinalis squamosa*** (Figure 5) cultured in early May at 14º and 20°C required 18 days to germinate from tiny (10 µm), early season green spores. Capsules collected at the same time and stored at 10°C until late May provided spores that were larger (25 µm) and germinated under the same conditions in as few as 5 days (Glime & Knoop 1986). Capsules stored at 3°C until late May provided spores that generally failed to germinate, and those that did required a minimum of 15 days, failing to develop further.

In this case, spores shed prematurely apparently developed externally and took longer to germinate. Such adjustments suggest that under natural conditions at different latitudes the moss would have different responses, with the ones at colder temperatures being able to germinate more quickly when the critical temperature was reached, but at very cold temperatures, germination would generally not occur, thus protecting the protonema from potential freezing.

Figure 5. ***Fontinalis squamosa*** spore germinating. Photo by Janice Glime.

In a latitudinal study on ***Meesia triquetra*** (Figure 6), Montagnes and Vitt (1991) found that morphology varied in a linear way with latitude, with variances in characters decreasing with increasing latitude. Among the characters that decreased were annual growth increment, number of leaves produced each year, and leaf length. As leaf length decreased, leaf width increased with increasing latitude, and the tip was less acute, making a shorter, broader, more ovate leaf. However, unlike the insect larvae that are able to adjust their life cycles "on the fly," these morphological changes persisted in a **common garden** (where different populations are grown together with the same conditions), therefore suggesting that they are genetically controlled (Montagnes 1990) and most likely a product of natural selection.

Figure 6. ***Meesia triquetra***. Photo by Michael Lüth, with permission.

Polytrichum strictum (Figure 7) (Longton 1974) likewise had decreased leaf length as it grew farther north, and as expected, less annual growth in length and weight, and fewer leaves per annual growth increment (Figure 8). These factors seemed to be under both external and genetic control.

Figure 7. ***Polytrichum strictum*** from the temperate zone. Photo by Jan-Peter Frahm, with permission.

Figure 8. ***Polytrichum strictum*** from Alaska, USA, showing shorter plants and smaller leaves. Photo by Andres Baron Lopez, with permission.

Hylocomium splendens (Figure 9) varies so much that subspecies and varieties have been named. On the west coast of Canada, it grows in **wefts** (loosely interwoven, often ascending growth form), earning it the subspecies designation ***giganteum***, and has the typical stair-step frond (Figure 10; Montagnes & Vitt 1991). North of the tree line, where it is designated var. ***obtusifolia***, it lacks the stair-step character. The variety ***splendens*** is intermediate to these two taxa.

Figure 9. ***Hylocomium splendens*** in its typical weft form. Photo by Michael Lüth, with permission.

Figure 10. ***Hylocomium splendens*** showing stair-step growth form typical of the north temperate and boreal region. Photo by Janice Glime.

In summary, as demonstrated in ***Meesia***, ***Polytrichum***, and ***Hylocomium***, increasing latitudes can select for mosses with **shorter leaves**, cause **reduced annual growth**, **reduce** the **number of leaves** produced per year, and **change growth form** and **branching patterns**. These differences can be under environmental or genetic control, or both.

Life Cycle Importance

Bryophyte life cycles have stimulated the curiosity of botanists for centuries. Their simple representation of two clearly visible generations makes them choice organisms for introducing the concept of a life cycle to students. Because of their ease of expressing genetic effects, bryophytes have provided the laboratory material for pioneering breakthrough research in several areas of genetics and molecular biology, permitting us to understand not only bryophyte development, but paving the way for understanding tracheophyte development as well (Reski 1998; Schumaker & Dietrich 1998; Christianson 2000b). The first sex (X & Y) chromosomes were found in bryophytes, in ***Sphaerocarpos*** (Figure 11). The continuity of chromosomes during mitosis was elucidated in bryophytes. Discovery of non-Mendelian inheritance was first found in bryophytes. Furthermore, the haploid generation permits us to isolate gene mutations in order to determine their developmental roles.

Figure 11. ***Sphaerocarpos michelii***. Photo by Michael Lüth, with permission.

The moss ***Physcomitrella patens*** (Figure 3) has become the experimental rival of *Arabidopsis*, *Nicotiana*, and *Brassica*. Its most recent advantage is in **reverse genetics** (genotype-driven technique in which genes are either knocked out or added to see the effect on phenotypic expression), enabling geneticists and physiologists to understand gene function by targetting specific genes. Because the moss is haploid, it is much easier to isolate a mutant gene and determine its function. As this new information becomes available, understanding the role of the environment in regulating gene function, and ultimately in influencing development, will become much clearer.

We should expect a variety of geographic differences in the life cycle as well as differences influenced by the weather in a given year in one location. To understand and predict these differences, we must first understand the developmental ecophysiology. This requires that we understand the functions of hormones.

Growth Regulators

Hormones, or growth regulators, were originally defined for animals as substances that are produced in one part of the organism and move to another where they carry out their action, in very small quantities. This definition works less well for plants, wherein ethylene always and others sometimes are produced in their final step at the site of action. But plant hormones differ from those of animals in other major ways as well. They have a much wider array of actions than the limited action ability of most animal hormones (Christianson 2000a). (Or do those animal folks just not understand their hormones as well as the botanists understand theirs?) Rather, in plants the hormones usually act in combinations that present a wide array of possible outcomes. In plants, as in animals, every aspect of development involves hormones.

If hormones are within the organism, why should an ecologist even care to understand their nature and action? Hormones are often leaked into the environment by other

organisms and those external sources may even be necessary to the development of the plants. Plants both excrete hormones and are affected by external hormones (Beutelmann & Bauer 1977). Bryophytes are no exception to these external regulators. This makes the role of the environment of far more importance than for most animal hormone functions (human contributions not withstanding). While the number of hormones known in plants is small (Table 1), the importance of external hormones is poorly known, especially in bryophytes.

Consider for a moment what the bryophytes have been doing for their 400-million-year history. Limited in structure by their lack of lignin, they were not limited in any discernible way regarding their biochemical evolution. This has afforded them three times as long to perfect their development and biochemical adaptations compared to the Magnoliophyta (flowering plants) (Christianson 2000a). In fact, the very absence of large morphological adaptations has increased the selection pressures for cellular level biochemical ones (Christianson 2000a). Here we will examine what we do know about the hormones found in bryophytes.

Auxins

Auxins have long been known as plant growth hormones, and were conclusively demonstrated in bryophytes in 1985 (Law *et al*. 1985), but their mode of action is still not clearly understood. They are amino-acid based hormones, and through studies with Venus flytrap (*Dionaea muscipula*), we have discovered that they have a role in cell extension. This extension seems to be mediated by an efflux of H^+ that accumulates between the cells, breakage of the calcium pectate bonds that glue cell walls together, and appearance of Ca^{++} inside the cells in the area of rapid growth. Concomitant with these events, the auxin **IAA** (indole-3-acetic acid) increases in the region of growth (in this case, the lower side of the midrib). Using the moss ***Funaria hygrometrica*** (Figure 12*)*, Kapoor and Bhatla (1998) suggest that the influx of Ca^{++} to the cells may be induced by the IAA, although in this case it is in callose (complex, branched polysaccharide) synthesis that precedes the differentiation of chloronema (youngest part of protonema) to caulonema (part of protonema giving rise to leafy plants). IAA has a known role in this chloronema to caulonema transformation (Decker *et al*2006).

Table 1. Classes of growth regulators affecting bryophytes, their known presence in mosses and liverworts, and their known functions in that group.

Class	Specific Regulator	Presence	Function
auxins	IAA	mosses, liverworts	membrane transport (esp. Ca), cell elongation, protonema differentiation, stem elongation (promote at low, inhibit at high), rhizoid initiation, seta elongation, tropisms, apical dominance
cytokinins	zeatin	mosses	cell division, aging, bud initiation, archegonium initiation, gametophore production
	isopentenyladenine	mosses	
	Factor H?	mosses	inhibition of caulonema growth, bud initiation, gemma formation
	analogs	mosses, liverworts	promote thallus growth, slow aging, increase Ca in cell
gibberellins	gibberellin-like	?	development, promote growth, enhance antheridial development, decrease archegonial production
dormancy hormones	lunularic acid (LA)	liverworts	growth regulator, dormancy, drought tolerance, antiherbivory?
	abscisic acid (ABA)	mosses hornworts?	drought tolerance, growth form, capsule stomatal closure, gametophore bud inhibition; controls cytokinin response
ethylene	ethylene	mosses liverworts	development, leaf morphology, epinasty, cell elongation, color changes, response to substrate, senescence, suppression of 3rd row of leaves in liverworts, increased number of antheridia, chloronema to caulonema, inhibits seta elongation, may control gametophore bud development
acetylcholine		mosses, liverworts?	light response?; antiherbivory?; cellular regulation?
cryptochromes		mosses, liverworts?	protonema branching, gametophore induction, development, auxin control, photoperiodic responses

While the Venus flytrap provides the advantages of knowing where and when the growth response will occur, the number of responses of a single plant is limited, and the response is extremely rapid, making it difficult to obtain large amounts of data. The moss system provides a slower response that can be controlled by the researcher through externally applied auxin. As a single-cell-thick response system (leaf or protonema, Figure 12), the moss offers strong advantages over leaves or buds of tracheophytes, where any externally applied auxin must slowly penetrate the epidermis or other protective cells and substances. Because of these advantages, we are beginning to understand the role of IAA and calcium through the use of moss models.

Auxin activity seems to be an ancient character present when liverworts first emerged on land (Ishizaki *et al*. 2012). Ishizaki and coworkers demonstrated auxin activity at the bottom of gemma cups and junction of gametophyte and sporophyte in ***Marchantia polymorpha*** (Figure 13), suggesting its importance in actively dividing cells.

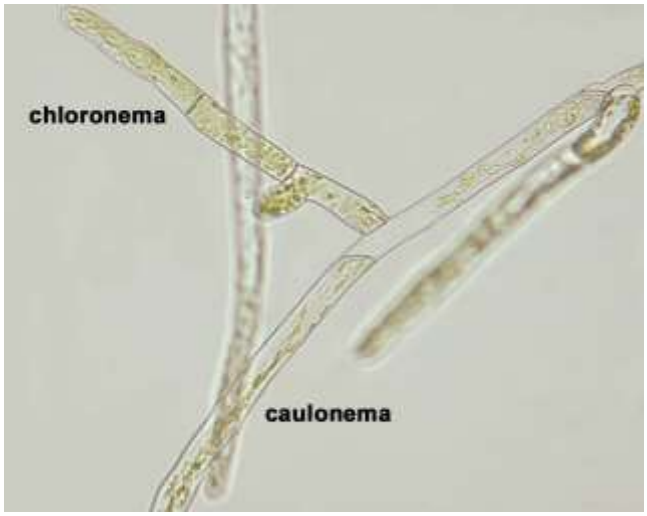

Figure 12. ***Funaria hygrometrica*** protonema with differentiation into chloronema (perpendicular cross walls) and caulonema (diagonal cross walls). Photo by Janice Glime.

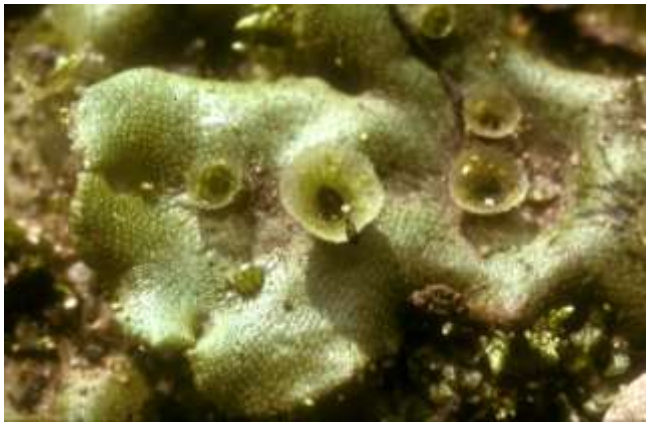

Figure 13. ***Marchantia polymorpha*** vegetative thallus with gemma cups. IAA moves basipetally (away from the tips) in this species. Photo by Janice Glime.

Our knowledge of the role of IAA in moss gametophores is still limited. We do know that the maximum concentrations are at the stem apex and base (Decker *et al.* 2006). The IAA seems to respond to changes in light quality, with red light retarding growth of protonemata but causing elongation of the gametophores, nevertheless making leaves shorter and narrower. Far red light enhances these responses (Bierfreund *et al.* 2003).

Thomas *et al.* (1983) demonstrated that IAA controlled seta elongation in the liverwort ***Pellia epiphylla*** (Figure 14). Although this and other studies provided indications of the presence of IAA in bryophytes, the first definitive HPLC (high-performance liquid chromatography) demonstration of its presence was published in 1985 by Law and coworkers in sterile culture of the liverwort ***Plagiochila asplenioides*** (Figure 15) subsp. ***arctica***. The natural auxin is **indole-3-acetic acid** (IAA), which is produced in the stem and branch tips of higher plants, and among bryophytes the same apical production is indicated in ***Marchantia*** (Maravolo 1976; Gaal *et al.* 1982). Due to its polarity, IAA moves **basipetally** (toward the base), as demonstrated in ***Marchantia polymorpha*** (Figure 13) by Maravolo (1976, 1980), where it travels in the midrib. Its transport is inhibited by aging and ethylene.

Figure 14. ***Pellia epiphylla***, a species in which IAA controls seta elongation. Photo by Malcolm Storey, through Creative Commons.

Figure 15. ***Plagiochila asplenioides***, a liverwort in which the presence of IAA has been demonstrated. Photo by Dick Haaksma, with permission.

In mosses, we know that early development is triggered by the auxin IAA working with **cytokinin** (another hormone) and requiring light that acts through the mediation of **phytochrome** (pigment sensitive to photoperiod) and a blue light receptor (Reski 1998), possibly **cryptochromes**. Auxins respond to light and gravity and thus provide a means for plants to grow in the right direction relative to the Earth. Their mode of action is still controversial, despite extensive research into their movements within plants and plant responses.

IAA seems to be essential for normal stem elongation (Bidwell 1979). When researchers removed the tips of actively growing tracheophytes, growth stopped. If they applied IAA, growth continued. On the other hand, at least in flowering plants, removal of the stem apex can promote growth of the branches, which were heretofore inhibited by the IAA during its downward movement. Similar reactions seem to occur in at least some mosses, as exhibited by the **innovations** (new ascendant branches near the shoot tip; Figure 16) of mosses following **gametangial senescence** (*i.e.* loss of gametangial function with aging), but experimental evidence of the IAA connection in bryophytes seems to be lacking.

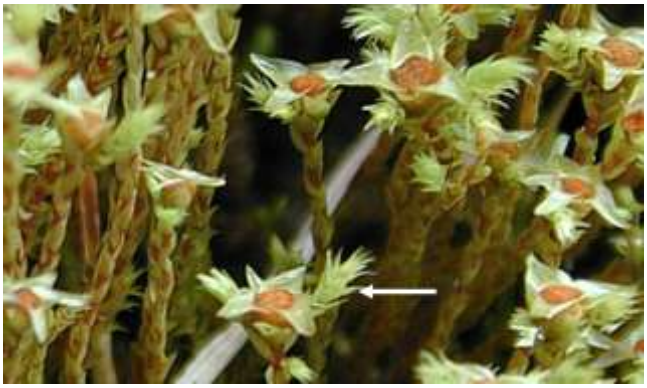

Figure 16. Innovation (arrow) beneath senescing antheridial head of ***Philonotis caespitosa***. Photo by Michael Lüth, with permission.

Auxins play major metabolic roles. IAA, in particular, seems to play a role in membrane transport; Lüttge and coworkers (1972) demonstrated that IAA can enhance leaf uptake of potassium by ***Mnium*** from both KCl and K_2SO_4. Inhibition of IAA by TIBA (2,3,5-triiodobenzoic acid; polar auxin transport inhibitor) reduces starch accumulation at night and disrupts meristem polarity in the thallose liverwort ***Riella helicophylla*** (Figure 17) (Stange 1985). The role of IAA in cell extension is still unclear, but perhaps it again plays a metabolic role in the transport of substances across the cell membrane, particularly calcium, thus increasing the osmotic potential of the cell.

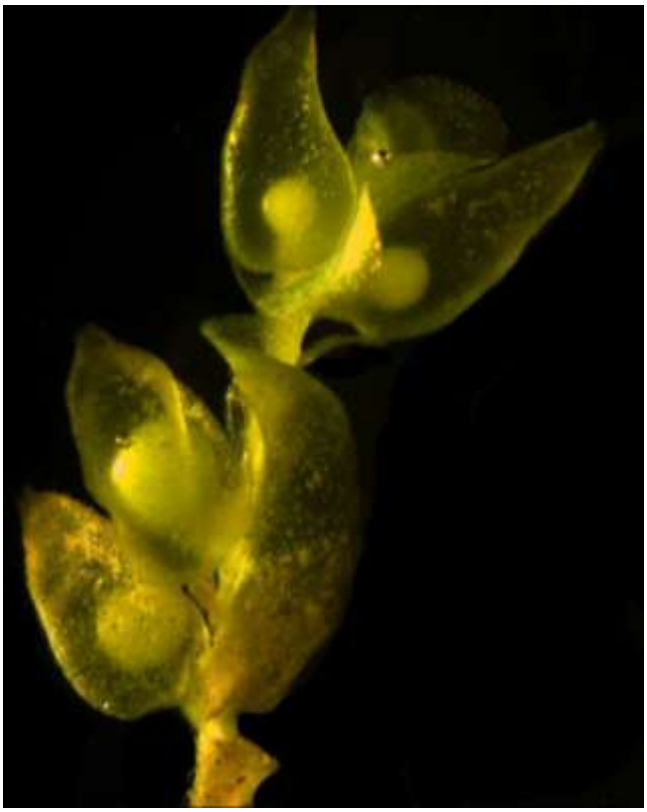

Figure 17. ***Riella helicophylla***, a liverwort where polar auxin transport causes reduction in nighttime starch accumulation and disruption of meristem polarity. Photo from NACICCA, through Creative Commons.

Auxins promote stem elongation at low concentrations and inhibit it at high ones, presumably due to induction of ethylene (Goodwin & Mercer 1983), and concentrations that promote growth in one part of a plant may inhibit it in another. In reviewing the body of literature on auxins in both non-tracheophytes and tracheophytes, Cooke and coworkers (2002) were surprised to find bryophytes exhibited most of the same physiological mechanisms for regulating IAA and for IAA-mediated responses as did the tracheophytes. These responses include **tropisms**, **apical dominance**, and **rhizoid initiation**. Both charophytes (the likely progenitors of bryophytes) and liverworts synthesize IAA via the tryptophan-independent pathway, regulating IAA levels through a balance between the rates of IAA biosynthesis and IAA degradation. All other land plants use the same pathway, but seem to have more precise spatial and temporal control through additional hydrolysis reactions. Although charophyte tips are apparently not sensitive to polar IAA transport inhibitors, both moss and liverwort gametophytes and moss sporophytes carry out polar transport, but sensitivity to the transport inhibitors differs within these groups.

The small quantities in which auxins are present in plants, combined with the small size of bryophytes, have made detection difficult. Their presence was indicated at least as early as 1963 when Cox and Westing demonstrated it in peat extracts. Despite its nanoconcentrations, Bhatla and Dhingra-Babbar (1990) report the presence of IAA in the protonemata of ***Funaria hygrometrica*** (Figure 12), ***Physcomitrella patens*** (Figure 3), and ***Polytrichastrum formosum*** (Figure 18), where it seems to be involved in differentiation. Many researchers (Cove *et al*. 2006; Von Schwartzenberg 2009) consider ***Physcomitrella patens*** to be a potential model system for study of this and other hormones because we now know its genome and can use gene knockout to determine the functions of the genes and ultimately the functions of the hormones.

Figure 18. ***Polytrichastrum formosum***, a moss in which IAA seems to be important in differentiation. Photo by David T. Holyoak, with permission.

Cytokinins

Cytokinins are important in bud formation. Using ***Physcomitrella patens*** (Figure 3) as a model, we can observe that the apical cell of the protonema divides (Reutter *et al*. 1998). When bud development begins, some of the subapical cells produce three-faced apical cells. These are the buds that will develop into the **gametophores**

(leafy shoots). Application of cytokinin enhances bud formation, but the buds often do not develop further. The moss ***P. patens*** produced isopentenyl-type cytokinins, whereas the zeatin-types produced by **tracheophytes** (non-bryophyte plants) were absent.

Cytokinins in bryophytes remained elusive until very recently because of their low concentrations. Cytokinins form another class of hormones that generally cause cell division (mitosis). Higher plants contain various **endogenous** cytokinins (produced within plant), such as zeatin, and scientists have identified many other compounds that act as cytokinins, such as kinetin and benzyl adenine. Unlike IAA, cytokinins travel to the tip of the protonema and accumulate there. Only two cytokinins (zeatin, isopentenyladenine) had been identified in bryophytes by 1979, both from protonemata (Cove *et al*. 1979, Gerhauser unpubl.). By 1990, there were indications that a third exists (Bhatla & Dhingra-Babbar 1990). Now we know that at least 20 of the 40 known cytokinins exist in the moss ***Physcomitrella patens*** (Figure 3), the most abundant of which are cis-Zeatin-riboside-O-glucoside, N6-(Δ2-isopentenyl)adenosine-5′-monophosphate (iPRMP), and trans-zeatin-riboside-O-glucoside as intracellular hormones (von Schwartzenberg *et al*. 2007).

The ability of cytokinins to affect Developmental changes in gametophores has been demonstrated experimentally. Chopra and Sood (1973) have shown that the cytokinin analog **kinetin** promotes growth of thalli in ***Riccia crystallina*** (Figure 27), but it also enhances archegonial formation. Vashistha (1987) likewise found that three different cytokinins applied to the liverwort ***Riccia frostii*** (Figure 19) stimulated vegetative growth and archegonial induction. Besides cell division, this hormone group can prevent or slow aging and cause changes in sex expression in higher plants (Kahn 1971). Cytokinins seem to cause the increase of calcium in the cell and together with calcium may cause an increase in ethylene. Magnesium ions seem to antagonize this calcium transport.

Figure 19. ***Riccia frostii***, a liverwort that responds to cytokinin in the medium. Photo by Rosemary Taylor, with permission.

Mosses respond differently to different concentration levels of cytokinins (Reski & Abel 1985). Among protonemata, only the chloronemata respond to low cytokinin concentrations, At high concentrations, only the caulonemata responded by increased bud formation. Hence, there is a specificity among cells in the concentrations to which they respond.

Reutter *et al*. (1998) were able to connect specific genes with their functions by using transgenic ***Physcomitrella patens*** (Figure 3). Using mutants that were unable to accomplish specific developmental tasks, they showed that cytokinins were able to supply the necessary signals for these events to occur (Figure 20).

In some cases, an outside source is needed to catalyze the production of cytokinins. For example, ***Agrobacterium tumefaciens*** (Figure 21) has the isopentenyl transferase gene that is needed to catalyze the first step in the biosynthesis of cytokinin (Decker *et al*. 2006). For some mosses, this bacterium is needed for development to go from the protonema to gametophore stage. Reutter *et al*. (1998) found that the moss ***Physcomitrella patens*** (Figure 3) responds differently to the same cytokinin when it is internal (**endogenous**) vs external (**exogenous**), and that most of both cytokinin and auxin is outside the moss (Reutter *et al*. 1998; Ralf Reski, pers. comm. 19 September 2013). Reutter *et al*. (1998) suggest that this external presence may permit translocation of the hormones in the bryophytes.

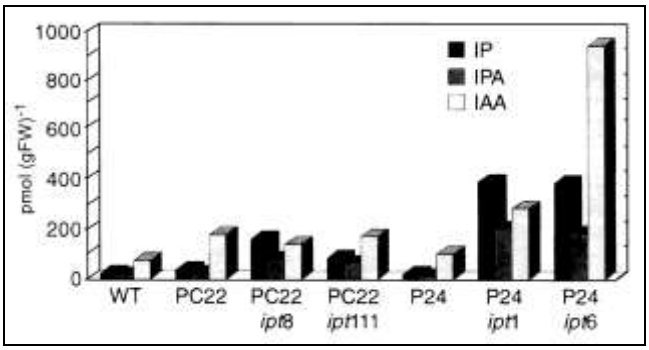

Figure 20. ***Physcomitrella patens*** hormonal contents. WT=wild type, PC22 = mutant defective in gametophore development and plastid division, P24=mutant that does not produce buds, *ipt*=gene of respective transgenic plant. Y axis is the immunoreactive IP, IPA, and IAA equivalent [pmol $(gFW)^{-1}$] in 9-day-old plants in liquid culture. Note that hormone levels are elevated in all the *ipt* transgenics. Redrawn from Reutter *et al*. 1998.

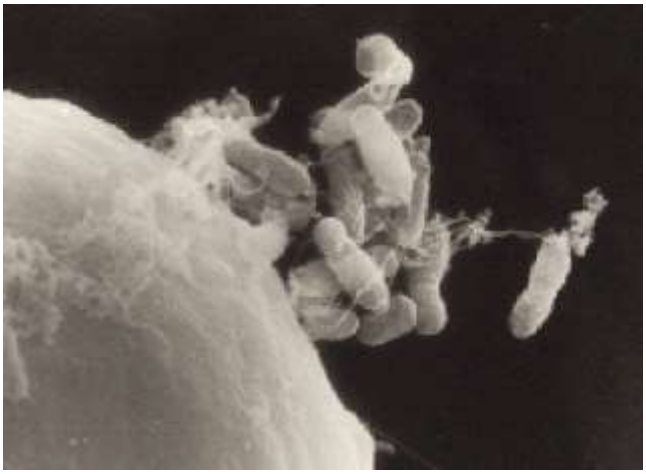

Figure 21. ***Agrobacterium tumefaciens*** on a carrot, a species known to provide hormones to mosses in nature. Photo through Creative Commons.

External application of cytokinins cause ***Physcomitrella patens*** (Figure 3) to develop abnormally, causing bud production without leafy gametophore development and becoming necrotic (Reutter *et al*. 1998). On the other hand, transgenic mutant mosses with the added bacterial ipt gene were able to develop normally with the internal production of cytokinins.

Cytokinins may have important roles in responding to the environment (Lorenz *et al*. 2003). For example, it seems to have a role in the change from juvenile tissue growth to sexual reproduction under high-energy conditions (exogenous carbohydrates or bright light). Thelander *et al*. (2005) found that high-energy conditions resulted in pronounced caulonema formation. Low energy conditions, resulting from low light, short days, or low temperatures, stimulate development of gametangia and subsequent development of sporophytes (Hohe *et al*. 2002).

The limited number of cell types, ability to regenerate from small fragments, and ease of cultivation of the entire life cycle in the laboratory makes bryophytes good experimental organisms for study of the functioning of cytokinins (von Schwartzenberg 2006). And the fully mapped genotype of ***Physcomitrella patens*** (Figure 3) provides us with an ideal study organism. Von Schwartzenberg *et al*. (2007) found that the nucleotide iPRMP is the most abundant extracellular cytokinin in ***Physcomitrella patens***. By using cytokinin oxidase/ dehydrogenase (CKX)-overexpressing plants, von Schwartzenberg and coworkers observed reduced and retarded budding, absence of sexual reproduction, and abnormal protonema cells. Extracellular IP and IPR seem to be the primary cytokinins responsible for inducing buds in ***P. patens***. Control of levels is undoubtedly important.

^{14}C-labelled adenine has also shown up in cytokinin in the culture medium of ***Physcomitrella patens*** (Figure 3), indicating a similar role of adenine in production of cytokinin (Bhatla & Dhingra-Babbar 1990). A similar, perhaps same, substance in ***Bryum klinggraeffii*** (Figure 22) inhibits growth and stimulates gemma formation. Because it leaks into the medium, this substance could have interactive effects on other species of mosses and even control its own population size. More recently, Proust *et al*. (2011) found that **strigolactones** regulate the branching of protonemata in ***Physcomitrella patens*** and act as **quorum sensors** – a way of signalling that no more bryophytes should be added there. Hence, the strigolactones inhibit the growth of both that protonema and that of neighboring colonies.

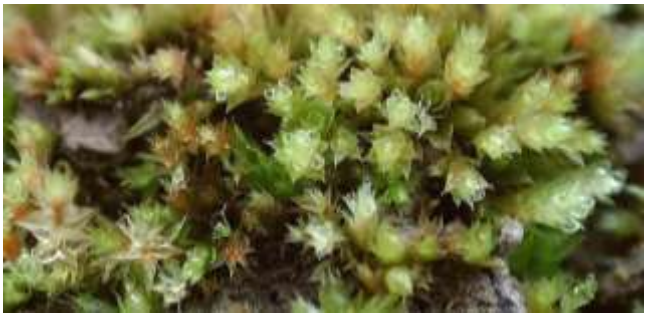

Figure 22. ***Bryum klinggraeffii***, a moss in which a cytokinin-like substance leaks into the environment and inhibits growth while promoting gemma formation. Photo by Michael Lüth, with permission.

Based on the work of Bopp (1963, 1968), Watson (1981) suggested that it could be the inhibitory properties of a hormone (Factor H – see below) that caused differing aggressive patterns among juvenile ***Polytrichum*** *s.l.* (Figure 7-Figure 8; Figure 18) species, thus affecting ultimate community structure. Perhaps more important is the effect of controlling simultaneous production of buds in the population so that they develop together and conserve moisture by creating a smooth surface. This same control would prevent them from over-shadowing one another, avoiding intra-specific light competition.

It seems that the moss need not produce its own cytokinin. Rather, it may serve as host to bacteria that produce this hormone. In ***Funaria hygrometrica*** (Figure 1), the bacterium ***Methylobacterium*** (Figure 23) is epiphytic on the moss, inhabiting leaf surfaces, especially in the grooves between adjacent leaf **lamina** cells (cells of the blade portion of the leaf, exclusive of costa) (Hornschuh *et al.* 2002). In the presence of these bacteria on agar cultures, the protonema produces buds just as it would in the presence of cytokinin, and the exudate also stimulates the growth of the protonemal filaments. Glime and Knoop (1986) suggested a similar relationship in ***Fontinalis squamosa*** (Figure 24), wherein the only protonemata cultures that produced buds on a mineral nutrient medium were the ones that became contaminated with bacteria and fungi.

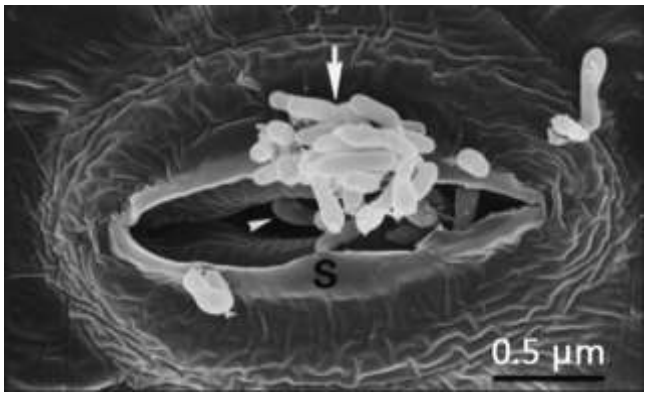

Figure 23. ***Methylobacterium*** sp., a possible source of cytokinins for mosses, on sunflower stoma. Photo by Kutschera U., through Creative Commons.

Figure 24. ***Fontinalis squamosa*** protonema. Photo by Janice Glime.

One aspect of the life cycle that will be discussed in other chapters is the production of asexual structures, a feature that is rare among **tracheophytes** (non-bryophyte plants). One example of this unique phenomenon is the discovery of protonemal gemmae in the aquatic moss ***Fontinalis antipyretica*** (Ares *et al*. 2014). In this species, where capsule production is relatively rare, vegetative shoots are important dispersal units. These dispersal units can come from detached cortical cells, margins and abaxial (away from the stem) surfaces of leaves, leaf laminae, and stems with leaves removed. Likewise, the protonema can continue growth from the filament or its rhizoids. But the discovery by Ares *et al*. is that these protonemata can also produce filamentous gemmae and spherical brood cells. These occur as the cultures age or dry out. Thus in nature they are produced as streams dry and water levels drop, providing a means of surviving these unfavorable periods. It is interesting that bacteria and fungi in the cultures (and in nature) seem to play a role in this development. but at this point in time we do not know what that biochemical interaction may be or how the drying of the environment may trigger the formation of propagules on the protonema.

One of the cytokinins that is effective on bryophytes is produced by the bacterium ***Agrobacterium*** (Figure 21). It appears that flowering plants lack the gene for this cytokinin, but evidence suggests that mosses may in fact possess it, and furthermore, ***Agrobacterium*** in the environment may supply it to some mosses. Addition of ***Agrobacterium tumefaciens*** (Figure 21) to the medium can stimulate the production of gametophores in ***Pylaisiella selwynii*** (Figure 25; Spiess *et al*. 1971), an epiphyte. The presence of this bacterium with the moss on tree bark suggests its possible role in the development of ***Pylaisiella selwynii*** in that habitat.

Figure 25. ***Pylaisiella selwynii*** growing on bark where it encounters the bacterium ***Agrobacterium tumefaciens***, which most likely contributes to its production of gametophores there. Photo by Janice Glime.

Factor H

A possible cytokinin known as **Factor H**, an adenine derivative (Bhatla & Dhingra-Babbar 1990)**,** has been known for much longer as a stimulant for increasing the number of gametophore buds (Klein 1967; Brandes & Kende 1968). **Factor H** has been isolated from the culture medium of ***Funaria hygrometrica*** (Figure 1) and from tissue extracts of several other mosses (Bhatla & Dhingra-Babbar 1990). Its roles in inhibiting caulonema growth and promoting bud formation are clear, thus resembling the behavior of a cytokinin. Christianson (1998b) discovered that not all mosses have the same "Factor H."

Although the experiments mentioned above suggest that mosses respond to this hormone from other species, ***Ceratodon purpureus*** (Figure 26) is not affected by this substance from ***Funaria hygrometrica*** (Figure 1), nor is it able to affect the development of ***Funaria hygrometrica***, but ***Ceratodon*** does exhibit interspecific regulation. Its growth substance does not pass through a dialysis membrane, whereas factor H does.

Figure 26. ***Ceratodon purpureus***, a species that is not affected by "factor H" from neighboring species. Photo by Michael Lüth, with permission.

In 1980, Bopp determined that Factor H not only is not a cytokinin, it is not a cytokinin-like substance. But in 2013, Ralf Reski assured me it is most likely a cytokinin. Its identity seems still to be unknown. It does seem to carry out some of the functions we might attribute to a cytokinin.

The Factor H that has made medical news lately (Büttner-Mainik *et al*. 2011) should not be confused with the natural Factor H produced by bryophytes. The moss ***Physcomitrella patens*** (Figure 3), through recombinant DNA, is able to make the human complement regulatory serum protein Factor H – a substance that can assist in treatment of human diseases, including severe kidney and retinal disorders. It is a cheaper solution that does not involve the need for animals to manufacture the compound.

Gibberellins

Gibberellins (GA) are terpenoid-based hormones (Harborne 1982) that can stimulate stem elongation as well as cell division, depending on the species involved (Bidwell 1979). Gibberellins, unlike auxins, are non-polar and free to move about all over the plant. In studying ***Marchantia polymorpha*** (Figure 13) Melstrom and co-workers (1974) isolated three gibberellin-like substances from the thalli. Chopra and Sood (1973) found that gibberellins could enhance antheridial formation while promoting normal growth in the thallose liverwort ***Riccia crystallina*** (Figure 27). Chopra and Kumra (1986) later found that GA_3 not only enhanced normal growth of ***Riccia gangetica***, but also increased the production of antheridia while causing a decrease in archegonial production.

Figure 27. ***Riccia crystallina***, a species in which gibberellins can enhance antheridial formation while promoting normal thallus growth. Photo by David T. Holyoak, with permission.

However, Bhatla & Dhingra-Babbar (1990) reported that gibberellins still are not confirmed in mosses, although GA-like substances are known in both mosses and liverworts (Chopra & Kumar 1988). Even recent studies have failed to confirm the presence of GA in bryophytes, with the "lab rat" ***Physcomitrella patens*** (Figure 3) failing to respond to gibberellic acid (Hiranoa *et al*. 2007). It appears that GID1/DELLA-mediated GA signaling arose in tracheophytes after they diverged from the bryophyte lineage (Hiranoa *et al*. 2007; Yasamura *et al*. 2007). On the other hand, Ergün *et al*. (2002) demonstrated that at least some bryophytes can produce not only IAA, ABA and zeatin, but also GA_3. Furthermore, the production of GA in mosses should be expected, since its presence is known in algae (Radley 1961; Mowat 1965; Tietz & Kasprik 1986; Tietz *et al*. 1989; Hirch *et al*. 1989).

Gibberellic acid is the hormone responsible for giant growth. I can remember that in my high school years Burpee was experimenting with it on horticultural flowers and encouraged seed buyers to try it and report the results. It didn't do much for my poor flowers in terrible soil. Could the absence of this hormone be part of the reason bryophytes have remained small?

Even if GA is absent in bryophytes, that does not necessarily mean that mosses cannot respond to it. Indeed, the fungi could deliver GA to the mosses and thus facilitate or interfere with development, perhaps accounting for bryophyte specificity to certain habitats. Certainly the presence and use of gibberellins in bryophytes is worthy of further exploration.

Abscisic Acid

Abscisic acid (ABA) is known not only in plants, but also in bacteria, animals, and elsewhere (Hartung 2010; Takezawa *et al*. 2011). It is therefore an important hormone to understand. The moss ***Physcomitrella patens*** (Figure 3) once again provides a suitable organism in which to study its functions. In this, and other bryophytes, it is known to respond to stress, including desiccation (Mayaba *et al*. 2001) and cold tolerance (Minami *et al*. 2003, 2005). In ***Atrichum androgynum*** (Figure 29) this desiccation tolerance seems to be accomplished by increasing the concentration of soluble sugars. In ***Physcomitrella patens*** (Figure 3), 22 genes are activated by ABA, and part of its role appears to be in the period of recovery from desiccation (Khandelwal *et al*. 2010).

The role of ABA in development seems to be ambiguous (Hartung 2010). Nevertheless, high levels of ABA seem to be present in organs of bryophytes that produce sporophytes.

Abscisic acid (**ABA**) is a sesquiterpenoid (15-C compound) that is partially produced via the mevalonic pathway in chloroplasts and other plastids. Therefore, synthesis occurs primarily in the leaves. It appears to be an indirect product in the synthesis of **carotenoids** (yellow to red lipid-soluble pigments). It has a variety of roles in both tracheophytes and bryophytes. In tracheophytes, it is important in regulating transpiration, stress responses, germination of seeds, and embryogenesis. Its most widespread function is in signalling water stress and regulating water loss. Interaction with other hormones gives it a role in most plant developmental processes.

ABA has been confirmed relatively recently in bryophytes, in the protonema of ***Funaria hygrometrica*** (Figure 12) (Bhatla & Dhingra-Babbar 1990; Werner *et al*. 1991). Its presence was unknown in liverworts (Gorham 1990) until 1994 (Hartung *et al.* 1994). However, there are indications that it is present in all bryophytes – at least all that have been tested (Hartung *et al*. 1994). It is known to inhibit the cytokinin-stimulated response of bud induction in the moss ***Funaria hygrometrica*** (Figure 1), making cytokinin a useful bioassay tool for detecting not only the presence but also the concentration of **ABA** (another hormone), since the inhibition is concentration dependent (Christianson 2000b).

The highest concentrations in bryophytes occur in species adapted to dry environments, and conversely, the lowest concentrations in aquatic species, suggesting it had a role in drought tolerance (Hartung *et al*. 1994). For example, in ***Funaria hygrometrica*** (Figure 12) , it makes the protonema drought resistant and in the Marchantiales it induces drought tolerance in the thallus. Burch and Wilkinson (2002) used it to ensure drought tolerance for long-term storage of ***Ditrichum cornubicum*** (Figure 28) protonemata, reducing membrane damage suffered during dehydration and freezing, and providing 100% recovery upon rehydration.

Figure 28. ***Ditrichum cornubicum***, a moss in which ABA has been used to ensure drought tolerance for long-term storage, apparently through accumulation of sugars. Photo by David T. Holyoak, with permission.

The use of ABA for cryopreservation reduces both labor and loss of plant material in ***Ceratodon purpureus*** (Figure 26), ***Funaria hygrometrica*** (Figure 1), ***Physcomitrella patens*** (Figure 3), and ***Sphagnum*** spp. (Christianson 1998a). There are likewise genetic implications for its presence, with 11 expressed sequence tags matching up with tracheophyte stress response genes, "including responses which may involve ABA" (Machuka *et al*. 1999). In ***Atrichum androgynum*** (Figure 29), application of ABA prior to desiccation reduces membrane leakage (Beckett 1999). It appears that this drought tolerance mechanism may be similar to that in higher plants under stress, with ABA reducing membrane damage by reducing the changes in membrane lipids (Guschina *et al*. 2002). On the other hand, ABA does not endow all bryophytes with desiccation tolerance. ***Plagiochila*** (Figure 15) shows no response, and ***Marchantia polymorpha*** (Figure 13) requires both ABA and encapsulation in alginate (sticky gum) beads for successful cryopreservation (Pence 1998). Furthermore, in the desiccation-tolerant ***Syntrichia*** (Figure 30), desiccation tolerance is not under ABA control, despite a large number of desiccation-response genes (Oliver 1996).

But what is the role of ABA in development? Decker *et al*. (2006) found that under the influence of ABA the protonematal subapical cells differentiate into round, short cells (**brachycytes**) or **tmema** cells (short-lived abscission cells), the latter being nearly free of cytoplasm (Figure 31). Thus, ABA has a role in asexual reproduction of the protonema. We know that in ***Funaria hygrometrica*** (Figure 1), when the ABA is removed, these short, round cells (**brachycytes**) germinate and form new protonemata (Schnepf & Reinhard 1997). The role of ABA is at least in part that of restructuring the cell walls of the protonema (Schipper *et al*. 2002; Decker *et al*. 2006).

Figure 29 ***Atrichum androgynum***, a moss in which membrane leakage is reduced by ABA application. Photo by Tom Thekathyil, with permission.

One interesting role of ABA is its ability to convert the aquatic (floating) forms of ***Riccia fluitans*** (Figure 32) and ***Ricciocarpos natans*** (Figure 34) into their terrestrial forms (Figure 33, Figure 35; Hartung *et al*. 1994). In ***Riccia fluitans***, ABA causes changes in the gene expression that cause the nearly filamentous floating form to become the broadly thallose soil form (Hellwege *et al*. 1996). This mechanism may be similar to that seen in the aquatic fern *Marsilea quadrifolia* in which ABA induces changes from aquatic to aerial leaf forms (Hsu *et al*. 2001).

Figure 30. ***Syntrichia ruraliformis*** on sand dunes at Harlech, Wales. This is a desiccation-tolerant moss whose tolerance is not controlled by ABA. Photo by Janice Glime.

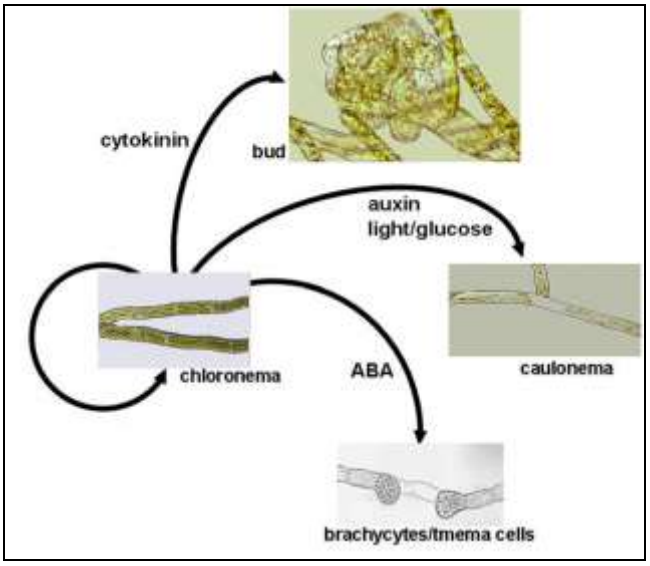

Figure 31. Hormone pathways in the cell cycle for protonemata. Modified from Decker *et al*. 2006.

Figure 32. ***Riccia fluitans*** floating form. Photo by Janice Glime.

Figure 33. ***Riccia fluitans*** soil form. Photo by Michael Lüth, with permission.

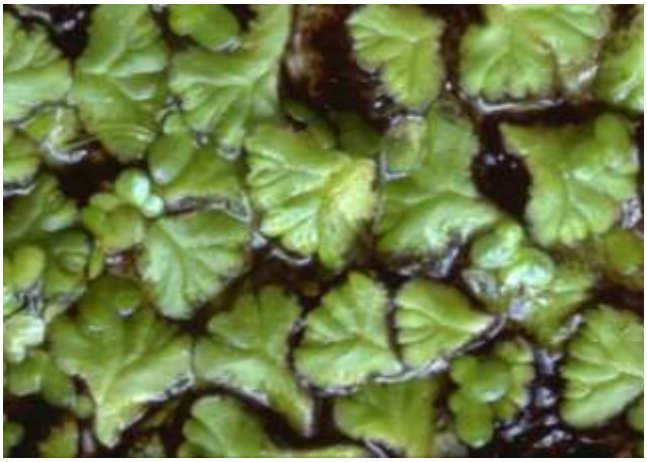

Figure 34. ***Ricciocarpos natans*** floating form. Photo by Jan-Peter Frahm, with permission.

One more important role of ABA in tracheophytes is the movement of K^+ out of guard cells of leaves, causing them to close, and suggesting that it might control membrane permeability. It is interesting that it likewise induces the closure of stomata in capsules of mosses and in Anthocerotophyta (hornwort) sporophytes (Hartung *et al*. 1994). ABA also seems to play a role in regulation of extracellular protein secretion (Decker *et al*. 2006).

Figure 35. ***Ricciocarpos natans*** soil form. Photo by Janice Glime.

It is not unusual for desiccation-tolerant species to also be cold/freezing tolerant. Nagao *et al*. (2005) found that The transformation from starch to sugar in chloroplasts is associated with ABA-induced freezing tolerance in protonemata of ***Physcomitrella patens*** (Figure 36), changing the LT50 from -2°C to -10°C. Compared to untreated cells, ABA-treated cells had more slender chloroplasts and a reduced starch grain content. Instead of one central vacuole, the treated cells often had multiple segmented vacuoles. At -4°C the untreated cells had lesions in the cell membranes; the treated cells did not. Osmotic concentration increased as sugars accumulated.

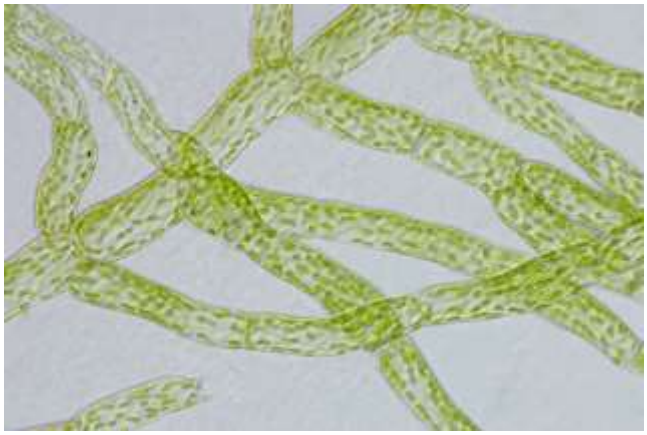

Figure 36. ***Physcomitrella patens*** protonema, a common research organism for hormone studies. Photo from Ralf Reski Lab, through Creative Commons.

Wang *et al*. (2011) found 65 proteins that responded to ABA in ***Physcomitrella patens*** (Figure 3; Figure 36). This involved down regulation of 13 proteins and upregulation of 52 proteins, 4 of which were newly induced. The roles of these proteins included material and energy metabolism, defense, protein destination and storage, transcription, signal transduction, cell growth/division, transport, and cytoskeleton. Most of the up-regulated proteins functioned as molecular chaperones, transcriptional regulators, and defense proteins. Thus the ABA was able to trigger responses that served in defense and protection from oxidative damage. They also learned that ***Physcomitrella patens*** responds to exogenous (applied externally) ABA. This latter response permits them to respond to other organisms in the environment. They found that ABA could inhibit photosynthesis, a phenomenon that suggests we should look at the ability of other organisms to compete with them with this hormone. Such an inhibition may prevent spores from germinating on leaf litter that is leaking ABA. This would seem like an interesting field for ecological research.

Lunularic acid

We know that a hormone similar to ABA, **lunularic acid** (LA), first discovered in ***Lunularia cruciata*** (Figure 37; Schwabe & Nachmony-Bascomb 1963), is present in at least the 47 genera (80 species) of liverworts examined by Gorham (1975, 1990) and is important in dormancy and growth regulation (Schwabe 1990). More recently it has been identified in ***Plagiochila spinulosa*** (Figure 38) (Connolly *et al*. 1999), ***Ricciocarpos natans*** (Figure 34-

Figure 35) (Kunz & Becker 1992), ***Frullania convoluta*** (Flegel *et al*. 1999), and ***Marchantia polymorpha*** (Figure 13) (Friederich *et al*. 1999).

Figure 37. ***Lunularia cruciata*** with gemmae pockets. Photo by Martin Hutten, with permission.

Figure 38. ***Plagiochila spinulosa***, a leafy liverwort known to produce lunularic acid. Photo by Michael Lüth, with permission.

Although reputedly unique to liverworts, this hormone has recently been found in *Hydrangea macrophylla*, a flowering plant (Eckermann *et al*. 2003). In liverworts, the largest amounts of LA occur in dormant and desiccation-resistant thalli (Chopra & Kumar 1988) and its presence confers drought resistance (Schwabe & Nachmony-Bascomb 1963; Schwabe 1972), reminiscent of ABA. Part of this resistance is the initiation of dormancy, an effect that is greater at higher temperatures (Schwabe 1990). Nevertheless, Gorham (1990) found that lunularic acid does not affect stomatal conductance, suggesting that its effect on cells is different from that of abscisic acid.

Lunularic acid is compartmentalized (localized) within cells, hence restricting its function (Gorham 1977), although Imoto and Ohta (1985) found that it is equally distributed between vacuoles and cytoplasm in ***Marchantia polymorpha*** (Figure 13), and that it does not accumulate in chloroplasts, mitochondria, or peroxisomes. Gorham (1977) found it in all organs of ***Marchantia*** and ***Preissia*** (Figure 39), in sporophytes of ***Pellia epiphylla*** (Figure 14), and in the greatest concentration (more than 600 µg/g fresh weight) in young thallus tips of ***Conocephalum conicum*** (Figure 40) grown in continuous light. Higher light intensities increased its concentration; age decreased it. Continuous light caused a greater production of both growth and lunularic acid in thallose liverworts than in any photoperiod interrupted by darkness, creating a condition in which lunularic acid was not inhibitory. Leafy liverworts of the Jungermanniales contained smaller quantities (1-50 µg/g fresh weight) than did the thallose species tested.

Figure 39. ***Preissia quadrata***, a liverwort known to have lunularic acid in all its organs. Photo by Jan-Peter Frahm, with permission.

Figure 40. ***Conocephalum conicum*** showing growing tips where concentration of lunularic acid. Photo by Jan-Peter Frahm, with permission.

Because of its dormancy effect, lunularic acid could act as a growth inhibitor. However, compared to its analogs, this hormone is less effective in inhibiting growth of the liverwort ***Marchantia polymorpha*** (Figure 13) and the flowering plants *Nasturtium officinale* (water cress) and *Phleum pratense* (timothy grass) (Nakayama *et al*. 1996), but is known to inhibit growth in ***Lunularia cruciata*** (Figure 37) (Yoshikawa *et al*. 2002).

Lunularic acid forms a variety of conjugates (Kunz & Becker 1992). Among these are glycosides, suggesting an antiherbivory role as well. This suggestion is supported by Wurzel and coworkers (1990) who found, in ***Ricciocarpos natans*** (Figure 34-Figure 35), molluscicidal behavior against *Biomphalaria glabrata*, a snail that carries schistosomiasis (parasitic disease caused by blood fluke).

Research on lunularic acid in this century is scarce, but we still have much to learn about its role in liverworts.

Ethylene

Ethylene (C_2H_4) is important in every step of the developmental process of higher plants (Abeles 1973), and has been demonstrated in both liverworts (Fredericq *et al*. 1977; Thomas *et al*. 1983) and mosses (Rohwer & Bopp 1985). It is known from the sporophyte of ***Pellia*** (Figure 14), especially during rapid seta elongation (Thomas *et al*. 1983) and from the thallus of ***Marchantia*** (Figure 13) (DeGreef *et al*. 1981). However, Stange and Osborne (1989) found that the liverwort ***Riella*** (Figure 17) appears to have a different pathway for ethylene synthesis from that of higher plants.

Ethylene is an unsaturated hydrocarbon synthesized in tracheophytes via the following pathway:

$$\text{Methionine} \rightarrow \text{SAM} \rightarrow \text{ACC} \rightarrow C_2H_4$$

IAA is possibly the catalyst for the conversion of SAM (S-adenosylmethionine) to ACC (1-aminocyclopropane-1-carboxylic acid) (Bradford & Yang 1980a), as suggested by the 10-fold increase in ethylene obtained when 10^{-6} IAA is supplied in the medium (Bhatla & Dhingra-Babbar 1990). O_2 is required for the conversion of ACC to C_2H_4 (Bradford & Yang 1980b), suggesting that there might be interesting environmental responses for mosses that live part of their lives in water.

Although ethylene is a gaseous substance, it has been termed a growth hormone. It is important in **senescence** (aging) and its presence can cause **epinasty** (leaf and stem curling). In the aquatic moss ***Fontinalis squamosa***, treatment with its precursor ACC causes color changes, wavy leaves, and curled tips (Figure 41), as well as inhibiting growth at high concentrations (Glime & Rohwer 1983). It is likely that these responses are actually to ethylene produced in response to the ACC application.

Figure 41. **Left:** ***Fontinalis squamosa*** grown with ACC, the precursor of ethylene, demonstrating the contorted leaves and curved tips. **Right:** ***Neckera pennata*** exhibiting undulate leaves that could prove to be the result of genetically controlled ethylene behavior. Photos by Janice Glime.

Ethylene production coincides with that of the change from chloronema to caulonema and is probably tied to the increase in auxins (Rohwer & Bopp 1985). We know that ethylene and IAA can work together in both bryophytes and higher plants (Mignone & Basile 2000). In bryophytes, we know that an additive effect exists in at least some, for example ***Riella helicophylla*** (Figure 17), causing "super" cell elongation (Stange & Osborne 1988). Chopra and Sood (1973) demonstrated that ethrel, which produces ethylene in water, causes the production of more antheridia in ***Riccia crystallina*** (Figure 27).

IAA and ethylene often work in tandem, controlling each other's concentrations. For example, in ***Pellia epiphylla*** (Figure 14), IAA results in seta elongation, whereas ethylene inhibits it (Thomas *et al*. 1983). In the leafy liverworts, ethylene works together with auxin and certain arabinogalactan-proteins to suppress the third row of leaves by suppressing development of every third leaf primordium (Basile & Basile 1984; Mignone & Basile 2000). Mignone and Basile considered that ethylene played a suppression role in three processes. It is able to cause reductive development by causing failure in development of primordia to mature organs. It modulates the size and shapes of leaves. And it facilitates the change from diffuse growth to polar/apical growth. Nevertheless, ethylene remains largely a mystery.

The ACC pathway seems to work somewhat differently in bryophytes (Osborne *et al*. 1996). Lower plants seem unable to convert ACC to ethylene, nevertheless producing ethylene continuously. Although the ***Riella helicophylla*** (Figure 17) they studied seemed to take up the ACC easily, no ethylene gas was released. Nevertheless, in ***Fontinalis*** (Figure 41) ACC causes symptoms consistent with those expected from ethylene (Rohwer & Bopp 1985).

Acetylcholine

Acetylcholine – a compound better known for its role in nerve cells, has been conclusively shown in bacteria, protists, and mosses (Hartmann & Kilbinger 1974; Wessler *et al*. 1999), and more recently, in corn (Momonoki 1992). Interestingly, the original report (Hartmann & Kilbinger 1974) found it only in a hybrid of ***Funaria hygrometrica*** (Figure 1) and ***Physcomitrium pyriforme*** (Figure 3), whereas its hydrolyzing enzyme cholinesterase was not found in either (Fluck & Jaffe 1974). Later, however, Gupta *et al*. (2001) found cholinesterase in 30 out of 39 species of bryophytes tested, including five liverworts, with the highest activity in the moss ***Anoectangium bicolor***.

In non-animal organisms, the production of acetylcholine (ACh) is always accompanied by cholinesterase activity, thus preventing it from behaving as a hormone (Wessler *et al*. 1999). Nevertheless, its activity and the activation of acetylcholine receptors can interfere with ion channels and key enzymes – the cellular signalling pathways. In this role, it appears to play a part in regulating such cellular functions as mitosis, cell differentiation, organization of the cytoskeleton, cell-to-cell contact, secretion, and absorption. Furthermore, it appears to contribute to the regulation of immune functions.

But the role of acetylcholine in bryophytes is still unclear (Bhatla & Dhingra-Babbar 1990). Light quality certainly affects its production in at least some bryophytes, with 56 times as much produced in red light as in red/far-red (Bhatla & Dhingra-Babbar 1990). The red/far-red response is indicative of regulation by phytochrome (pigment that measures day length), but researchers disagree on the mechanism. As a growth regulator, it could

have an important role in habitat response and spore germination as a means of interpreting light quality.

In lactic acid bacteria, acetylcholine can be produced in response to osmotic stress (Kets *et al*. 1997). In a moss that is often desiccated by dust and other solutes on the surface, as well as being subjected to frequent desiccation due to weather, perhaps the acetylcholine might respond similarly.

Cryptochromes

Cryptochromes – This almost colorless yellow plant pigment has both enlightened and dumbfounded the plant physiologists since its discovery. Although we know that it responds to light and somehow signals to IAA in a way that affects plant development, its mechanism has remained elusive. Then entered the moss, of course the lab rat of all mosses, ***Physcomitrella patens*** (Figure 3). In 1999, Imaizumi and coworkers posted the identification of a cryptochrome homologue from this moss. ***Physcomitrella patens*** is more than just a convenient, small organism for testing things. It is unique. It is the only plant found thus far in which gene replacement is predictably reliable due to the high frequency of homologous recombination. In plain English, that means that instead of one chance in a million for a transplanted gene entering the genome, it is a predictable certainty.

Hence, to discover how cryptochromes function in plants, researchers (Imaizumi *et al*. 2002) created a moss [a strain of ***Physcomitrella patens*** (Figure 3)] with a defective genome, one that had disruptants for the two known genes for cryptochromes (CRY1 & CRY2). The moss could not make its cryptochromes. The results were rather astounding. They revealed that cryptochrome signals regulate induction of side branching of the protonema, gametophore induction, and development. Furthermore, disruption of these cryptochromes altered the induction of the auxin-inducible genes. Since these modified mosses were more sensitive to external auxin than their unmodified relatives in blue-light responses, it appears that the cryptochromes provide the signal to repress auxin signals that control plant development. This breakthrough in discovering the utility of ***Physcomitrella patens*** in delineating gene function could have astounding contributions to the entire field of plant physiology! In fact, it already does.

Summary

All aspects of development are influenced not only by the internal environment, but also by the external environment. These signals trigger responses in the bryophytes that permit them to survive and take advantage of the ever-changing conditions of their environment, from growth forms to drought resistance to dormancy.

These responses are typically mediated by hormones. Known bryophyte hormones include **auxins** (IAA) that regulate growth and gametangial production, **cytokinins** (**isopentenyladenine**, **zeatin**, and most likely **Factor H**) that regulate protonemal bud formation and branching, **gibberellin**-like compounds that inhibit cytokinin responses, **lunularic acid** and **ABA** (abscisic acid) that regulate dormancy and drought resistance, and **ethylene** that controls antheridial production and triggers **senescence**; **acetylcholine** and **cryptochromes** (photo-receptive pigments) also play a role in controlling bryophyte growth and development. The modes of control of these growth regulators are poorly understood in bryophytes, although in most cases they seem to act similarly to their mode of action in tracheophytes.

Some hormones may be supplied **exogenously**, that is, supplied by other organisms in the environment such as bacteria and fungi. And some of the hormones may be moved from place to place in the bryophyte by external conduction.

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Yoshikawa, H., Ichiki, Y., Sakakibara, K. D., Tamura, H., and Suiko, M. 2002. The biological and structural similarity between lunularic acid and abscisic acid. Biosci. Biotechnol. Biochem. 66: 840-846.

CHAPTER 5-2

ECOPHYSIOLOGY OF DEVELOPMENT: SPORE GERMINATION

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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.

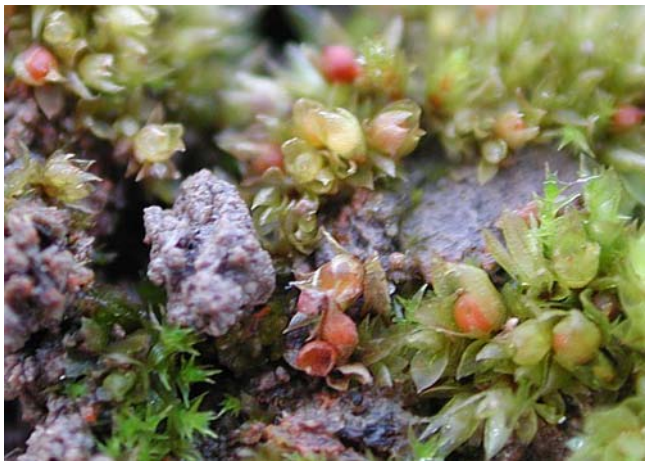

Figure 2. ***Physcomitrella patens*** with capsules. Photo by Michael Lüth, with permission.

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?

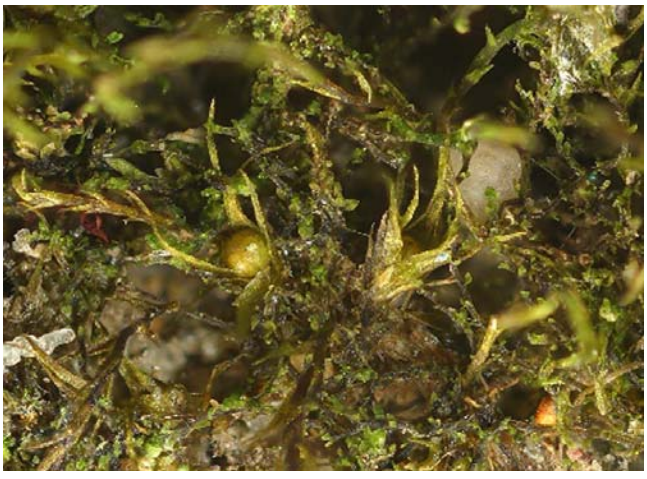

Figure 5. ***Archidium alternifolium*** with capsule. Michael Lüth, with permission.

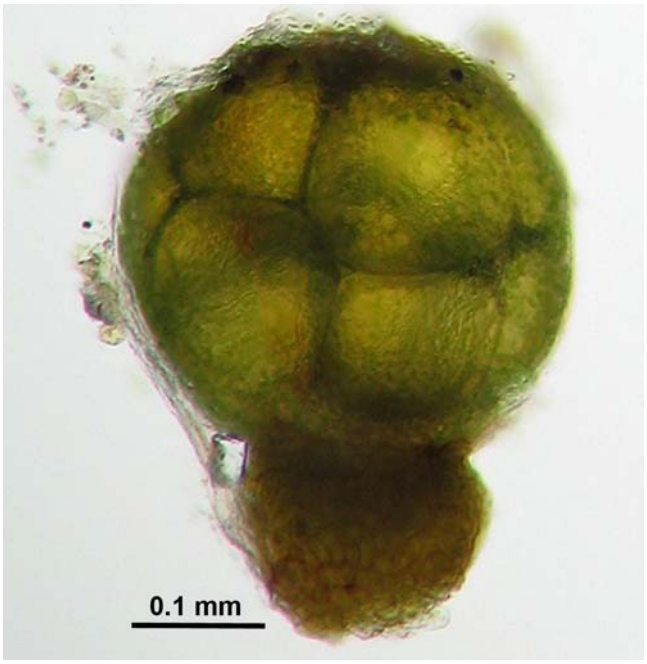

Figure 6. ***Archidium alternifolium*** capsule showing the large spores inside. Photo by Norbert Stapper, with permission.

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.

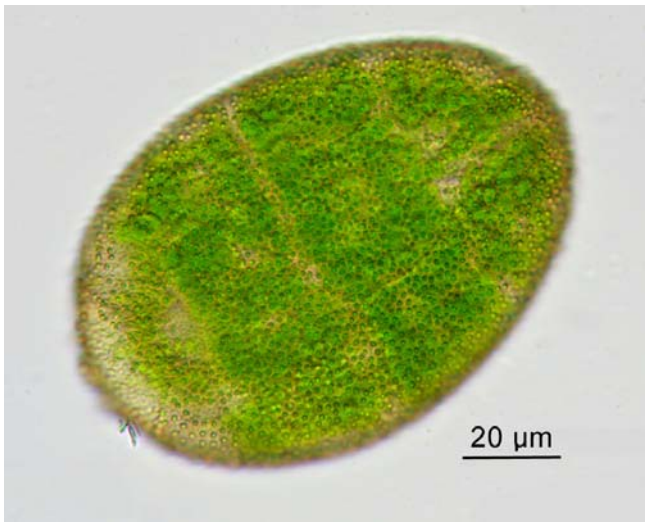

Figure 10. ***Pellia epiphylla*** spore showing endosporous development that occurs within the capsule. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

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.

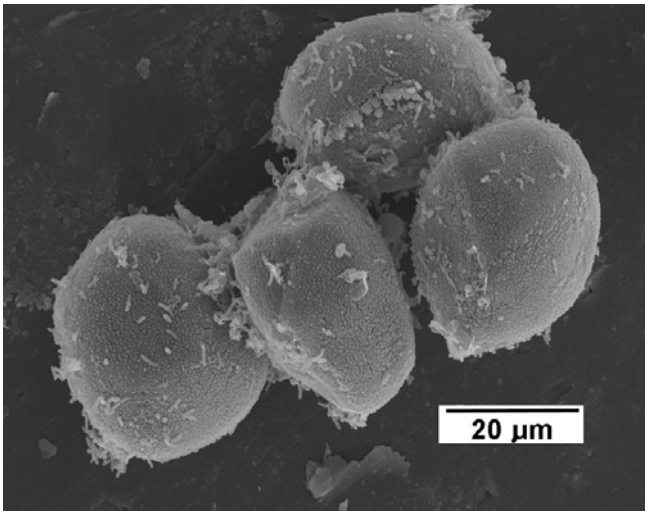

Figure 13. SEM of ***Andreaea rothii*** spores before germination. Photo courtesy of Karen Renzaglia.

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?

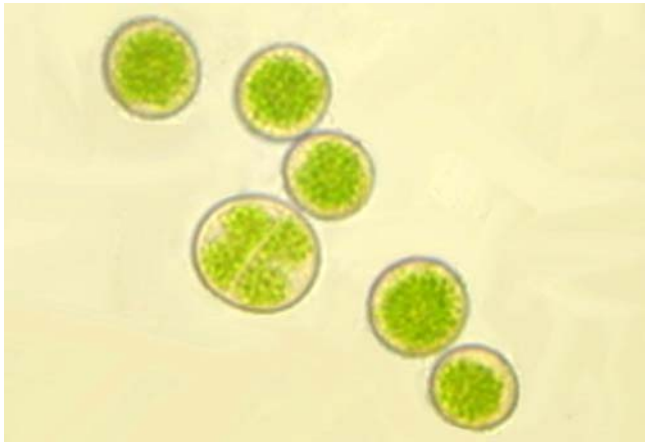

Figure 17. ***Radula recubans*** spores showing one with endosporic development. Photo by Adaíses Simone Maciel da Silva, with permission.

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

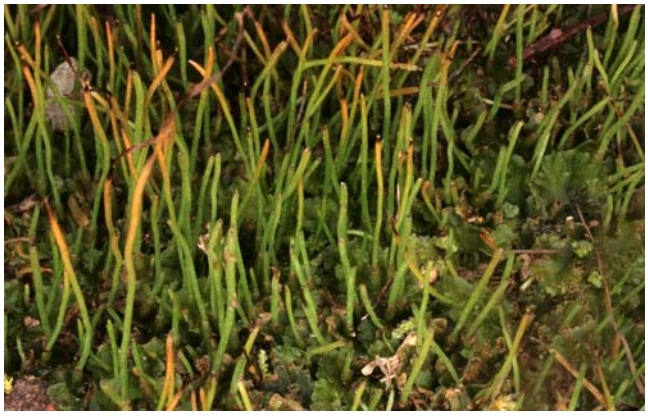

Figure 19. ***Anthoceros fusiformis*** with sporophytes. Photo by Jan-Peter Frahm, with permission.

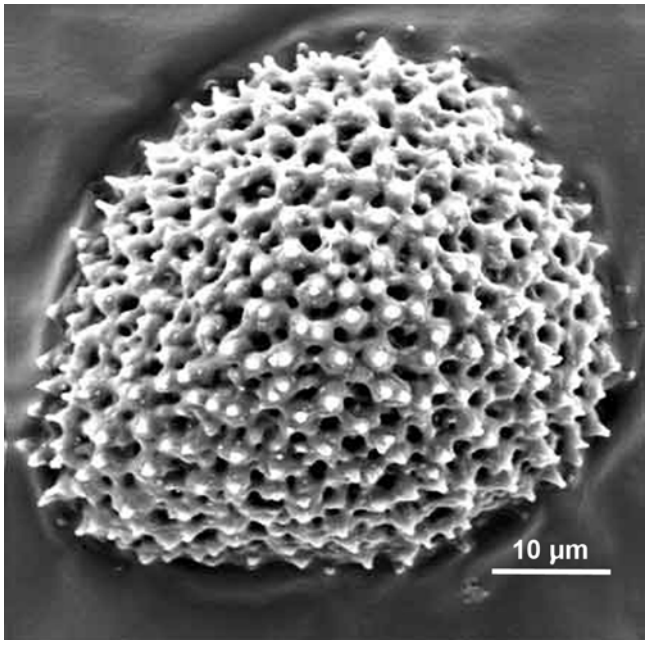

Figure 20. ***Anthoceros fusiformis*** spore distal view, SEM. Photo by Bill Doyle, with permission.

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.

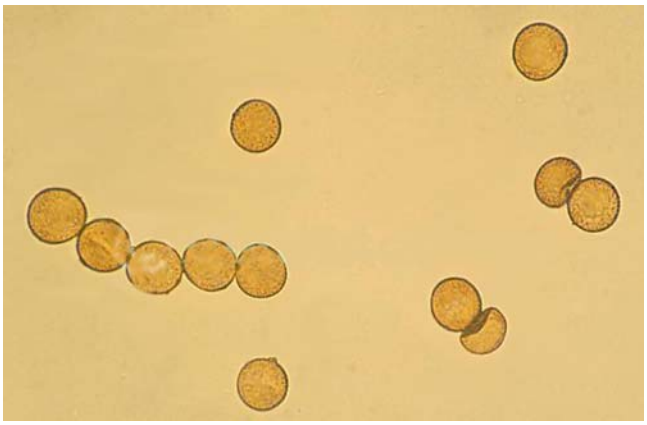

Figure 23. ***Funaria hygrometrica*** spores. Photo by Eugenia Ron and Tom Sobota, with permission.

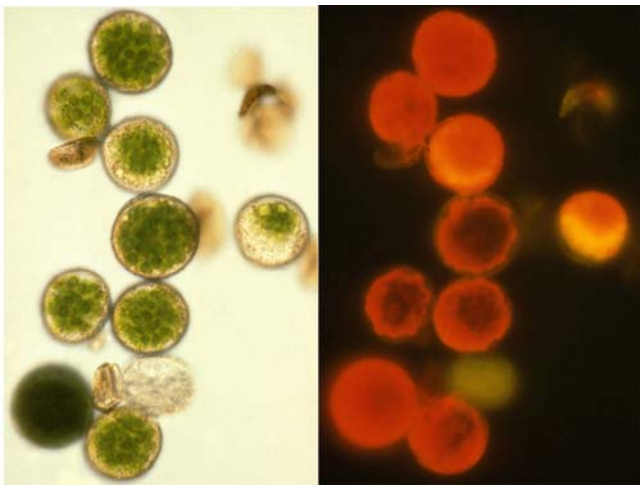

Figure 24. Comparison of chlorophyll in white light (**left**) and chlorophyll fluorescence in UV light (**right**) in large and small spores of ***Fontinalis squamosa***. Note dark gray areas in the photo on right are those areas lacking chlorophyll, and smaller spores tend to disappear in UV light.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

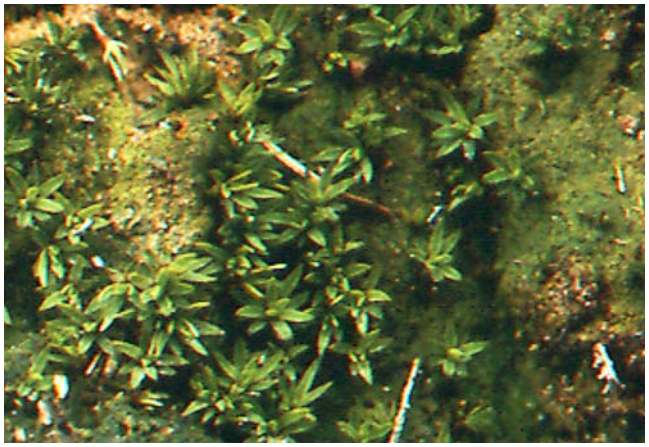

Figure 25. ***Pogonatum aloides*** with persistent protonemata. Photo by Janice Glime.

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.

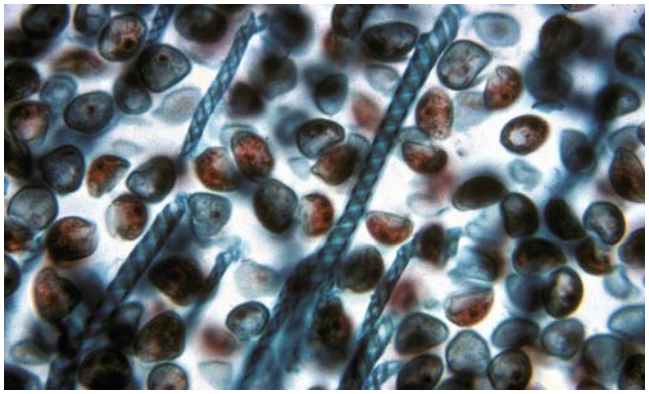

Figure 26. ***Marchantia polymorpha*** spores & elaters (stained) as they would appear in the capsule. Photo from Michigan State University teaching collection, with permission.

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.

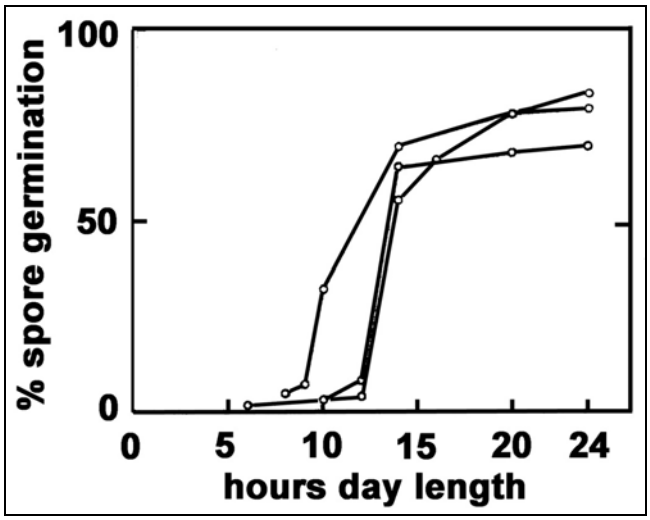

Figure 29. Effect of day length on germination of spores of ***Bryum pseudotriquetrum***. Redrawn from Kinugawa & Nakao 1965.

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.

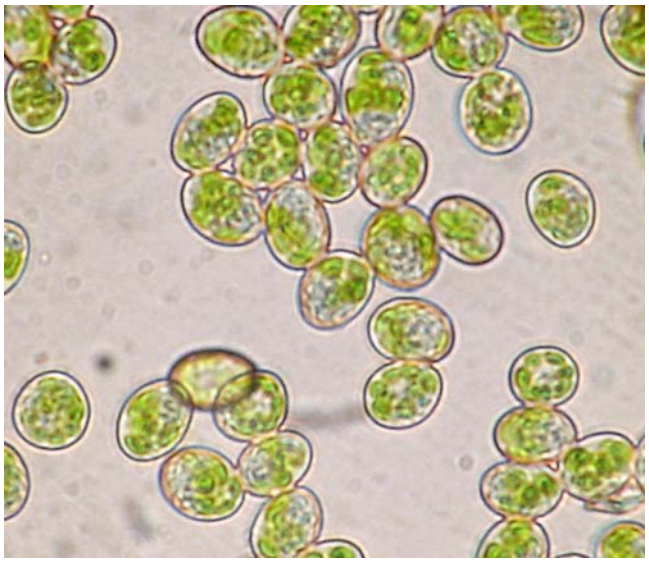

Figure 30. ***Schistostega pennata*** spores, a species where spores germinate in the dark and under snow. Photo by Misha Ignatov, with permission.

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.

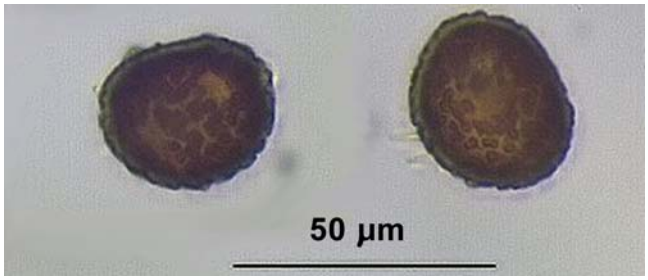

Figure 40. ***Weissia longifolia*** spores, a species that differs among populations in optimum germination temperatures. Photo by Kristian Peters, with permission.

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).

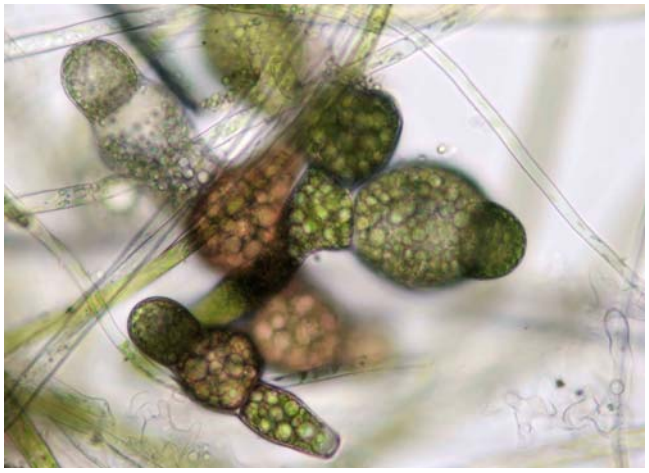

Figure 41. ***Dicranella heteromalla*** protonemata that survived winter in an acid mine lake. Photo by Jan Fott, with permission.

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.

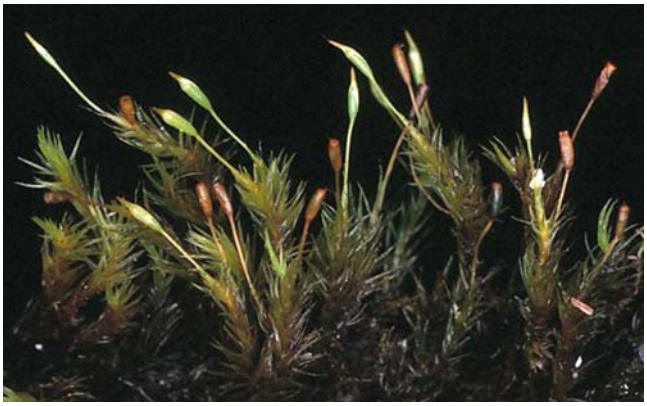

Figure 52. ***Eucamptodon perichaetiale*** with capsules. In this genus, spores germinate within the capsule. Photo by Jan-Peter Frahm, with permission.

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.

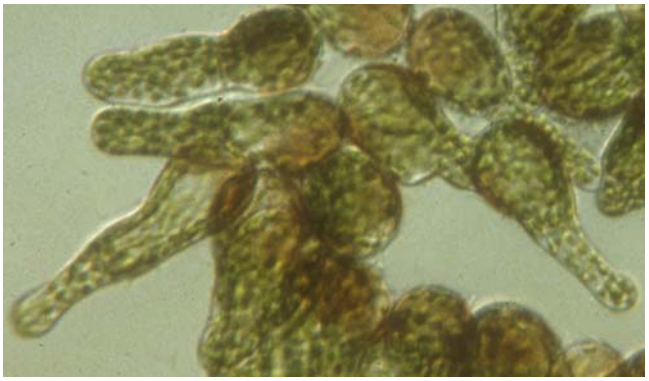

Figure 67. ***Funaria hygrometrica*** germinating spores. Photo by Yenhung Li, with permission.

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.

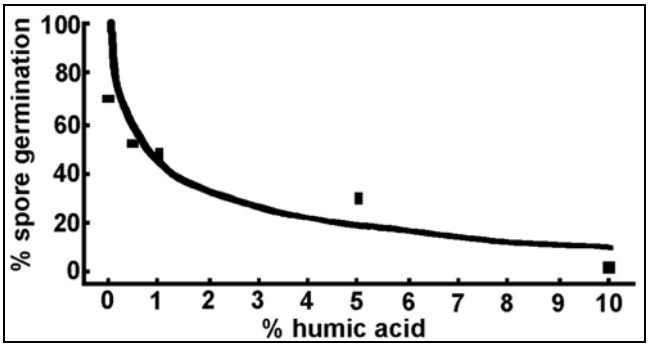

Figure 68. Effects of humic acid concentration on mean percent spore germination in ***Funaria hygrometrica***. From Raeymaekers, unpublished data.

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.

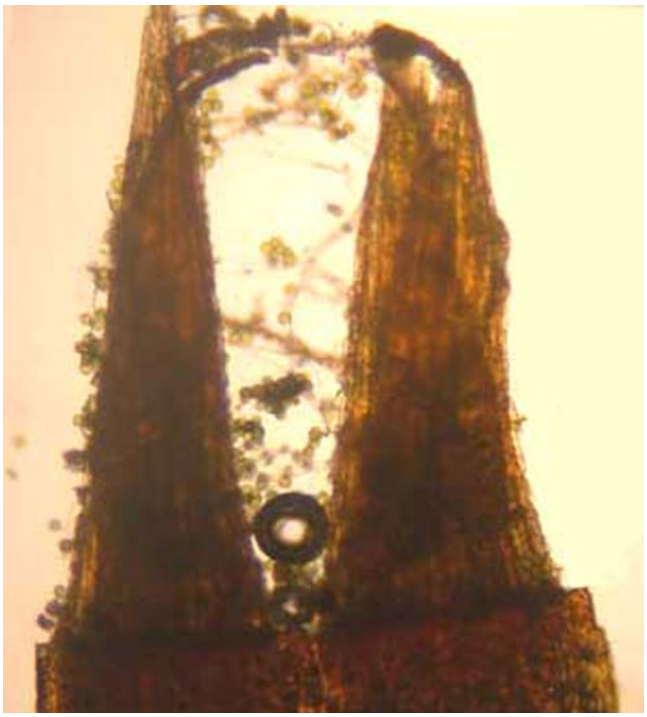

Figure 72. ***Tetraphis pellucida*** teeth and spores. Photo from Botany website, UBC, with permission

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.

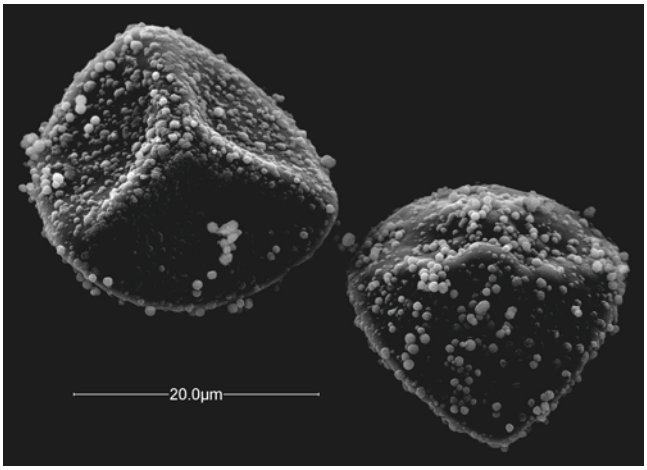

Figure 81. ***Sphagnum*** spore SEM. Photo by Whitaker & Edwards 2010, with permission.

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.

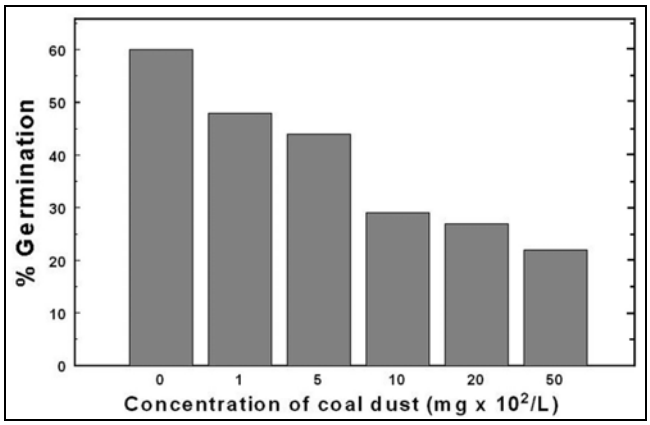

Figure 85. Inhibition of germination of ***Platyhypnidium riparioides*** spores resulting from suspended coal particles. Redrawn from Lewis (1973).

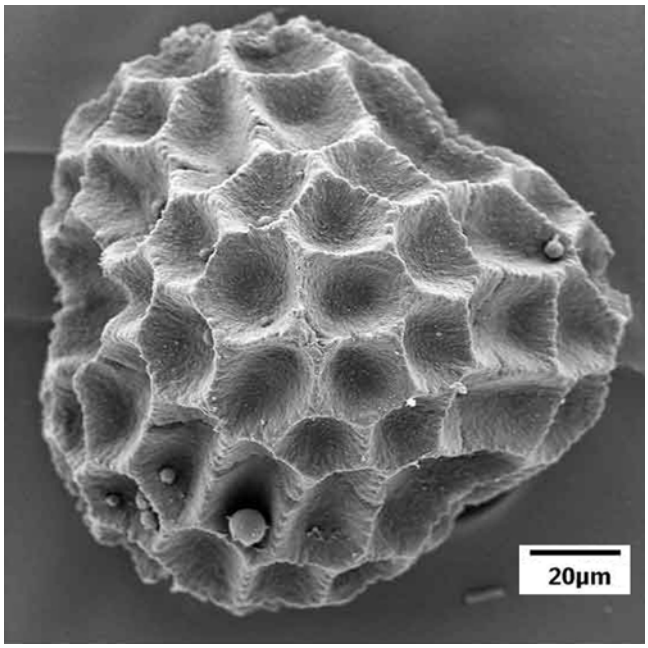

Figure 86. SEM of ***Sphaerocarpos texanus*** distal spore wall. Photo by William T. Doyle, with permission.

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.

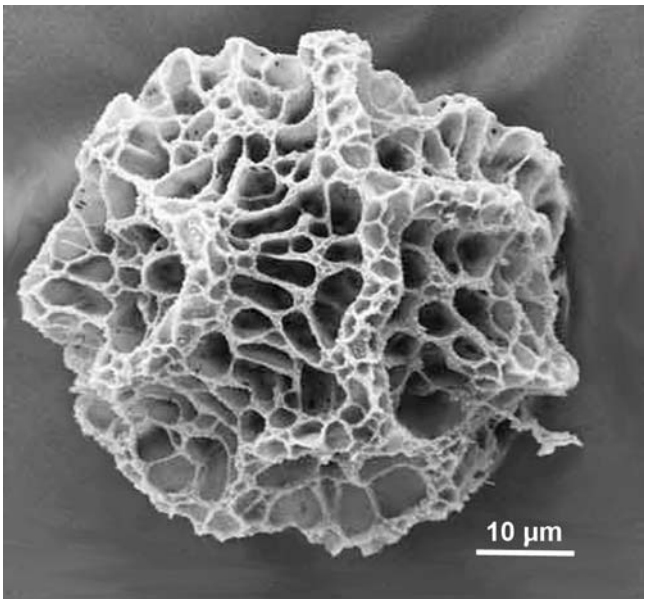

Figure 90. ***Targionia hypophylla*** distal spore wall SEM. This genus sometimes has unequal spore sizes. Photo by William T. Doyle, with permission.

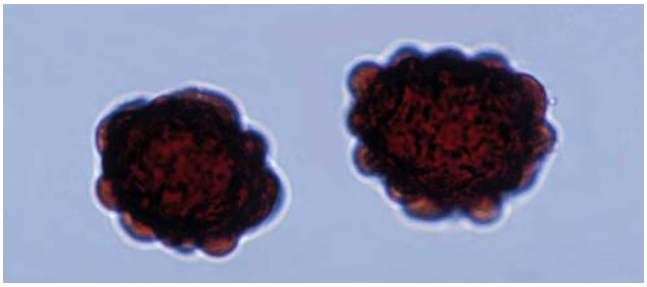

Figure 91. Spores of ***Cyathodium***, where spores are sometimes of unequal size. Photo courtesy of Noris Salazar Allen.

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.

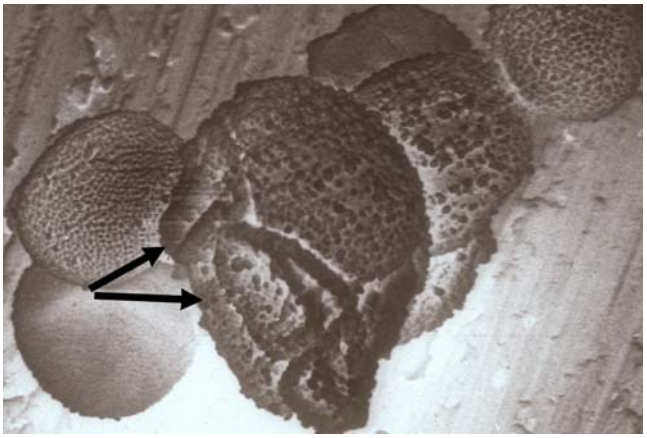

Figure 92. SEM of spore tetrad of ***Fontinalis squamosa*** showing one normal and at least two aborted spores (**arrows**) in the middle tetrad. The remaining visible spore is larger than nearby spores. Photo by Janice Glime.

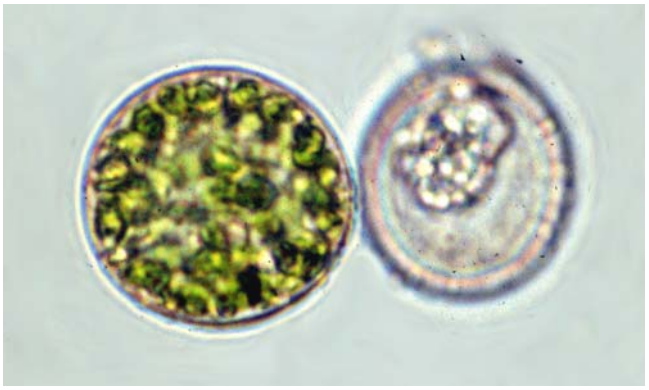

Figure 93. Normal (**left**) and aborted (**right**) spores of ***Fontinalis squamosa***. Photo by Janice Glime.

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.

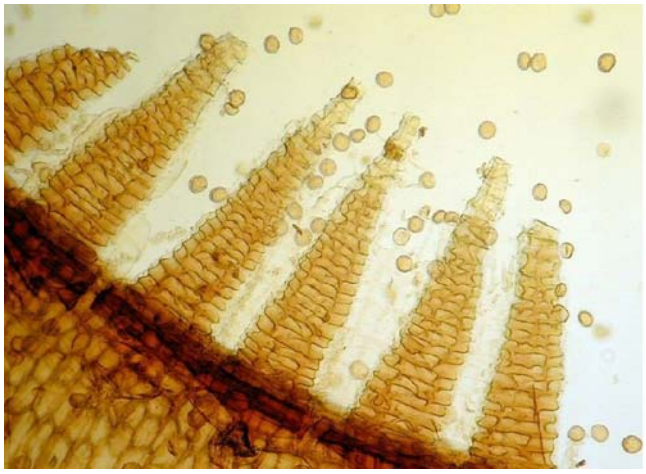

Figure 108. ***Bryum algovicum*** peristome and spores. These spores germinate in the light in three days. Photo by Michael Lüth, with permission.

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.

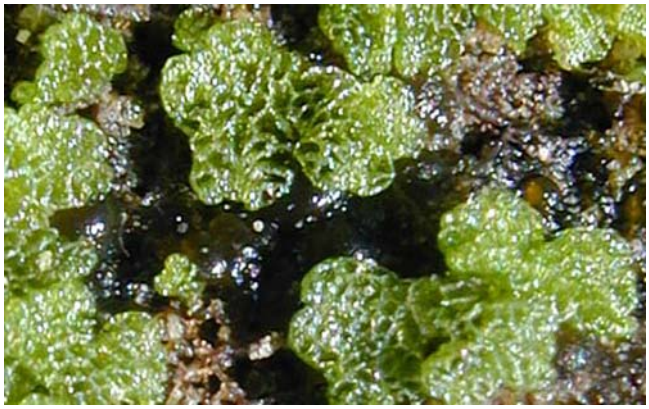

Figure 114. ***Riccia cavernosa*** on mud. Photo by Michael Lüth, with permission.

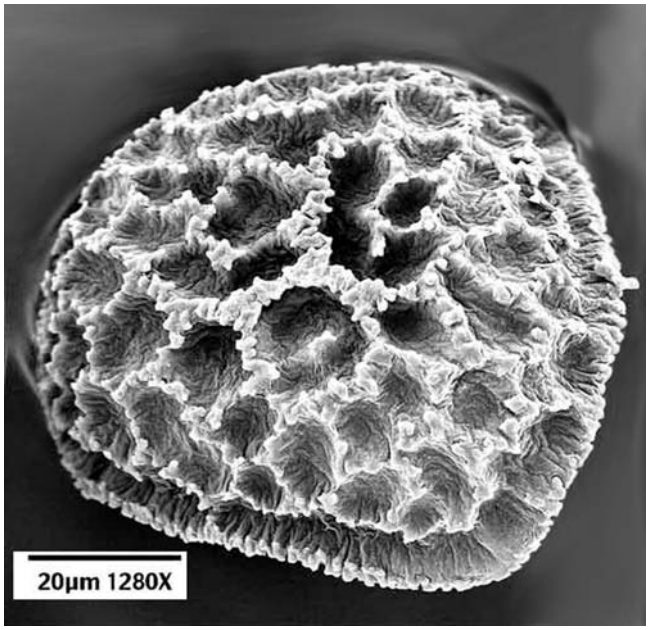

Figure 115. SEM of ***Riccia cavernosa*** spore SEM. Photo by William T. Doyle, with permission.

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.

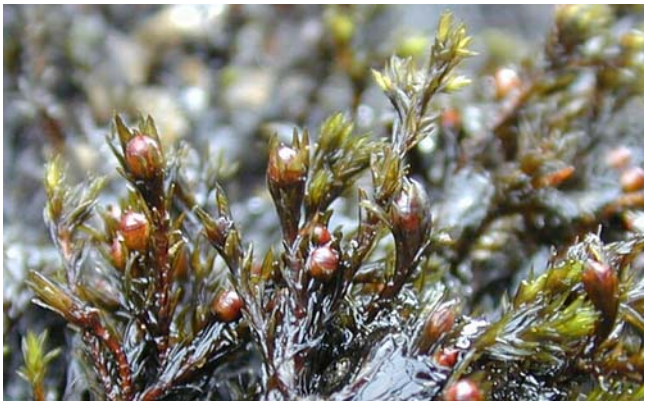

Figure 120. ***Schistidium rivulare*** growing on a wet, emergent rock. Photo by Michael Lüth, with permission.

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.

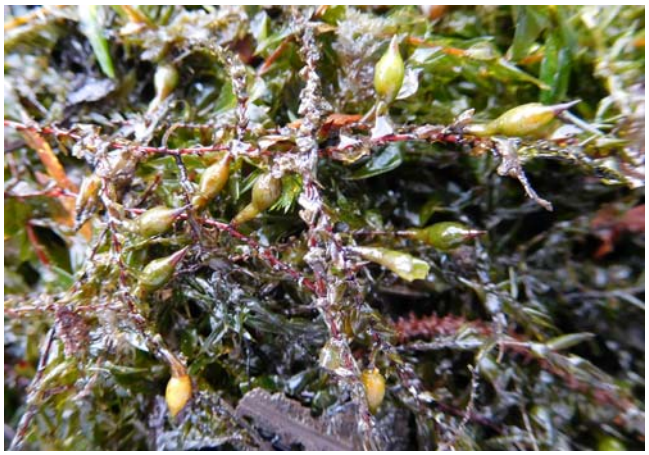

Figure 133. ***Fontinalis antipyretica*** with capsules, a species with very small annulus cells that do not dehisce. Photo courtesy of Rienk-Jan Bijlsma (per Joop Kortselius).

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).

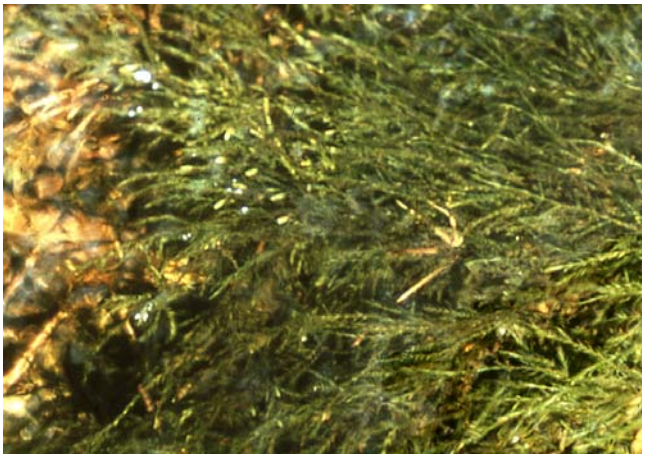

Figure 134. ***Fontinalis novae-angliae*** with capsules, a species that seems to fail in operculum dehiscence. Photo by Janice Glime.

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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CHAPTER 5-3

ECOPHYSIOLOGY OF DEVELOPMENT: PROTONEMATA

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CHAPTER 5-3

ECOPHYSIOLOGY OF DEVELOPMENT: PROTONEMATA

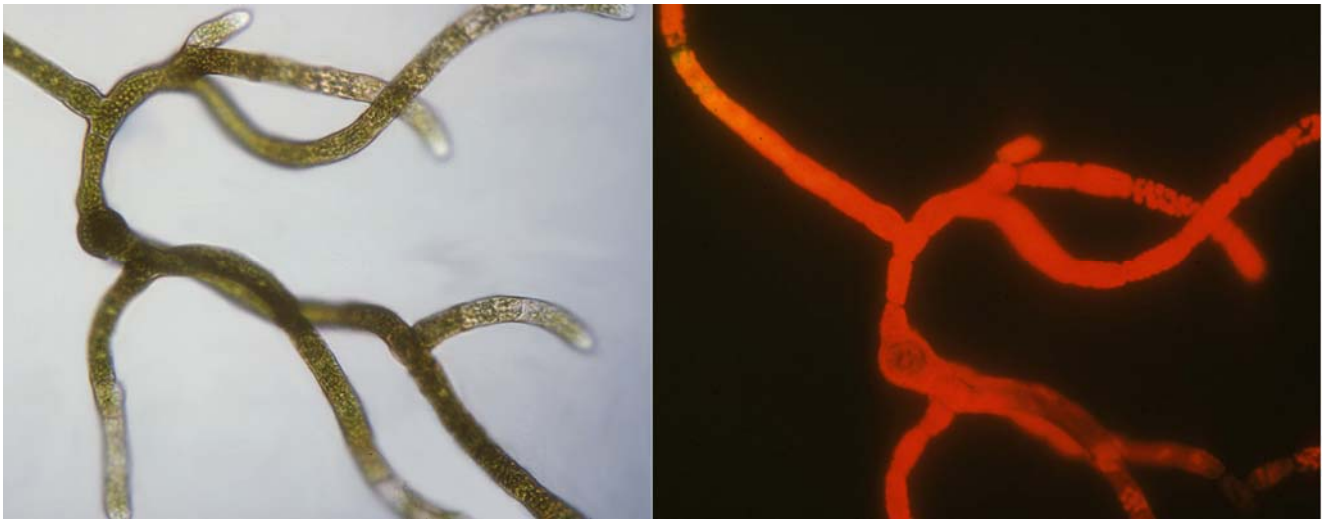

Figure 1. Protonema of ***Fontinalis squamosa***. **Left:** white light. **Right:** UV light showing chlorophyll fluorescence. Photo by Janice Glime.

The Protonema

The **protonema** is an elongate, thread-like structure that develops from the germinated spore of mosses and some liverworts. In most liverworts it is thalloid.

It was Sironval (1947) who defined two clear stages in protonema development. All mosses have the **chloronema** stage (Figure 2), which is the one that develops first from the germinating spore. The **caulonema** (Figure 2) stage is second and in some mosses it is not distinguishable from the chloronema.

The moss protonema typically branches (Figure 1) and can develop into **chloronema**, **caulonema**, or **rhizoids** (Figure 2), depending on the species, conditions, and developmental stage. The **chloronema** is the first thread formed by the germinating spore and is distinguished by its perpendicular crosswalls, short cells, numerous chloroplasts, colorless cell walls, and irregular branching. The **caulonema**, when present, develops later and is the source of gametophore buds in those species with both types of protonemal segments. It is distinguished by its distal position relative to the spore, longer cells with diagonal cross walls, usually brownish cell walls, and fewer, less evenly distributed, smaller, spindle-shaped chloroplasts. The chloronema, at least in culture, is able to grow vertically as well as horizontally, but the caulonema grows only horizontally (Bhatla 1994).

The protonemal stage is the best-studied part of bryophyte development. Due to its relative ease of culture and one-cell-wide structure, it has been the subject of numerous physiological studies to elucidate basic physiological mechanisms in plants.

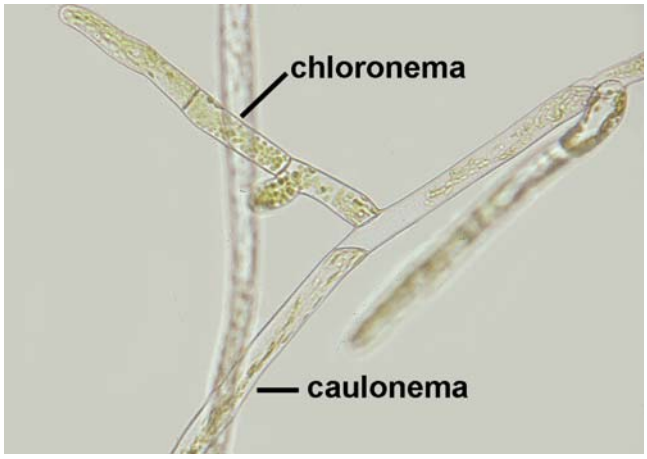

Figure 2. Distinction of chloronema and caulonema on the protonema of ***Funaria hygrometrica***. Photo by Janice Glime.

As discussed earlier with life cycles, spores in most true moss (**Bryopsida**) germinate to form filamentous protonemata, whereas **Sphagnopsida** has a thalloid form, **Andreaeopsida** a massive one, and liverwort protonemata may range from filamentous to thalloid (Mishler & DeLuna 1991). In the **Bryopsida**, non-filamentous protonemata occur in the **Schistostegales**, **Tetraphidales**, and some

genera in the **Grimmiales**, **Dicranales**, **Orthotrichales**, **Hypnobryales**, and **Isobryales** (Nishida 1978, Nehira 1983).

Fulford (1956, in Watson 1974) identified 10 protonemal types in the leafy liverworts, but Nehira (1966) and Schuster (1966) warn us that the protonema form is plastic and can be strongly modified by the environment. Nevertheless, Nehira (1966) identified 24 liverwort sporeling types.

The protonema, simple as it is, has a variety of forms. For example, in ***Lindbergia brachyptera*** (Figure 3), there is no caulonema (Zhao *et al*. 2004). The rhizoids and buds develop from the chloronema. And it takes only three days for the spore to germinate, with 95% of the spores germinated by 8 days.

Figure 3. ***Lindbergia brachyptera***, a species that does not develop a caulonema. Photo by Bob Klips, with permission.

But the environment can likewise cause modifications to the protonema. Such characters as cell shape, growth polarity, rate of mitosis, differentiation of chloronema into caulonema, and branching frequency of filamentous protonemata can change in response to changes in response to light quality and intensity, photoperiod, temperature, hydration, *p*H, hormonal levels, and interaction with microorganisms (Chopra and Kumra 1988; Mishler & DeLuna 1991). Nevertheless, Anderson and Crosby (1965) found that the basic thalloid and massive forms of the Sphagnopsida and Andreaopsida remained unchanged.

Even in mosses such as ***Funaria hygrometrica*** (Figure 2, Figure 8) with well-developed caulonemata, culture in liquid media can inhibit formation of caulonema, resulting in reduced bud formation – suggesting very wet conditions would be detrimental to development of gametophores in these taxa (Johri & Desai 1973). Furthermore, high cell densities cause failure of caulonema differentiation, suggesting some sort of self-inhibition. This might be another adaptive mechanism that prevents gametophores from competing with each other and that permits the protonema time to revert to chloronema, spread to a wider area, or partially die off before putting forth upright plants.

By contrast, ***Tetraphis pellucida*** (Figure 6; **Tetraphidopsida**) produces a bladelike structure from the protonema, described as **protonemal flaps** (Figure 4-Figure 5). Gemmae can develop at the base of the flap. The changes from distended spore to protonema growth to gametophore buds can require increasingly more specialized conditions in this and other species. For example, Forman (1964) found that spore germination in ***Tetraphis pellucida*** (Figure 4-Figure 5) requires a *p*H of 3.0-7.3 whereas growth of the leafy shoot occurs in the much narrower *p*H range of 5.1 to 5.8. This has limiting implications for species that arrive as spores.

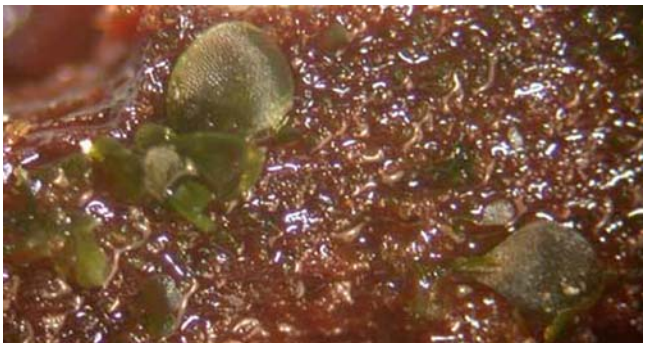

Figure 4. Protonemal flaps of the moss ***Tetraphis pellucida***. Photo from botany website and University of British Columbia, Canada, with permission.

Figure 5. Protonema and protonemal flaps of the moss ***Tetraphis pellucida***. Photo from Botany Website, University of British Columbia, Canada, with permission.

Figure 6. ***Tetraphis pellucida*** with gemmae cups, a species that develops protonemal flaps. Photo by Andrew Spink, with permission.

Temperature requirements, on the other hand, are broader for the leafy shoot, but as the humidity drops, the viable temperature range narrows. Furthermore, the change from chloronema to caulonema can be delayed by inappropriate environmental conditions. Bopp (1961) found that the caulonema stage, and thus the bud stage, can be delayed by low temperature, submersion, or low light.

There seems to be controversy over the degree of difference between chloronema and caulonema, with Bopp (1959) contending that they are distinct stages, and Kofler (1958) and others finding no consistent distinction, even in ***Funaria hygrometrica*** (Figure 2, Figure 7-Figure 8), for which Bopp first made his claim. Several factors appear to lead to these disagreements (Watson 1974). The plasticity of the protonema permits it to respond differently to the varying environmental conditions. The distinction is exhibited more strongly in some species than others, and in some species, apparently no distinction exists. And, Kofler contended that genetic differences are more likely to be expressed in the protonema than in the gametophore or sporophyte because the environment has less time to exert selective pressure on the protonema. Hmmm...

Figure 7. ***Funaria hygrometrica***, a species for which the protonemal physiology has been extensively studied. Photo by Michael Lüth, with permission.

Figure 8. ***Funaria hygrometrica*** spore with branch protonema developing from a chloronema cell. Photo by Janice Glime.

Application of IAA induces the switch from chloronema to caulonema side branches (Johri & Desai 1973; Christianson 2000) and inhibits the further growth and initiation of chloronema branches (Johri & Desai 1973). Application of ABA to chloronema instead results in cell division and the formation of asexual reproductive cells, but not in caulonemata (Christianson 2000). Inadequate calcium causes the chloronema cells to divide unevenly and to form **tmema** (abscission cell that ruptures to release moss gemmae; see below), but not in caulonemata. Cytokinin stimulates the formation of gametophore buds in the caulonema, but not in the chloronema. Perhaps even more surprising, chloronemata exhibit positive phototropism, whereas caulonemata exhibit negative phototropism, much like the differences in response to IAA in stems vs roots of tracheophytes.

But are these applied hormone responses initiated by moss hormone productions? In the well-studied ***Physcomitrella patens*** (Figure 9-Figure 10), we do know that transition from chloronema to caulonema cells is under control of auxin (Gonneau *et al.* 2001). Since IAA concentrations seem to be under environmental influence, variability and inconsistencies may be explained in the near future as we unravel the cryptochrome/IAA complex of reactions in this moss, and plants in general, using gene knockout techniques.

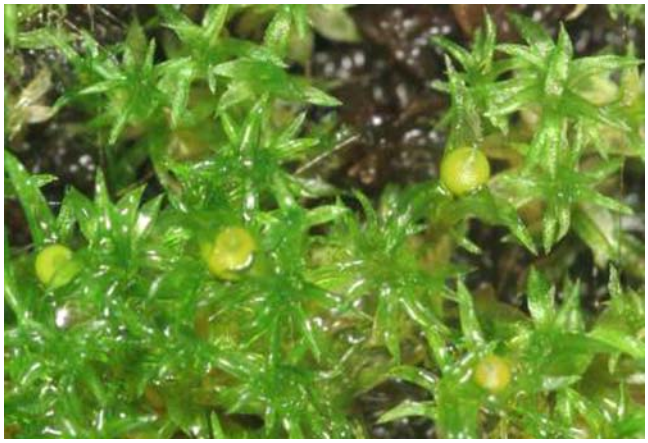

Figure 9. ***Physcomitrella patens*** with capsules, a common research organism because of the ease with which its genes can be manipulated. Photo by David Cove, with online permission.

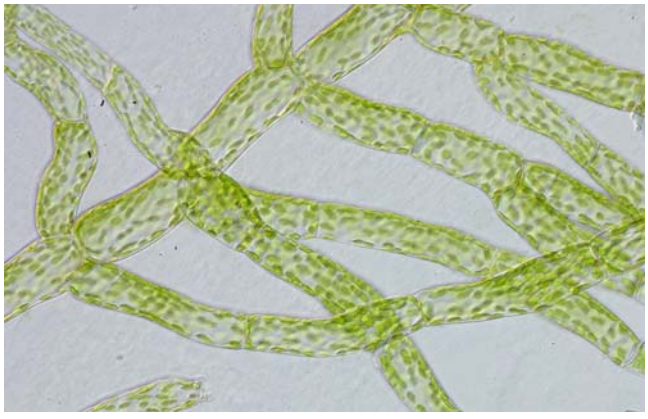

Figure 10. ***Physcomitrella patens*** protonema. Photo from Ralf Reski Lab, through Creative Commons.

Bittisnich and Williamson (1989) identified H^+ efflux at the tips of the chloronema (Figure 2) in ***Funaria hygrometrica*** (Figure 2, Figure 7-Figure 8) and elaborated

the role of acid flux in the extension of the protonema. However, unlike fungal hyphae, pollen tubes, and root hairs, the growth of the moss protonema is slow (Bhatla 1994) and is not confined to the apex. Growth apparently occurs in accordance with the acid growth mechanism, in which H^+ ions, induced by light and IAA, loosen the cell wall. In ***Funaria hygrometrica***, acidification of the medium to *p*H 5.5 increases the extension of the tip cells (Figure 8), whereas buffering to a *p*H of 6.8 prevents it. Calcium seems necessary for the acquisition of new materials to the wall and the ability to extend the wall.

The development of protonemata has not been widely studied, and those studies have concentrated on the changes in morphology resulting from cytoskeletal aspects of tip growth and production of asexual propagules (Pressel *et al*. 2008). Pressel *et al*. set out to remedy the situation by examining the differentiation of the caulonemata and rhizoids. This comprehensive study included more than 200 moss species! They found that the differentiation of caulonemata and rhizoids results in fully differentiated cells that have a remarkable resemblance to the moss food-conducting cells. In both rhizoids and caulonemata, the cytology is dependent on having an intact microtubule cytoskeleton. The vacuole disappears during the differentiation process, a phenomenon that Pressel *et al*. consider to be related to the solute transport functions of the caulonemata and rhizoids.

Figure 11. ***Grimmia orbicularis*** with capsules in its dry rock habitat. Photo by Michael Lüth, with permission.

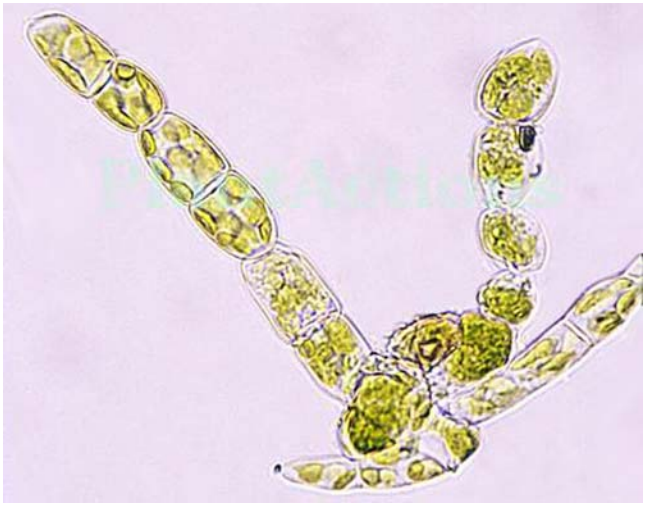

Figure 12. ***Grimmia orbicularis*** protonema. Photo by Eugenia Ron and Tom Sobota, with permission.

Water Relations

We have often assumed that the protonema stage is the most susceptible to desiccation damage. However, this is not always true. During (pers. comm.) found that unsuccessful cultures of xerophytes such as ***Grimmia*** (Figure 11-Figure 12) produced gametophores only after being put aside and forgotten, *i.e*., after desiccation. But it is surprising that Glime and Knoop (1986) found that after cultures of the aquatic moss ***Fontinalis squamosa*** (Figure 1) had dried out, added water caused the protonemata to swell and again become active. This is further supported by observations on protonemata that dried overnight on a microscope slide. When I added water to observe them for fluorescence, they produced vivid red chlorophyll fluorescence and regained their normal shape. It appears that protonemata may have considerable desiccation tolerance.

Further evidence that the protonema is desiccation tolerant can be gleaned from their dispersal period. As seen in the chapter on phenology, dispersal in spring is commonplace. It would seem, therefore, that the protonema must be growing in summer, when desiccation is most likely. The other period of high spore dispersal is fall, again preceding the dry season of winter in many temperate regions. Although we have insufficient evidence to show that the protonemata are present during these two relatively dry seasons, it appears likely that they are in at least some, if not many, species. Figure 13 shows a hydrated protonema in the field.

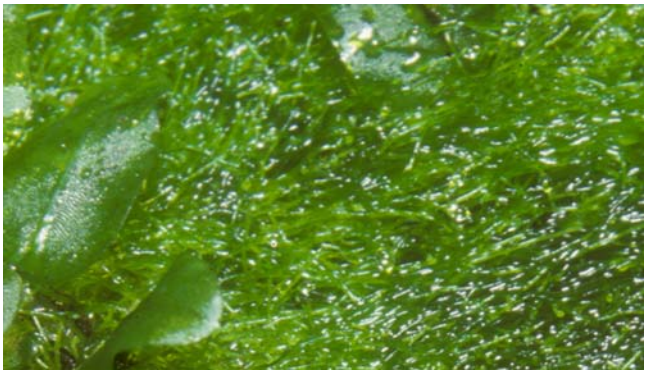

Figure 13. Protonema of ***Plagiomnium*** sp. in the field. Photo by Janice Glime.

Seasonal Light/Temperature Changes

It is hard to talk about light without also considering temperature, since brighter light generally means greater exposure and higher temperatures. Higher temperatures and brighter light are also usually coupled with a longer photoperiod. Knowledge of their effects on protonemal growth and development is based on laboratory cultures.

Light, coupled with temperature, seems to play a role in the pattern of development of protonemata in the aquatic

moss ***Fontinalis***. ***Fontinalis squamosa*** (Figure 15) spores germinated throughout the range of 40 to 3000 lux, and cultures exhibited unipolar, bipolar, tripolar, and one tetrapolar germination (Figure 14, Figure 15) (Glime & Knoop 1986). The number of germ tubes was generally consistent within a single plate, despite having bands of spores from three different capsules. At 3ºC and 120 lux, germination required four weeks, and only distended spores with a single protrusion were present (Figure 16). At 14ºC, 1200 lux, two plates of spores had single threads (Figure 14), one had double threads, and one had short single and double threads. At 20ºC, 2100 lux, two plates had only single germ threads that formed weak spirals and two had many spores with two or three germ threads and no spiral growth (Figure 15); branching was much more extensive than at 14ºC and 1200 lux. Although effects of temperature cannot be separated from those of light intensity, they mimic environmental conditions as they change from winter to summer. Such environmental controls can prevent spores from germinating or protonemata from developing too early in the season. The high degree of branching at higher light and temperatures could afford more self-protection from desiccation by providing overlapping threads (Figure 17). Bipolar and tripolar germination is also likely to be a response to the greater ability to photosynthesize with more light and provide energy for the developing germ tube.

Figure 14. Single-thread protonemata of ***Fontinalis squamosa*** formed at 14ºC and 1200 lux. Photo by Janice Glime.

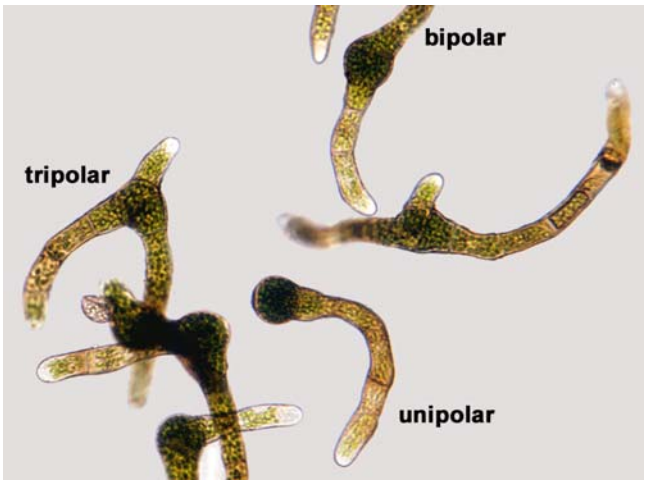

Figure 15. Protonemata of ***Fontinalis squamosa*** showing unipolar, bipolar, and tripolar germination typical at 20ºC and 2100 lux. Photo by Janice Glime.

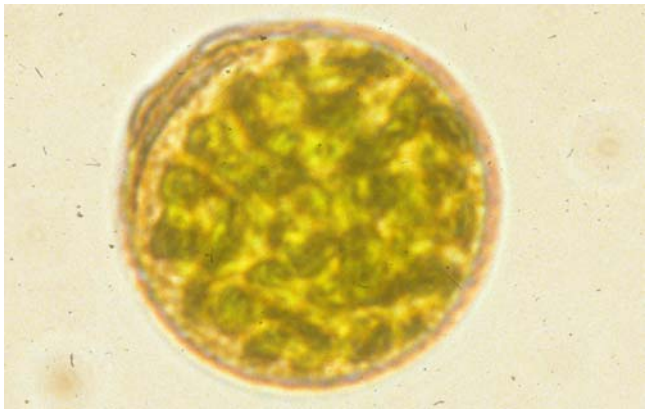

Figure 16. Distended spore of ***Fontinalis squamosa*** as one might find at 3ºC and 120 lux. Photo by Janice Glime.

Figure 17. Dense growth of overlapping protonemata of the moss ***Plagiomnium*** sp., a strategy that can help to conserve water and produce multiple leafy gametophytes. Photo by Janice Glime.

Light

Light Intensity

High light intensity can promote protonemal growth, as in ***Microdus*** (Figure 18), ***Hymenostylium*** (Figure 19), and ***Campylopus*** (Figure 20) (Mehta 1988). In the ephemeral ***Physcomitrella patens*** (Figure 9-Figure 10), high light intensities promote branching of the caulonema, thus proliferating the potential bud sites (Cove *et al*. 1978, 1979). By contrast, ***Bartramia ithyphylla*** (Figure 21) can exhibit branching from the first cell emerging from the spore (Figure 22) (Cove *et al*. 1978, 1979), as can ***Brachythecium velutinum*** (Figure 23) (Herguido & Ron 1990). ***Gymnostomum*** sp. *s.l*. (Figure 24) can branch from multiple caulonemal cells (Figure 25) (Cove *et al*. 1978, 1979). These multiple branches can produce multiple buds, forming a colony or cushion of plants (Figure 26) that help each other to maintain moisture. In species like ***Atrichum altecristatum*** (Figure 27), a large mat of protonemata commonly forms before buds develop, ensuring a colony of plants to protect each other (Figure 28).

Figure 18. ***Microdus brasiliensis***, a species in which high light intensity promotes protonemal growth. Photo by Jan-Peter Frahm, with permission.

Figure 19. ***Hymenostylium recurvirostrum***, a species in which high light intensity promotes protonemal growth. Photo by Michael Lüth, with permission.

Figure 20. ***Campylopus*** sp., a genus in which high light intensity promotes protonemal growth. Photo by Blanka Shaw, with permission.

Figure 21. ***Bartramia ithyphylla*** in a typical habitat. Photo by Michael Lüth, with permission.

Figure 22. ***Bartramia ithyphylla*** protonema showing branching in the cell just outside the spore. Photo courtesy of Eugenia Ron and Tom Sobota at Plant Actions, with permission.

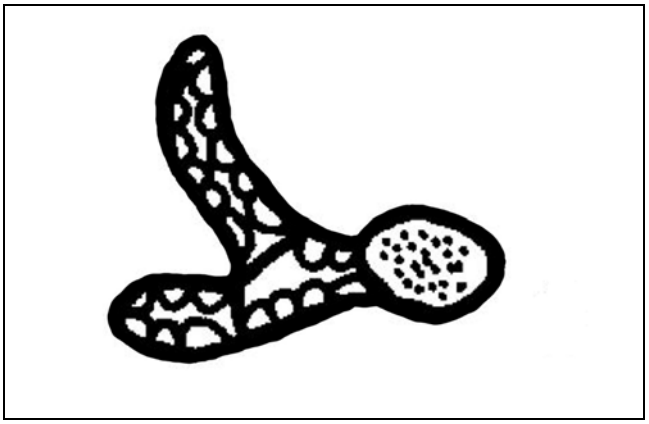

Figure 23. ***Brachythecium velutinum*** protonema branching Redrawn from Herguido & Ron 1990.

Figure 24. ***Gymnostomum aeruginosum*** with capsules, a species that can branch from multiple caulonema cells. Photo by Michael Lüth, with permission.

Figure 25. A species of ***Gymnostomum*** *s.l.* showing multiple branches from caulonema cells. Note the diatom living on it in its rock wall habitat. Photo by Janice Glime.

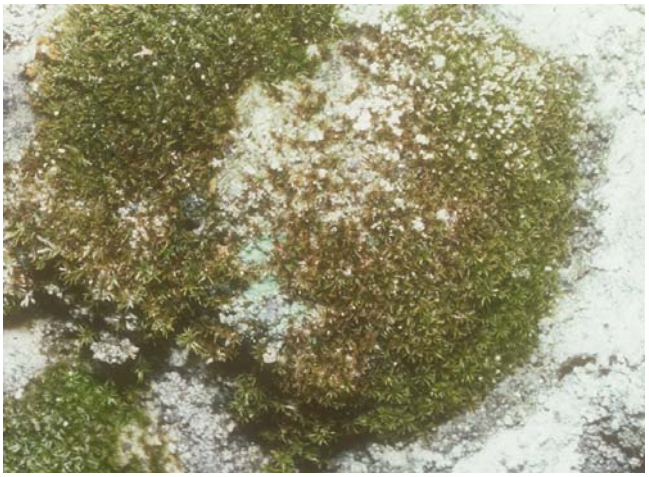

Figure 26. ***Gymnostomum*** forming colony, possibly from multiple buds from one protonema. Photo by Janice Glime.

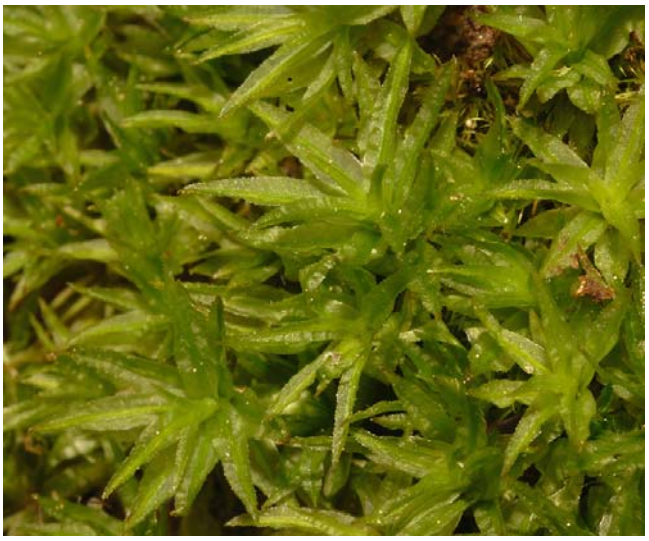

Figure 27. ***Atrichum altecristatum*** drying in an exposed habitat. Photo courtesy of Eric Schneider.

Figure 28. ***Atrichum altecristatum*** mat of protonemata with buds and young gametophores. Photo courtesy of Eric Schneider.

Continued high light promotes secondary caulonemata instead of bud formation. Is this adaptive by extending the plant to a darker location? Or is it merely a way of measuring all the available illuminated space for successful gametophores? Sood (1975) also observed an effect of light intensity on the number of germ tubes arising from the spore in ***Pogonatum aloides*** (Figure 29-Figure 30). At 1000 lux germination was unipolar, increasing at 3000 lux. At 6-8000 lux some spores swelled but failed to germinate. In germinating spores of ***Polytrichum commune*** (Figure 31) and ***P. juniperinum*** (Figure 32), there was a lag in synthesis of chlorophyll, being longer in ***P. commune*** (Karunen 1973). The chlorophyll *a*/*b* ratio at that time in ***P. commune*** was 1.4-1.8, thus providing little antenna effect by chlorophyll *b*. The low concentration of chlorophyll in general and the reduced relative amount of light-gathering chlorophyll *b* would force the gametophyte to require food reserves during early development.

Figure 29. ***Pogonatum aloides*** with protonemata and buds. Photo by Walter Obermayer, with permission.

Figure 30. ***Pogonatum*** protonema. Photo by George Shepherd, through Creative Commons.

Figure 31. ***Polytrichum commune*** showing the extensive turf it can form. Photo by Christopher Tracey, through Creative Commons.

Figure 32. ***Polytrichum juniperinum***, a species that exhibits a lag in chlorophyll production after the spore has germinated. Photo by Janice Glime.

High temperatures required for the protonemata can force a species into a narrow geographic range despite the ability of the spores to germinate at cooler temperatures. For example, ***Anisothecium molliculum*** has an optimum temperature of 25°C, not only for protonemal growth, but also bud formation (Kumra & Chopra 1985), preventing it from living in polar regions.

Although light generally seems to be necessary for spore distension, in some cases the protonema can even grow in the dark. In ***Ceratodon purpureus*** (Figure 33) darkness first induces an increase of starch grains in the chloroplast (Valanne 1971). This is followed by disappearance of starch and an increase in the number of grana lamellae.

Figure 33. ***Ceratodon purpureus*** with capsules, a species in which protonemata can grow in the dark despite its typical exposed habitat. Photo by Michael Lüth, with permission.

At least for ***Fontinalis squamosa***, higher light intensity and temperatures result in more germ tubes arising from the spore, suggesting that more sugars might be available, both for energy and for creating a high osmotic potential. The increased number of protonematal branches at higher light intensities and temperatures could provide a thicker mat to decrease evaporative losses and to increase self-shading against UV light damage.

Protonemata can form numerous branches, leading to numerous buds. When these buds develop into upright gametophores, the presence of many in close proximity permits them to protect each other from desiccation.

Light Quality

It is clear that light quality affects the growth and development of at least some protonemata. Light quality shift from white light to green and far red, as found in the forest, resulted in reduced protonemal growth in ***Pohlia nutans*** (Figure 34), with the least growth occurring in green light (Mitra *et al*. 1959). Giles and von Maltzahn (1967) found that red light stimulates mature leaf cells of ***Plagiomnium affine*** (see Figure 13) to regenerate by protonemata, and they suggested that phytochrome was most likely involved. Although liverworts seem to lack any consistent kind of photoregulation (Hartmann & Weber 1990), mosses respond differently to different wavelengths. Their best chloronema growth seems to be in white light (Bhatla 1994), but we must question whether this is true for all species that grow only under a canopy of green. In ***Funaria hygrometrica*** (Figure 2), the red range stimulates normal growth, whereas the blue range leads to the development of caulonema-like cells. It is possible that these shifts in light quality response could help to signal the time to develop gametophores as the protonemal mat thickens from extensive growth, changing the light quality of underlying strands.

Figure 34. ***Pohlia nutans*** with capsules. This widespread species of open habitats has reduced protonema growth in green light as it might experience in a forest. Photo by Štěpán Koval, with permission.

Imaizumi and coworkers (2002) demonstrated that **cryptochromes** are sensitive to blue light in ***Physcomitrella patens*** (Figure 9-Figure 10). Their reception of blue light permits them to mediate the light response. This moss has two identified cryptochrome genes. Using disruptants of these genes permitted Imaizumi and coworkers to elucidate the method of action of the cryptochromes. Cryptochromes, it turns out, mediate many steps in moss development. These include the induction of side branching of the protonema and induction of the leafy gametophyte. Disrupting cryptochromes caused changes in the auxin responses and revealed that cryptochromes respond to light to repress auxin signals as a means of controlling the development of the bryophyte.

Light quality could also serve to signal that it is time to break dormancy. Both blue and red light will permit maintenance of normal chloroplasts in ***Ceratodon purpureus*** (Figure 33) protonemata, but blue light results in richer starch, denser stromata (colorless matrix of chloroplast in which packets of chlorophyll are embedded), and more mitochondria, whereas red results in a more effective use of lipids (Valanne 1971). Is there any adaptive value in this? Is the moss able to sense the decreasing cover by snow (Figure 35), as voles do, based on light quality and intensity?

Figure 35. ***Atrichum undulatum*** in melting snow. How do mosses sense the coming of snowmelt? Photo by Michael Lüth, with permission.

Photoperiod

In ***Ceratodon purpureus*** (Figure 33), long days stimulate elongation of the protonema, whereas short days result in protonemal branching (Larpent-Gourgaud & Aumaitre 1980). The two systems are antagonists. This relationship suggests that an IAA/cytokinin balance may be the important controlling factor, with long days promoting IAA, probably through phytochrome mediation.

In ***Bryum pseudotriquetrum*** (Figure 36) a day length of ten or more hours is required for germination and protonema growth (Kinugawa & Nakao 1965, Figure 37). However two minutes of light during a 16-hr dark period is sufficient to remove the inhibitory effect developed during the dark period and will likewise stimulate germination and growth. In other words, it is the length of a continuous dark period that is important. This further supports the hypothesis of a phytochrome response and is much like the photoperiodic control of flowering.

Figure 36. ***Bryum pseudotriquetrum***, a species that requires at least 10 hours of daylight for germination and protonema growth. Photo by David T. Holyoak, with permission.

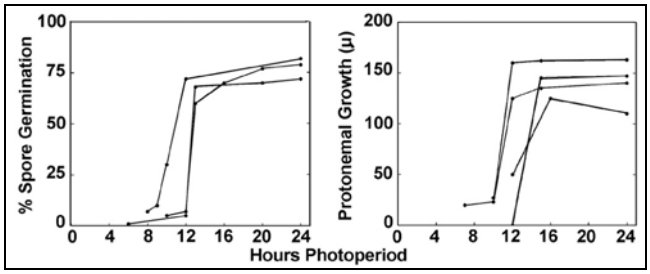

Figure 37. Effect of photoperiod on spore germination after 5 days (**left**) and protonema growth after 3 days (**right**) of ***Bryum pseudotriquetrum***. Redrawn from Kinugawa & Nakao (1965).

Hormonal Response

The complexity of these light responses and the implications of involvement by phytochrome is undoubtedly under the control of hormones. In the ephemeral ***Physcomitrella patens*** (Figure 9-Figure 10), light and hormonal combinations coordinate development (Cove *et al*. 1978, 1979). Bierfreund *et al*. (2003) supported this earlier conclusion by demonstrating that red light retarded the growth of protonemal filaments in ***Physcomitrella patens***. **Gametophores** (upright plants), on the other hand, responded by producing an elongated plant with shorter and narrower leaves. Responses of both protonemata and gametophores were even more pronounced when illuminated with far red light.

Cytokinin in the presence of auxin promotes buds (Gorton & Eakin 1957), and high concentrations inhibit caulonemata (Cove *et al*. 1978, 1979). This combination would therefore promote caulonema growth while the caulonemata are sparse, ensuring sufficient plants for a viable population and providing a sufficiently dense protonematal mat to help maintain moisture at the soil surface. When this mat becomes very dense, self-shading could stimulate the production of auxin and cytokinin and shift the development to bud formation. Once these self-shaded protonemata have shifted to bud development, they are likely to communicate this signal to the surface protonemata and induce buds throughout the mat. Figure 38 shows a developmental scheme modified from Cove *et al*. (1979) to include these environmental stimuli.

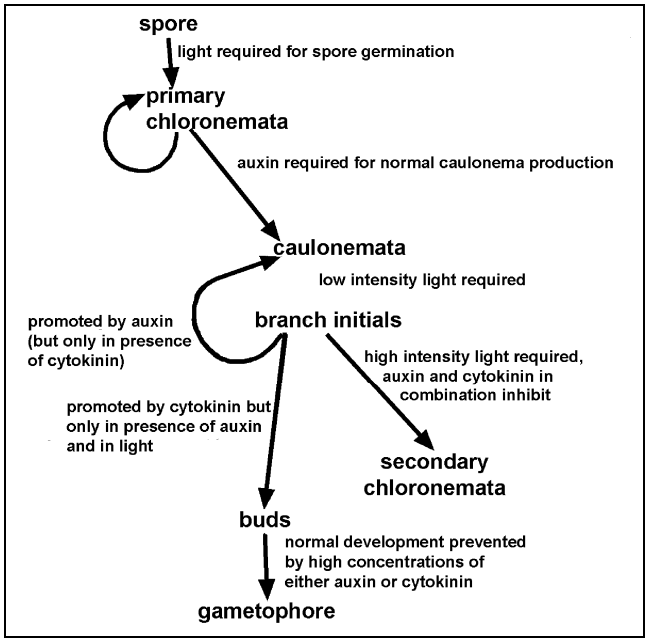

Figure 38. Effects of auxin and cytokinin on ***Physcomitrella patens***. Redrawn from Cove *et al*. (1979).

Bierfreund *et al*. (2003) used ***Physcomitrella patens*** (Figure 9-Figure 10) to determine the distribution of auxin (IAA) in the protonema. As in higher plants, the highest concentrations were in the dividing and young cells. Concentrations declined from the tip cells back to the basal cells of the protonema, supporting earlier work of Bopp and Atzorn (1992).

Auxin is important in the transition of chloronema to caulonema (Johri & Desai 1973; Figure 38) and the appropriate concentration maintains the caulonema state (Bopp 2000). Although we generally think that endogenous hormones from one plant cannot affect another, in ***Funaria hygrometrica*** (Figure 39) the minute quantity of 10^{-16} mol IAA/mg fw seems to be responsible for the change from chloronema to caulonema (Bhatla & Dhingra-Babbar 1990). Such a small quantity could surely leak from other members of the same species or from a different species to help coordinate behavior among individuals. In fact, as the protonema matures, the protonema can excrete most of its auxin to its substrate, as shown in ***Physcomitrella patens*** (Figure 9-Figure 10) (Reutter *et al*. 1998).

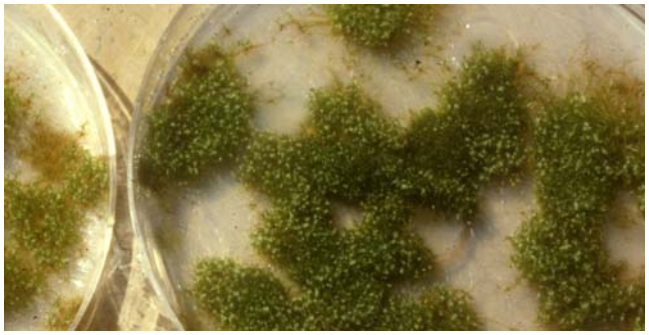

Figure 39. Culture of ***Funaria hygrometrica*** showing distinct colonies resulting most likely from hormonal interaction between clones at the protonemal stage. Each clump is the product of one spore. Photo by Janice Glime.

We already know that uptake of IAA by the protonema occurs; in the lab, uptake of IAA by protonematal cells is both passive and active. The passive component is *p*H-dependent, with the greatest increase in uptake occurring at *p*H 4.5-4.7, indicating a dissociation of the IAA molecule (*p*K = 4.7; ***pK*** is *p*H at which equal concentrations of acidic and basic forms of substance are present). The potential for an exogenous developmental regulator has enormous environmental implications not only for development, but for systematics and ecology as well.

Rose *et al*. (1983) used ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39) to show a strong *p*H dependence for the accumulation of auxins. The uptake of the auxin IAA increases when the *p*H is lowered from 7.6 to 4. The IAA appears to have influx and efflux carriers that help to determine the rate of this hormone in and out of the protonema. But these carriers seemed to be present only in low light intensities. At high light intensities (2.0-2.3 W m^{-2}) there was no evidence for them.

Physcomitrella patens (Figure 9-Figure 10) has become a widely used model for plant physiology. It is easy to grow and to standardize the cell culture protocol. Its complete genome is known. These characteristics make it useful to study plant physiological responses. And the protonema is an especially useful tool because it provides an isolated single cell type. ABA causes the subapical cells to form round **brachycytes** (short, thick-walled cells that are drought-tolerant brood cells) or nearly empty **tmema** (abscission cell) (Decker *et al*. 2006). When the cells are subsequently grown free of ABA, the brachycytes serve as propagules and germinate to form new protonema filaments (Schnepf & Reinhard 1997).

These brachycytes also occur in auxin-deficient mutants of ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39) (Schnepf & Reinhard 1997). Experiments in this species likewise confirm that ABA induces their production, and that it is concentration dependent. These brachycytes store lipids instead of starch and have altered chloroplast structure. This suggests that they provide a fallback mechanism to maintain the population if it becomes desiccated, a condition known to increase ABA production in mosses (Hajek & Vicherova 2014). Also, in ***Funaria hygrometrica***, application of auxin causes a change in development from the chloronema stage to the caulonema stage (Jayaswal & Johri 1980).

But having the right hormones isn't enough. There must be sufficient energy as well. We have seen that development of the protonema can occur in the dark, and in the early stages that energy is soon exhausted. To this end, the chloronemata are heavily endowed with chloroplasts (Thelander *et al*. 2005). The caulonemata, on the other hand, have more scattered chloroplasts and function to spread the colony by radial growth. The balance between the two protonema types is controlled by light and plant hormones. In ***Physcomitrella patens*** (Figure 9-Figure 10), caulonema formation is induced by high light, thus providing greater photosynthesis. External glucose also stimulates growth. But under low light conditions, the chloronema stage predominates, with chloronemal branching being stimulated by the low light (or perhaps high light suppresses chloronemal branching).

How widespread are these principles when we look at species outside the **Funariaceae**? In ***Hyophila involuta***

(**Pottiaceae**; Figure 40), **nurse protonemata** enhance the growth of other protonemata (Mehta 1990). This is the phenomenon in which substances diffused from an older protonema enhance the growth of the younger, developing protonema. It applies the rule of safety in numbers, in this case helping to protect the protonema and developing buds and gametophytes from desiccation.

Figure 40. ***Hyophila involuta***, a species that benefits from **nurse protonemata**. Photo by Bob Klips, with permission.

Tropisms

Tropisms, the bending, resulting from unequal growth on two sides of a stem, of a plant in response to a stimulus, are adaptive in orienting the plant into its most beneficial position. When the spore germinates, the developing protonema orients to gain the most light. When protonemal buds develop, they orient to obtain light. For the leafy gametophyte, this could mean extending away from gravity, as seen in acrocarpous mosses, or extending outward across the ground, as seen in pleurocarpous mosses. Both strategies of orientation have their advantages and disadvantages in obtaining sufficient light and consequent energy, and both are under control of hormones.

Phototropism

In bryophytes, protonemata are **positively phototropic** (bend toward light), whereas rhizoids are **photonegative** (bend away from light) (Heitz 1942). Although Kofler and coworkers investigated the effects of the environment on bryophyte tropisms as early as 1958 (Kofler 1958, 1971; Kofler *et al.* 1963), bryophyte tropisms have remained largely unstudied until recently. However, because of their simple protonemal structure, much of our current understanding of tropisms in plants has been learned from using bryophytes as model systems.

Yet bryophytes have different **phototropic** responses (directional growth in response to light) from those of tracheophytes. Rather than responding to blue light, as do the tracheophytes, most bryophytes seem to respond to red light, using **phytochromes** instead of **cryptochromes** as their sensory pigments (Wada & Kadota 1989; Esch *et al.* 1999). Jaffe and Etzold (1965) demonstrated that even spores (Figure 41) in ***Funaria*** (Figure 7-Figure 8, Figure 39) respond to red light, resulting in chloronema growth in the opposite direction from that of rhizoids. And even more intriguing is the ability of bryophytes to store a phototropic stimulus (Hartmann & Weber 1988), further suggesting the use of phytochromes. However, the expected dark reversal does not occur, indicating something else is involved (Christianson 2000). Phototropism will be discussed further under gravitropism because of the interaction of these two forces.

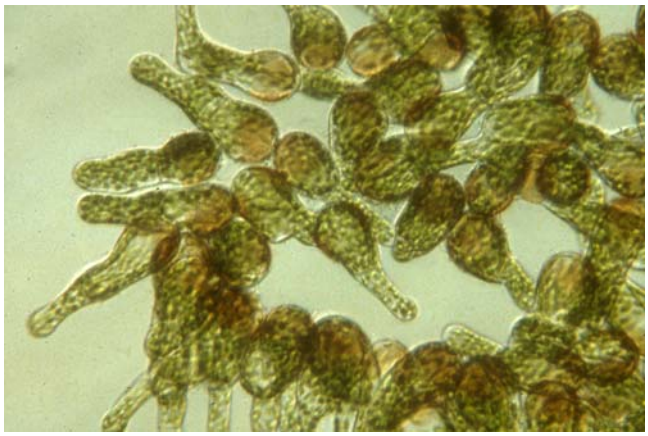

Figure 41. ***Funaria hygrometrica*** spore germination. Photo by Janice Glime.

Gravitropism

Gravitropisms respond to gravity, just as your spoon does when you drop it. But in plants, gravity has a different effect on different bryophyte plant parts and different life stages. In the protonema, it often is masked by the effects of light. Rhizoids are **positively gravitropic**, hence growing toward the earth, but for some species this is not the right position, so other responses have evolved. For acrocarpous mosses, the stems typically grow upward, as do the sporophytes. But like the rhizoids, stems may not always start in the right position. And likewise, the sporophyte might be pointed perpendicular to a vertical rock or tree trunk. For some species, there is a clear tropism in both gametophyte and sporophyte, for some only the sporophyte responds (Figure 42), and for some, both grow straight out from the vertical substrate (Figure 24), perpendicular to it.

Figure 42. ***Oligotrichum hercynicum*** showing a strong tropism in the seta but none in the gametophyte on this vertical surface. Photo by Michael Lüth, with permission.

Gravitropism is well documented in moss protonemata (Sack *et al*. 1998). Barlow (1995) suggested that the more evolutionarily advanced species will posses more systems for sensing gravity, arguing that if a system works, it is not likely to be discarded, thus being kept as new ones evolve. These multiple gravity-sensing systems permit gravity to be involved in a wider range of developmental responses. The sensing of gravity involves a membrane system to sense the gravity.

Schwuchow and Sack (1990) reported for the first time an effect of gravity on **microtubule** (essential protein filament of cell structural skeleton; Figure 43) distribution in plants, based on studies in protonemata of ***Ceratodon purpureus*** (Figure 33). In fact, this moss served as the model organism to demonstrate that microtubules help organelles to maintain their positions within the cell (Schwuchow & Sack 1994). Nevertheless, our understanding of **gravitropism** in protonemata is still in its early stages. We don't even have a very long list yet of mosses with demonstrated protonemal gravitropism, and we seem to know even less about liverworts. Schwuchow *et al.* (2002) have only recently found tropisms in protonemata of ***Barbula unguiculata*** (Figure 44), ***Fissidens adianthoides*** (Figure 45), ***Fissidens cristatus*** (Figure 46), and ***Physcomitrium pyriforme*** (Figure 47-Figure 48), despite the report of positive phototropism in ***Funaria*** protonemata in 1942 by Heitz.

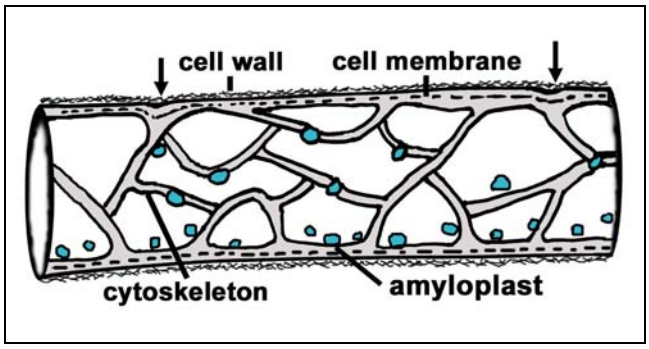

Figure 43. Schematic model of hypothetical relationship of **amyloplasts** (statoliths) of a protonema in response to gravity. Arrows denote pull of **cytoskeleton** on cell membrane. Drawing by Janice Glime.

Figure 44. ***Barbula unguiculata***, a species with tropisms in the protonema. Photo by Michael Lüth, with permission.

Figure 45. ***Fissidens adianthoides***, a species with tropisms in the protonema. Photo by Hermann Schachner, through Creative Commons.

Figure 46. ***Fissidens cristatus***, a species with tropisms in the protonema. Image ©Stuart Dunlop <www.donegal-wildlife.blogspot.com>, with permission.

Figure 47. ***Physcomitrium pyriforme*** with capsules in its soil habitat. Photo by Bob Klips, with permission.

Figure 48. ***Physcomitrium pyriforme*** protonema, a protonema that exhibits tropisms. Photo by Bob Klips, with permission.

The one-cell-thick protonema makes it easy to observe the **amyloplasts** (colorless plastids containing starch, sometimes referred to as statoliths) that respond to gravity. These statoliths are involved in **gravitropism** (directional growth in response to gravity). The ability to knock out or add genes that are easily expressed in the 1*n* plants (having only 1 set of chromosomes) has made the necessary manipulation much easier than in tracheophytes. Walker and Sack (1990) observed that **amyloplast sedimentation** occurred in horizontal protonemata of ***Ceratodon purpureus*** (Figure 33) grown in the dark. Protonemata grew straight up – away from the pull of gravity – at a rate of 20-25 μm h^{-1}, reaching an angle of 84° with the substrate by 24 hours. The tip cells exhibited a cluster of non-sedimenting amyloplasts, a zone free of amyloplasts, and a zone with pronounced amyloplast sedimentation. The sedimentation zone occurs only along lateral walls with some degree toward the horizontal and does not occur toward end walls regardless of their position. The beginnings of this gravitational rearrangement are visible within ~15 minutes of change in the direction of the gravitational pull. At this time Walker and Sack (and also Young and Sack 1992) suggested that the amyloplasts might act like the statoliths that help to orient crayfish and other organisms.

Young and Sack (1992) used time lapse photography to gain further understanding of the gravitropic response in ***Ceratodon purpureus*** (Figure 33). By this method, they observed that a "wrong-way" response occurred first. That is, the protonema initially curved downward in as little as 2 minutes after the protonemata were re-oriented. It required 30-45 minutes for upward curvature to begin. No amyloplast sedimentation occurred before the wrong-way response, but sedimentation seemed necessary for the onset of negative (correct) gravitropism.

But this brings to mind the question of their avoidance of the end walls when those walls are in the position closest to the gravitational pull. In succeeding experiments, Walker and Sack (1991) used centrifugation to displace all the amyloplasts in the apical cell to the end wall. In this position, the amyloplasts acted in the wrong way and the protonema curved downward, likewise in the wrong way. Upward curvature did not occur until sedimentation of amyloplasts occurred toward the lateral wall.

Later Wagner and Sack (1998) reported that the gravitropic response occurs within 1-2 cell divisions in the protonemal tip cells of ***Ceratodon purpureus*** (Figure 33), which grow upward in the dark (Wagner *et al*. 1997). Five mosses and four other species, representing five orders, support the hypothesis that amyloplast sedimentation probably serves in gravity sensing in moss protonemata. It appears that these amyloplasts tug on the **cytoskeleton** (structural support within cell), pulling down on it, much like trapped insects on a spider web. One theory is that this causes the cytoskeleton to pull on the cell membrane, creating larger holes in the membrane that facilitate the entry of Ca^{++}. This creates a higher concentration of Ca^{++} on the upper side of the cell, possibly causing it to inhibit the IAA on that side of the cell.

When auxin transport inhibitors were applied to ***Ceratodon purpureus*** (Figure 33), they strongly inhibited the gravitropic curvature of the apex of the protonema, suggesting the role of IAA in the process (Schwuchow *et al*. 2001). Reducing the concentration of inhibitors reduced the inhibition effect. Applications of high levels of IAA (40 μM) had no effect on the gravitropic response of the protonema apex, suggesting the mechanism differs from that in tracheophytes. But perhaps it is only the effective concentrations that differ. We know that roots respond to different levels from stems in tracheophytes, so we have no reason to expect bryophytes to respond to the same levels.

What little we thought we knew about gravitropisms in moss protonemata was further confused when growing protonemata of the moss ***Ceratodon purpureus*** (Figure 33) took a two-week trip in space on the space shuttle Columbia (Miller & Phillips 2003; Kern *et al*. 2005). On 16 July 2002, plant physiologist Fred Sack carefully opened a Petri dish that had spent the two weeks without gravity and without light. To his surprise, the protonemata had grown in a spiral pattern (Figure 49). This is quite different from the normal tangle of protonemata grown on Earth.

Figure 49. Spiral growth of protonemata of ***Ceratodon purpureus*** aboard space shuttle Columbia. Photo courtesy of Fred Sack.

According to Fred Sack (Miller & Phillips 2003), "These odd spirals mark the first time in space that a plant

normally oriented by gravity has grown in a non-random pattern." The puzzle begins with the **amyloplasts**. These starch bodies experience sedimentation in gravity and seem to tug on the cell skeleton. However, on the shuttle, with no gravity, this should not happen. Rather, they should float at random within the cell. Instead, they bunched together. This indicates a natural propensity for growing in a spiral that is overridden by the gravity of Earth. Perhaps Seifritz was right – all life does have a twist in it.

Another piece of this gravitropic puzzle is that a high-gradient magnetic field can substitute for gravity, causing curvature of tip cells in ***Ceratodon purpureus*** (Figure 33) (Kuznetsov *et al*. 1999). Genetically modified protonemata with larger plastids responded more strongly, supporting the hypothesis that plastids are involved in gravity sensing.

The caulonemata in ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39) are negatively gravitropic (Schwuchow *et al*. 1995). So in the dark, they grow upward. Such behavior can increase the opportunity to grow toward more light before there is light for them to sense. As in ***Ceratodon purpureus*** (Figure 33) and ***Physcomitrella patens*** (Figure 9-Figure 10), this upward curvature is temporarily reversed when the cell reaches its final stages of division. Tropism behavior in all three species indicates that subapical amyloplast sedimentation may be a common phenomenon in the protonemata of mosses.

Using ***Physcomitrella*** (Figure 9-Figure 10), Schwuchow *et al*. (1995) provided details of the gravitropic response within the cell. In the dark, a thin strip lacking amyloplasts was visible in the cytoplasm on the upper side of the cell. At this point, they suggested that amyloplast sedimentation might be a common gravitropic response in moss caulonemata. In 2002, Schwuchow *et al*. added ***Barbula unguiculata*** (Figure 44), ***Fissidens adianthoides*** (Figure 45), ***Fissidens cristatus*** (Figure 46), and ***Physcomitrium pyriforme*** (Figure 47-Figure 48) to the list of species with gravitropic protonemata that exhibited amyloplast sedimentation. Ultimately they demonstrated this sedimentation in nine species representing five different orders of mosses. Thus, we can conclude that this phenomenon is widespread among mosses and may be present in all of them.

This scenario is further explained by observations on ***Tortula modica*** (Figure 50-Figure 51) (Chaban *et al*. 1998). Amyloplast sedimentation occurs in the sub-apical zone. These amyloplasts seem to be important in signalling the direction of gravity and sedimentation is present before the tropic response occurs. Although spores require light for germination, the protonema is able to continue development in the dark, but both growth and number of filaments are limited (while resources last). Deprived of light, the protonemata are negatively gravitropic.

Secondary caulonemata, arising from a wound or fragment, likewise are strongly negatively gravitropic in the dark (Chaban *et al*. 1998). These are able to survive and grow well in the dark, most likely gaining resources from the wounded leafy gametophyte. In ***Tortula modica*** (Figure 50), these secondary caulonemata usually arise at the leaf bases. These tropic responses are rapid. When upright caulonemata are moved to make them horizontal or upside-down, the tropism can be seen within an hour and re-orientation to become vertical is completed in 1-2 days.

Figure 50. ***Tortula modica*** with capsules, a species exhibiting amyloplast sedimentation in the sub-apical zone of the protonema. Photo by Kristian Peters, with permission.

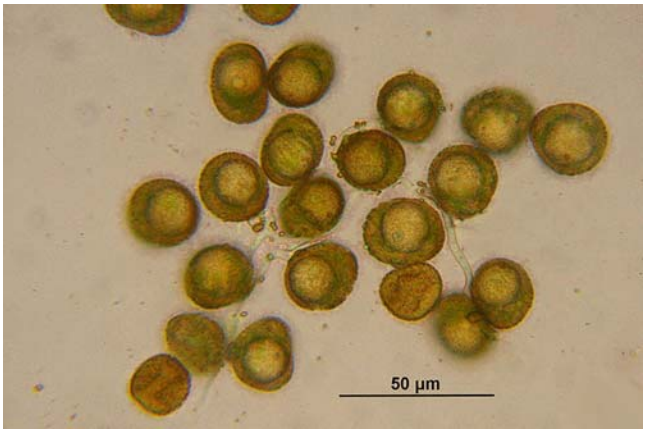

Figure 51. ***Tortula modica*** spores, a species exhibiting amyloplast sedimentation in the sub-apical zone of the protonema. Photo by Hermann Schachner, through Creative Commons.

We know that amyloplasts sediment in response to gravity (Walker & Sack 1992, 1997), just like sand grains dropped into a glass of water. So how do the plant organelles maintain their positions against the pull of gravity? The amyloplasts themselves may help us understand this. Using ***Ceratodon purpureus*** (Figure 33), several groups of researchers demonstrated that only some of the amyloplasts sediment along the length of the protonemal tip cell (Schwuchow & Sack 1993; Kern & Sack 2001; Kern *et al*. 2001). They reasoned that if gravity is the only or the major force determining the position of the amyloplasts, then they should be randomly distributed in space. But instead they are clustered in the subapical region when in **microgravity** (very weak gravity). The same occurs when the cells are rotated in a clinostat. But when controls are inverted and kept stationary, the distribution of the amyloplasts differs considerably due to sedimentation. This indicates that the amyloplast forces and mechanisms are normally masked in stationary cells. Kern and coworkers (2001) hypothesized that a "microtubule-based mechanism normally compensates for the drag of gravity, but at the same time it allows for the regulated amyloplast sedimentation." This basically agrees with the interpretation already put forth by Schwuchow *et al*. (1994) for ***Ceratodon***.

The foregoing research implies that gravity is not alone in controlling direction of growth. Using ***Ceratodon purpureus*** (Figure 33), Wagner *et al*. (1997) showed that in the dark, plastid sedimentation is more pronounced than in the light. In ***Ceratodon purpureus***, the apical protonema cells are negatively gravitropic in the dark, but in unilateral red light they are positively phototropic, thus overriding the gravitropic response (Kern & Sack 1999a, b). At light intensities of $\geq$140 nmol m^{-2} s^{-1}, the phototropism completely overrides the gravitropic response. Partial gravitropic response occurs at lower light intensities. In microgravity, phototropic responses occur. In normal gravity, gravitropism and phototropism compete and "winning" depends on the light intensity. ***Ceratodon purpureus*** demonstrates that phototropism is **phytochrome**-mediated (Lamparter *et al*. 1996, 1998; Kern & Sack 1999b). **Phytochrome** is a blue-green pigment in plants that regulates various developmental responses such as long-day and short-day responses.

Autotropism (tendency of plant organs to grow in a straight line when not influenced by external stimuli) occurs when no external stimuli (gravity, light) are present. Again using ***Ceratodon purpureus*** (Figure 33), Demkiv *et al*. (1997) determined that three stimuli are involved in the direction of protonema growth. In darkness, the protonemata have negative gravitropism. When illumination is uniform from all directions, they grow radially over the substrate, much like those in space or microgravity. In blue or far-red light the gravitropism is blocked, but in red light both gravitropism and autotropism are blocked. Green light (typical light in the forest) allows both gravi- and autotropism (Demkiv *et al*. 1998). Reversal of autotropism inhibition involves the phytochrome system, indicated by the red and far-red effects. Gravitropism occurs simultaneously with starch synthesis and amyloplast formation (Demkiv *et al*. 1997).

Using mutants of ***Physcomitrella patens*** (Figure 9-Figure 10), Jenkins *et al*. (1986) demonstrated that the genes that control gravitropisms of the caulonema do not appear to be involved in the control the tropisms of the leafy gametophyte.

Repp *et al*. (2004) used genetically modified ***Physcomitrella patens*** (Figure 9-Figure 10) to demonstrate the role of **cytokinin** signalling for gravitropism. When a knockout mutant lost its sensitivity to cytokinin, it had greatly reduced ability to respond gravitropically in the dark. Based on several studies, it appears that the cytokinins serve the protonemata primarily to induce gametophore buds (Lehnert & Bopp 1983; Bopp 1984).

Here you are, sitting in the dark, and you need light to continue life for long. What do you do? If you are a young protonema, you grow in the direction where you will most likely encounter light. And to do that, you exercise a **negative gravitropism**. That is, you grow away from gravity and toward the daytime sun. Once you reach sunlight, your **phototropism** takes over and you grow toward light.

Mosses may be "smarter" than seed plants. The moss protonemata apical cells can respond to both gravity and light, unlike most cell types (Kern & Sack 1999b). This permits these tiny structures to advance toward the most advantageous position. Even if they are anchored in a crevice, they can follow the path of light to reach the surface. For example, in ***Ceratodon purpureus*** (Figure 33), a species that is common in such cracks, the tips of the protonemata are negatively gravitropic in the dark and positively phototropic in unilateral red light. Thus, they would grow toward the opening in a crack.

It appears, based on our observations with protonemata, that the statoliths (**amyloplasts**) settle downward within the cell in response to gravity. This pulls on the **cytoskeleton**. The cytoskeleton is attached to the cell membrane, so this downward pull tugs on the membrane in the upper portion of the cell (Figure 43). A plausible theory is that this stretches the membrane, making it more permeable. This in turn permits more Ca^{++} to enter the upper side of the cell, where it inhibits the action of IAA, permitting the lower side of the cell to grow more.

Nutation

Under some circumstances, the protonema will exhibit **nutation** – a spiral or circular growth pattern that is displayed in time-lapse photography by apparent movements of the stem (or protonema) in a circle. In ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39), red light causes the protonema to grow into a ring (Simon & Naef 1981). I have observed the same nutation in contaminated cultures of ***Fontinalis squamosa*** (Figure 52) and in air-grown rhizoids of that species. Nutation appears to facilitate a kind of seeking – altering growth directions until a more favorable condition is located. It is beneficial when no directional stimulus is present, such as spiral growth of rhizoids until they contact a substrate, as observed in ***Fontinalis squamosa***. Although nutation is an IAA/ethylene response in higher plants (Morgan & Powell 1970), its occurrence as a response to red light suggests it results from a somewhat different mechanism here since red light is known to inhibit ethylene production. Could this be the same spiraling mechanism seen in the space-travelling ***Ceratodon purpureus*** (Figure 33) protonemata (Figure 49)? The curiosity there is that the entire population of protonemata grew in a spiral.

Figure 52. ***Fontinalis squamosa*** rhizoids showing spiral growth. Photo by Janice Glime.

Interactions

We have already implied that exogenous growth regulators could determine events in the development of the moss protonema. Protonemata in nature grow on substrata that are not sterile. Rather, they are teaming with fungi, bacteria, algae, and exudates of other plants. One might then predict that at least some of the protonemata respond in positive or negative ways to these companions.

One possible outcome of cohabitation is that bacteria, fungi, or other organisms may provide the growth substances needed to stimulate the next phase of development. Fungi commonly produce **gibberellic acid** that escapes into the environment. Vaarama and Tarén (1959) found that not only did 0.01% GA promote both spore germination and protonema growth in several mosses [***Dicranum scoparium*** (Figure 53), ***D. undulatum*** (Figure 54), ***Dicranoweisia crispula*** (Figure 55), and ***Pogonatum urnigerum*** (Figure 56)], but also inoculation with several fungi [***Aspergillus flavus*** (Figure 57), ***Penicillium martensii***, ***Mucor racemosus***, ***Fusarium scirpi***, and ***Rhodotorula mucilaginosa*** (Figure 58)] had even more effect than did the gibberellic acid.

Figure 53. ***Dicranum scoparium*** in a pine forest. In this species, spore germination and protonema growth are promoted by GA and fungi. Photo by Janice Glime.

Figure 54. ***Dicranum undulatum***, a species in which spore germination and protonema growth are promoted by GA and fungi. Photo by Michael Lüth, with permission.

Figure 55. ***Dicranoweisia crispula***, a species in which spore germination and protonema growth are promoted by GA and fungi. Photo by Michael Lüth, with permission.

Figure 56. ***Pogonatum urnigerum***, a species in which spore germination and protonema growth are promoted by GA and fungi. Photo by Michael Lüth, with permission.

Figure 57. ***Aspergillus flavus***, a fungus that interacts with the protonemata of mosses. Photo from Medmyco, through Creative Commons.

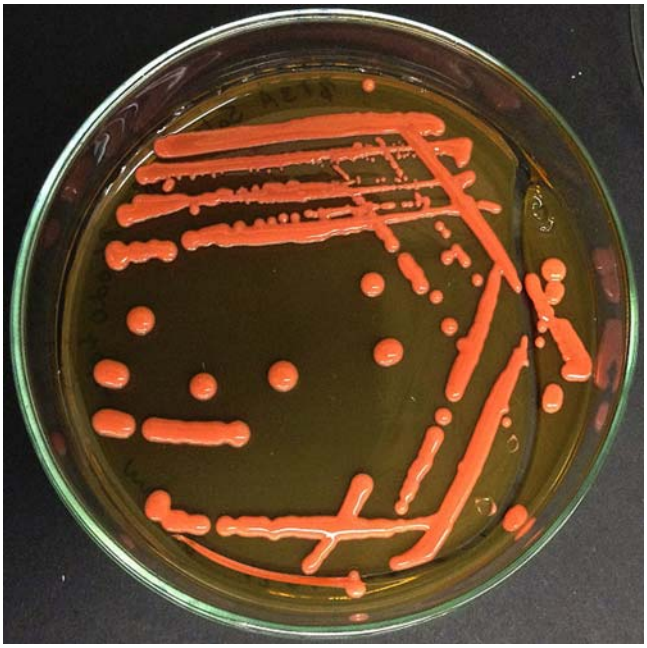

Figure 58. ***Rhodotorula mucilaginosa*** colonies, a yeast species that interacts with protonemata through Creative Commons.

In contaminated cultures of ***Fontinalis squamosa*** (Figure 1, Figure 15) most of the protonemata formed mature caulonemata in less than four weeks, whereas in uncontaminated cultures the chloronema state predominated (Glime & Knoop 1986; Glime, unpub data). And only the contaminated cultures ever produced buds. This suggests that at least some microbes might alter the developmental state of the moss.

Spiess *et al*. (1971) found that the bacterium ***Agrobacterium tumefaciens*** (Figure 59) influenced the development of ***Pylaisia selwynii*** (Figure 60). Spiess *et al*. (1986) found 48-68% of six groups of bacterial isolates (283 isolates) from separate samples [***Pylaisia selwynii***, ***Callicladium haldanianum*** (Figure 61)] increased the development of the moss species from which they were isolated but not that of ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39). There seemed to be both specificity and fidelity at nearby locations, but species differed between latitudes. Bacterial interaction may be important in bryophyte development.

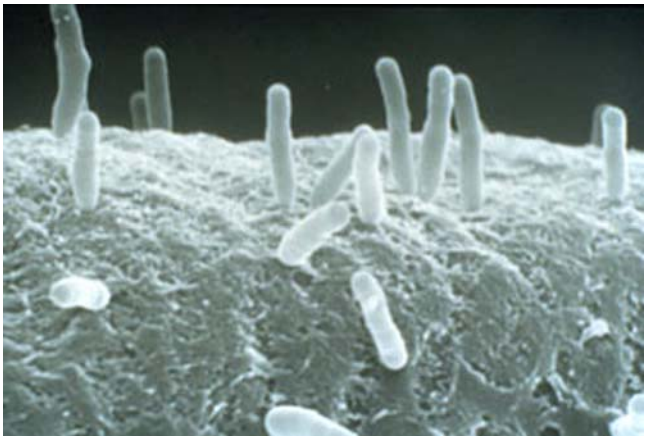

Figure 59. ***Agrobacterium tumefaciens*** on plant cell. Photo by Martha Hawes, University of Arizona.

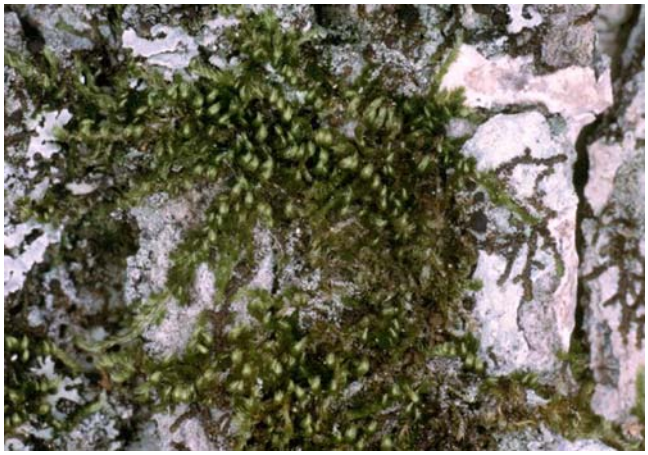

Figure 60. ***Pylaisia selwynii*** on tree bark. Protonema development in this species is enhanced by presence of ***Agrobacterium tumefaciens***. Photo by Jan-Peter Frahm, with permission.

Figure 61. ***Callicladium haldanianum***. Protonema development in this species is enhanced by presence of ***Agrobacterium tumefaciens***. Photo by Misha Ignatov, with permission.

Kutschera (2007) demonstrated a positive interaction between the methanol-using purple bacterium ***Methylobacterium*** [Figure 62; ***M. mesophilicum*** and two other unknown ***Methylobacterium*** species isolated from ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39) and sunflower achenes] and the protonemata of bryophytes [moss ***Funaria hygrometrica***; thallose liverworts ***Marchantia polymorpha*** (Figure 63) and ***Lunularia cruciata*** (Figure 64), but there was no benefit observed for the angiosperms studied. The same positive effect occurred for development from gemmae of the two liverworts. Methanol appears to be a waste product of the pectin metabolism of growing plant cell walls. Kutschera postulated that the ***Methylobacterium*** cells accomplished this protonemal developmental stimulation through their secretion of the plant hormones cytokinin and IAA (indole-3-acetic acid). Hence, the sequence seems to be:

1. Uptake and metabolism of plant waste products (methanol, amino acids, *etc*.) by the bacteria
2. Possible release of ammonium ions by bacteria
3. Secretion of cytokinins and IAA by bacterial "waste managers"

4. Bacterial hormonal signals may indicate to the plant that bacterial epiphytes are present and active
5. Hormones stimulate growth of the bryophyte gametophyte
6. Cross signals may help to regulate bryophyte growth.

This hormonal interaction may account for the success of bryophytes in some habitats in nature and the lack of success of at least some protonemata when grown in sterile culture.

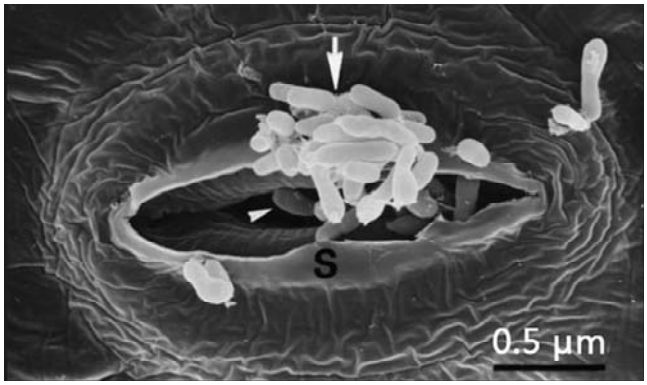

Figure 62. ***Methylobacterium*** in sunflower stoma, a bacterial species that has a positive interaction with protonemata of several bryophyte species. Photo by Kutschera U., through Creative Commons.

Figure 63. ***Marchantia polymorpha***, a species in which there is a positive interaction of the protonema with ***Methylobacterium*** spp. Photo by James K. Lindsey, with permission.

Figure 64. ***Lunularia cruciata***, a species in which there is a positive interaction of the protonema with ***Methylobacterium*** spp. Photo by David Holyoak, with permission.

Fungi have effects on other bryophyte protonemata as well. Hildebrand and coworkers (1978) found that fungal exudates promoted the growth of ***Atrichum*** (Figure 27-Figure 28), ***Funaria*** (Figure 7-Figure 8, Figure 39), and ***Brachythecium*** (Figure 65) protonemata (Figure 66) at low pH. As suggested above for spore germination, ***Splachnum ampullaceum*** (Figure 67) protonematal growth is promoted by several species of fungi (von Maltzahn & MacQuarrie 1958). Certainly growth hormones exuded by the fungi could be of importance here (see Bopp 1980).

Figure 65. ***Brachythecium velutinum*** with capsules, a species that has its protonematal growth promoted by fungi. Photo by Michael Lüth, with permission.

Figure 66. ***Brachythecium velutinum*** germinating spores and young protonemata, a species with fungal stimulation of protonemata. Photo by Eugenia Ron Alvarez & Tomas Sobota, with permission.

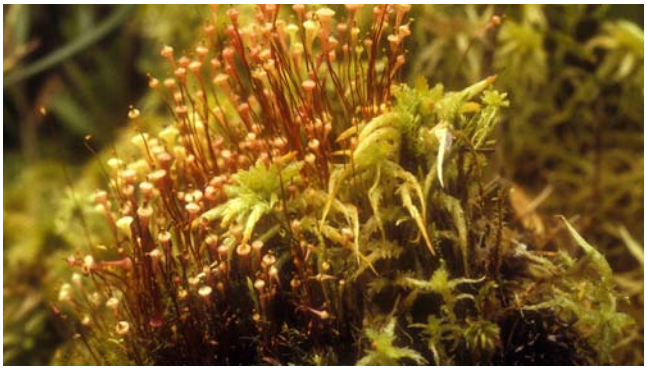

Figure 67. ***Splachnum ampullaceum*** growing among ***Sphagnum*** on dung, where changing dung conditions and fungal exudates influence development. Photo by Janice Glime.

In addition, contributions of vitamins from algae or amino acids or other organic compounds from bacteria might either be essential or promote a growth rate that is compatible with the seasons. Gibberellic acid, produced by many fungi, has a variety of effects, depending on the species of moss. It increases the number and length of protonemal cells in ***Dicranum*** (Figure 53-Figure 54) and ***Dicranoweisia*** (Figure 55), but it has no effect on ***Racomitrium fasciculare*** (Figure 68) (Vaarama & Tarén 1959). Since ***R. fasciculare*** grows on rocks where fungi are less likely to occur, and fungi are a natural source of GA, these differences in responses are consistent with habitat differences.

Figure 68. ***Racomitrium fasciculare***, a rock-dwelling species whose protonemata are not stimulated by GA. Photo by Janice Glime.

We know that the induction **Factor H** (an adenine derivative discussed in subchapter 5-1 on Hormones) is present in ***Funaria*** (Figure 7-Figure 8, Figure 39). It will induce not only other protonemata of ***Funaria***, but it can be induced by other species [*e.g.* ***Leptobryum pyriforme*** (Figure 69)] as well (Klein 1967; Bopp 1976). Such a factor is adaptive in insuring a sufficient breeding population, but perhaps more importantly it insures a community organization that offers resistance against desiccation, where middle plants are protected by outer ones in the population. In submerged mosses such as ***Fontinalis*** (Figure 70-Figure 71) species, on the other hand, moisture conservation is not so critical, and multiple gametophores would only offer competition for the limited substrate available for anchorage.

Figure 69. ***Leptobryum pyriforme***, a species whose protonemata can induce the protonemata of ***Funaria hygrometrica***. Photo by Michael Lüth, with permission.

Figure 70. ***Fontinalis squamosa*** on rock above water near Swallow Falls, Wales. Photo by Janice Glime

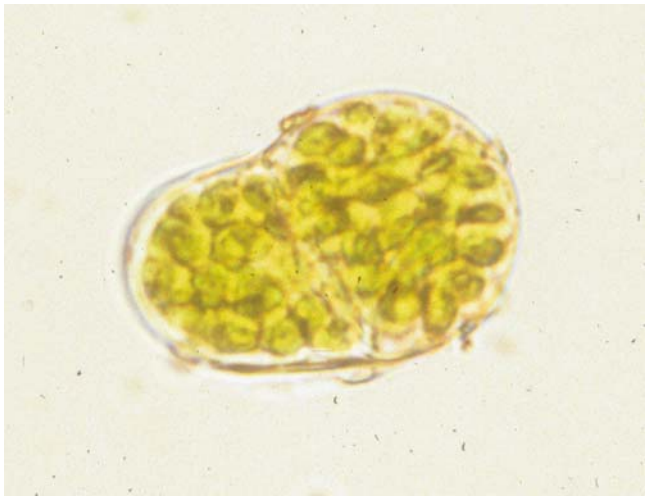

Figure 71. ***Fontinalis squamosa*** spore germination. Photo by Janice Glime.

Whereas some interactions can enhance growth of moss protonemata, others inhibit it, preventing the colonization of that substrate. Shrimal (1975) showed that bark extracts of several trees inhibited mitosis in onion root tips and caused non-separation of chromosomes. If these substances have the same effects on mosses, it could explain why some trees lack bryophytic epiphytes.

Inhibition can also occur within a species, as already suggested for ***Funaria*** (Figure 7-Figure 8, Figure 39). In this species, protonemata from several spores in one culture will not intersect (Watson 1981). The mat attains the same density when the protonemata are derived from many spores as when they are derived from only one. Watson also suggests that one species may inhibit another, thus making time an important factor in access to a habitat. And ***Funaria*** is not the only moss where some exudate of the protonema retards development of competing protonemata of the same species. This has been observed in culture in ***Physcomitrella patens*** (Figure 9-Figure 10) as well (Schween *et al*. 2003). It is perhaps a widespread phenomenon.

In ***Funaria*** (Figure 7-Figure 8, Figure 39), this factor of inhibition seems to break down in mature cultures. When I placed disks of agar from a mature culture onto fresh plates and inoculated the plates with spores, some of

the protonemata grew on the disks from the mature cultures. In no case did I find a zone of inhibition around the agar disk. This suggests to me that the substance preventing live protonemata from intersecting might be a gas produced by the growing protonemata. Gases are instrumental in maintaining maximum distance among sporangia of some slime molds, and one gas that could accomplish this in mosses is ethylene. Since ethylene is known to affect ***Funaria*** protonemata (Rohwer & Bopp 1985) and it is a known inhibitor of cell division (Abeles 1973), small concentrations produced by the tips could easily signal their presence to neighbors. Ethylene production is stimulated by the action of IAA on S-adenosylmethionine (SAM), so we might expect the tip (where there is the most IAA) to have the highest ethylene concentration. The longest branches will interact first, and these are the ones most likely to be IAA-rich and apically dominant.

Hormones produced by other organisms in the environment can affect the development of protonemata, and in some cases these may be required to take the bryophyte to its next developmental stage. Among these, GA (gibberellic acid) is a likely candidate. It is produced by many fungi and readily enters the environment. It is known to increase the number and length of protonematal cells in some soil-inhabiting species, but may have no effect on rock-dwelling taxa that normally would have much less contact with soil fungi. Bark exudates may also inhibit growth of some bryophyte protonemata, and some bryophytes may inhibit each other, both of different species and of other clones of their own species.

Nutrients

In some mosses, the form of the protonema is dependent on available nutrients. For example, in nature ***Sphagnum*** (Figure 72-Figure 74) normally has a thalloid protonema (Figure 73-Figure 74). However, in a medium with high potassium, the protonema becomes filamentous (Schofield 1985). Since ***Sphagnum*** normally grows in habitats very low in potassium, this filamentous growth form is not observed in nature.

Figure 72. ***Sphagnum***, a genus with a thalloid protonema. Photo by Janice Glime.

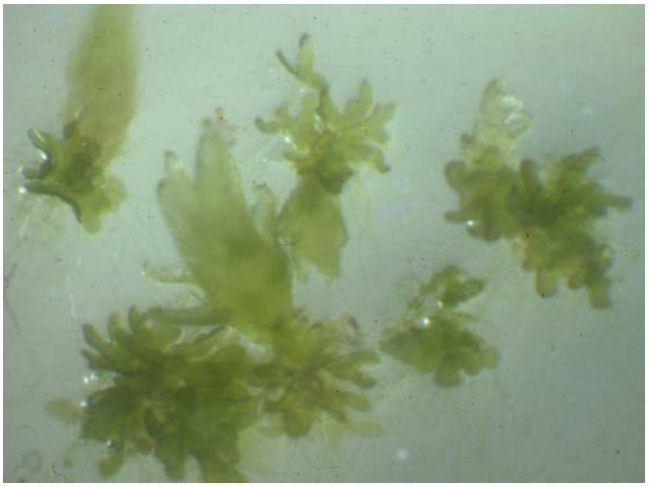

Figure 73. Thalloid protonemata of ***Sphagnum papillosum***. Photo courtesy of Yenhung Li.

Figure 74. ***Sphagnum*** protonemata on the stem of a mature ***Sphagnum*** plant. Photo by Andras Keszei, through Creative Commons on Flickr.

Sucrose may not be a problem in nature, but in culture added sucrose enhances growth, provided the culture does not become contaminated. Yu *et al*. 2008 pointed out that sucrose effects vary among species. The optimal sucrose concentration for the moss ***Microdus brasiliensis*** (Figure 18) was 1-2% (Sarla 1992), whereas both ***Splachnum ampullaceum*** (Figure 75-Figure 76) and ***Atrichum undulatum*** (Figure 35) grew better with no added sucrose (Sabovljević *et al.* 2005; González *et al*. 2006). One problem is that when the concentration of sucrose is too high it causes exosmosis, hence dehydrating the protonema (Fernández & Revilla 2003). Sabovljević *et al*. (2006) demonstrated that a 3% sucrose concentration inhibited the protonemal growth of the moss ***Atrichum undulatum***. Yu *et al*. (2008) tested sucrose:nitrogen effects on protonemata of ***Polytrichum commune*** (Figure 31) at sucrose levels of 0, 10, and 40 g L^{-1} and ammonium nitrate of 0, 0.2, and 0.4 g L^{-1}. The best growth of those protonemata were at ratios of sucrose to nitrogen of 10:0.2, 40:0.2, and 40:0.4.

Figure 75. ***Splachnum ampullaceum*** with capsules, a dung-dwelling species that grows better in culture with no added sucrose. Photo by Michael Lüth, with permission.

Figure 76. ***Splachnum ampullaceum*** peristome and spores that grow best on agar with no sucrose. Photo by Janice Glime.

Nitrogen in the medium can be detrimental to the protonemata at concentrations suitable for tracheophytes (see Chapt 8-1, pp. 1-4). Fangmeier *et al*. (1994) found that high concentrations of ammonium ions in plant cells can cause membrane dysfunction. It appears that established protonemata and plants can harbor sufficient nitrogen that they can be grown in the absence of nitrogen (Duckett *et al*. 2004). Nevertheless, Yu *et al*. (2008) found that when sucrose was added to the medium, growth was better in low concentrations of accompanying nitrogen as ammonium nitrate than with sucrose alone. In fact, the detrimental effects of high concentrations of sucrose can be counteracted by the addition of nitrogen (George 1993; González *et al*. 2006), and for ***Polytrichum commune*** (Figure 31) Yu *et al*. found that even at 4% sucrose there was a positive effect on protonemal growth when sucrose was combined with the appropriate level of ammonium nitrate.

Sundberg and Rydin (2000) showed that ***Sphagnum*** (Figure 73-Figure 74) establishment from spores was limited by the amount of phosphate released by underlying litter. Added moose dung likewise promoted establishment. They concluded that cover of other plants and nutrient release from litter provided safe sites where ***Sphagnum*** spores could germinate and establish new plants.

Calcium seems important to protonema development in some species and may be the actual factor where *p*H affects viability. For ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39), Reiss and Herth (1979) suggest that a calcium gradient is responsible for protonemal tip growth. The calcium concentration is highest at the tip where fluorescence is strongest. It is likely that calcium is involved in transport of substances across cell membranes.

Nutrient availability is affected by *p*H. Thus *p*H could affect success of protonemata. In ***Physcomitrella patens*** (Figure 9-Figure 10, Figure 77, Figure 78), changes in pH in the range of 4.5 to 7.0 influenced differentiation of protonemata but did not have any negative impact on growth rate (Hohe *et al*. 2002). In another example, ***Anisothecium molliculum*** has an optimum *p*H of 5.5 for not only protonemal growth, but also for bud formation (Kumra & Chopra 1985). The *p*H may not only alter the ability of bryophyte protonemata to obtain nutrients, but also affect their susceptibility to exudates from other plants and fungi. Following fire, invasion by bryophytes onto the charred substrate seems to be likewise influenced by both *p*H and residual chemicals (Thomas *et al*. 1994). Germination success in the moss ***Campylopus pyriformis*** (Figure 79) is positively influenced by increases in the *p*H in the range of 3.5-6.4.

Figure 77. ***Physcomitrella patens*** in its natural habitat where *p*H and moisture can change considerably as spring flooding recedes. Photo by Michael Lüth, with permission.

Figure 78. ***Physcomitrella patens*** plants with protonemata on the wet soil. Photos by Michael Lüth, with permission.

Figure 79. ***Campylopus pyriformis***, a species whose protonemata grow better as *p*H is increased in the range of 3.5-6.4. Photo by Michael Lüth, with permission.

Various heavy metals seem to alter protonematal form. Kapur and Chopra (1989) found that in the moss ***Timmiella anomala*** (Figure 80), when grown aseptically (conditions free of microorganisms), aluminum causes protonemal cells to become rounded and packed with chloroplasts and starch grains; the filaments themselves form bunches. Zinc and arsenic likewise cause rounded cells, with zinc-damaged cells becoming reddish; most arsenic effects are seen at the terminal and intercalary positions. Mercury causes cells to become broad with dense particles, whereas nickel results in long, thin protonemata with little branching. At 10^{-6} M, nickel increases protonemal growth slightly, but at 10^{-5} M it drastically decreases the number of gametophore buds. Cobalt inhibits protonemal growth but seems to have no effect on bud formation. What do these effects mean to development of the moss, and are they likely to occur in nature where soil chelators (organic compounds that bind metal by forming ring structure around it) may inhibit uptake, or concentrations never reach these levels? Could they actually affect appearance of mature gametophytes resulting from these anomalous forms and hence confound our understanding of the taxonomy?

Landing in the wrong place can inhibit spore germination, but it can also permit germination but inhibit protonema development. In some cases, these unfavorable conditions might cause the protonema to produce dormant cells that can act like gemmae to grow when favorable conditions are forthcoming. Such seems to be the case for protonemata of ***Dicranella heteromalla*** (Figure 81-Figure 82) that spent the winter in a lake with acid mine waste (Jan Fott, pers. comm.).

Figure 80. ***Timmiella anomala***, a species in which heavy metals alter the protonemal form. Photo by Michael Lüth, with permission.

Figure 81. ***Dicranella heteromalla*** with capsules, on a typical soil bank habitat. Photo by Michael Becker, through Creative Commons.

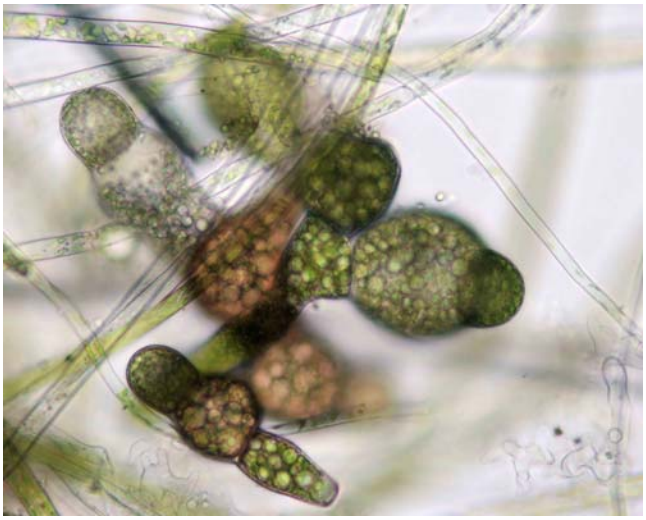

Figure 82. ***Dicranella heteromalla*** protonemata that survived winter in an acid mine lake. Photo courtesy of Jan Fott.

Our knowledge of nutrient requirements is based mostly on cultures of bryophytes and we know little of the generalities that might be important. For example, elevated potassium causes ***Sphagnum*** protonemata to become filamentous instead of thalloid, but in nature we have not observed protonemata in habitats where this condition exists. The level of phosphorus is often limiting and we can assume this plays a role in nature as well. An important observation is that heavy metals such as aluminum, zinc, mercury, and arsenic can cause abnormal protonemata with such symptoms as rounded cells with dense chloroplasts and starch. Elevated nickel, on the other hand, causes the protonemata to be thin. Calcium is undoubtedly important and its function may relate to membrane transport of other ions into the cell. All of these nutrient effects are likely to be affected by the pH because a lower (acidic) pH generally makes most nutrient ions more soluble.

Rhizoids

Botanists have traditionally considered rhizoids to function in anchorage only. In some cases they provide capillary spaces that aid in moving water externally to and even up the stem. But Duckett and Matcham (1995) discovered that the structure of rhizoids in ***Dicranella heteromalla*** (Figure 81-Figure 82) is cytologically similar to the food-conducting cells (**leptoids**) in many leafy mosses and moss sporophytes. This realization suggests that a major role of rhizoids may indeed be uptake, much like the root hairs of tracheophytes.

Rhizoids (Figure 83) form on the protonema at different stages, depending on the species and the growing conditions. On nutrient-free agar and in distilled water the first filaments to emerge from the spore are rhizoidal (Bhatla 1994). They are distinguished by their pigmented (usually brown) cell walls, oblique crosswalls, and discoid or cylindrical plastids. The rhizoids seem to depend on forced calcium entry (active uptake requiring energy) for growth and at least in those tested, respond positively to a calcium gradient (Bhatla 1994).

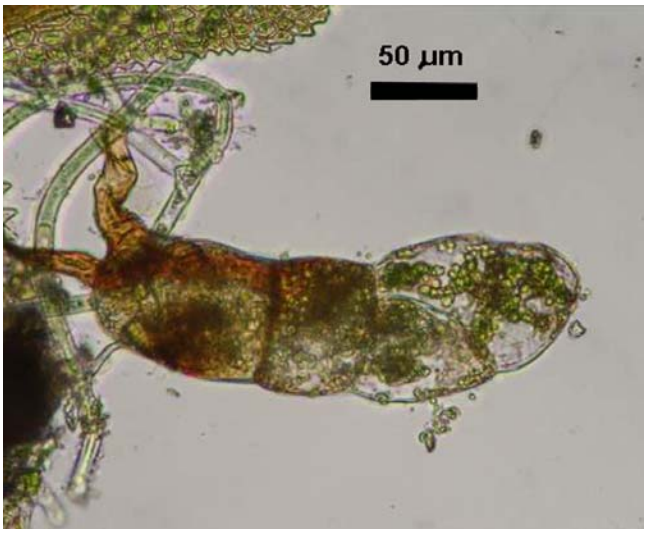

Figure 83. ***Fissidens tenellus*** bud with rhizoids at its base. Photo by Tom Thekathyil, with permission.

Rhizoids usually exhibit strong positive **gravitropism** (grow toward the center of gravity), negative **phototropism** (grow away from light), and **thigmotropism** (alter their growth upon contact), with the latter overriding the effects of the former once a substrate is contacted (Bhatla 1994). When growing in air, they often exhibit a spiral growth (**nutation**) until a substrate is contacted (Glime 1987). Upon contact, they may branch into short, fingerlike tips (Odu 1988), as noted in ***Lophocolea cuspidata*** (Figure 84) (Odu & Richards 1976) and ***Fontinalis squamosa*** (Figure 85) (Glime 1987). Among the liverworts, apical branching seems to be in part phylogenetically constrained, appearing commonly in the **Jungermanniales** (Figure 84) but only in the **Metzgeriineae** (Figure 86) of the **Metzgeriales** and not at all in the **Marchantiopsida** (Figure 87) (Pocock & Duckett 1985). Those liverworts with swollen rhizoids grow exclusively on peat and rotten wood associated with fungal hyphae. Pleurocarpous moss rhizoids become flattened near the tips, but in acrocarpous mosses these flattenings extend well behind the tips of the rhizoids (Odu 1988).

Figure 84. ***Lophocolea cuspidata***, a species in which rhizoids branch upon contact into finger-like tips. Photo from Botany Website, UBC, with permission.

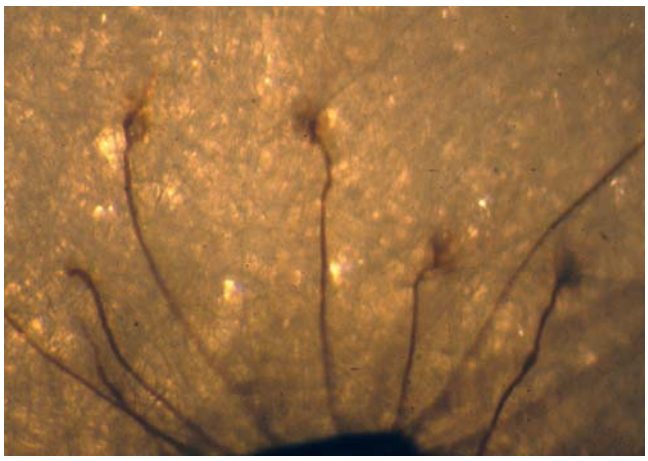

Figure 85. ***Fontinalis squamosa*** rhizoids forming fingerlike tips where they contact the filter paper. Photo by Janice Glime.

Figure 86. ***Metzgeria conjugata***, member of the **Metzgeriineae**, a genus that exhibits branched rhizoids. Photo by David Holyoak, with permission.

Figure 87. ***Cyathodium*** sp., representing the **Marchantiopsida** with the protonema lacking apical branching. Photo courtesy of Noris Salazar Allen.

Adhesion of rhizoids seems to be stimulated by the substrate itself (Odu 1988). Upon contact, rhizoids produce such extra-wall materials as sulfated mucopolysaccharides. These are highly viscous substances that serve as a sticky adhesive, also known in algae and other microorganisms.

But what controls the production of these rhizoids? Goode *et al*. (1992) were unable to get ***Tetraphis pellucida*** (Figure 6) to produce any protonemal rhizoids in culture, yet these occurred routinely in nature. They ascribed this difference to the limited nutrients and different irradiance in the wild. But hormones available from surrounding vegetation, bacteria, and fungi could play a role as well, as they apparently do for the protonemata.

Tmema

Tmema cells (Figure 88) are rounded cells that rupture, setting free a protonemal gemma (Figure 89) (Bopp *et al*. 1991). These cells result from a very unequal division of the cell near the proximal cross wall and divide the chloronema filaments into fragments of only a few cells. The tmema cells have few chloroplasts which soon become reduced in size, but the cell elongates in its proximal direction by expanding its newly formed wall, progressing in the opposite direction from normal cells. The new tmema wall forms inside the old lateral wall and the subsequent loosening of the old wall results in fragmentation of the protonema. This separation also occurs in older, untreated cultures of ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39) (>25 days) (Bhatla & Dhingra-Babbar 1990).

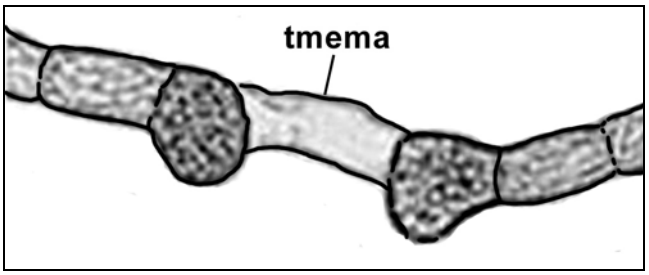

Figure 88. **Tmema** cell in protonema. Redrawn from Decker *et al*. 2006.

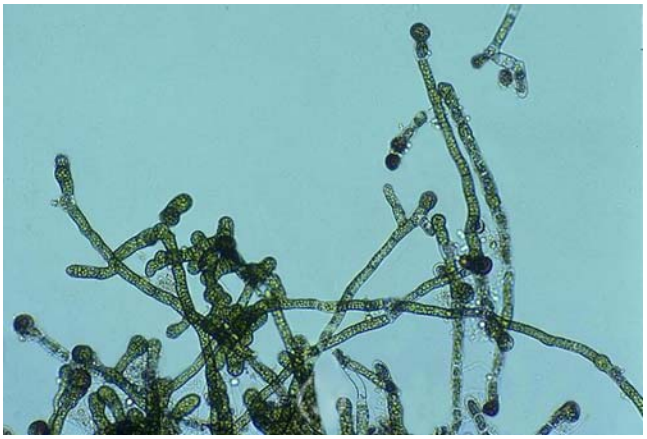

Figure 89. ***Bartramia ithyphylla*** with protonemal gemmae. Photo by Eugenia Ron Alvarez & Tomas Sobota – Plant Actions, with permission

In ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39), the ageing protonemata form tmema cells. Formation of these is inhibited by 10 µM IAA, indicating that they form when the protonema is auxin deficient (Bopp *et al*. 1991). Once formed, the cell elongates in the proximal direction by forming a new tmema cell wall, thus reversing its polarity compared to normal cells, which elongate distally. This new wall replaces the old lateral wall and also covers the tip of the tmema cell. The new wall is, however, lacking at the cross wall toward the sister cell of its division. The new wall contains a higher cellulose content and an array of microtubules and microfibrils compared to other cells in the protonema. The old lateral wall loosens and ruptures and the tmema disintegrates as its wall swells and dissipates.

But these are laboratory results. Does the tmema occur in nature? Is it adaptive? Could it permit small fragments of the protonema to have one more chance at dispersal before producing its upright gametophore, hence possibly allowing it to arrive at a place where it could indeed produce enough of its own IAA in a more favorable setting? How remarkable a survival mechanism if indeed it permits another chance at dispersal.

Tmemata seem to have received little attention among bryologists and we seem to have little knowledge of their occurrence in nature. In their cultures of ***Dicranella***

heteromalla (Figure 81-Figure 82), Duckett and Matcham (1995) found that tmemata had formed. These shortened cells were common on chloronemal side branches that produced both terminal and within-filament gemmae. The tmemata serve as abscission cells that permit the detachment of the gemmae. This occurs through the swelling of a new internal wall in the tmema cell, as seen in ***Funaria hygrometrica*** (Figure 7-Figure 8, Figure 39). If this species is grown on nutrient-free agar, the protonemata fail to produce gemmae, but rather produce filaments of different diameters, down to 4-5 μm, that make a spiral path through the medium or form knot-like aggregations if grown on cellophane-covered agar.

Goode *et al*. (1993) observed similar tmemata in cultures of ***Bryum tenuisetum*** (Figure 90). Ligrone *et al*. (1996) described a similar development for tmemata and gemmae in protonemata of ***Aulacomnium palustre*** (Figure 91). Edwards (1978) described tmemata associated with protonemal gemmae in collections of ***Schistostega pennata*** (Figure 92-Figure 93) and noted that this type of gemma with an associated tmema was rare among moss species. Based on my hunt in Google Scholar, I would conclude that they are either rare, or rarely reported.

Figure 90. ***Bryum tenuisetum***, a species that produces tmemata in culture. Photo by Michael Lüth, with permission.

Figure 91. ***Aulacomnium palustre***, a species that forms gemmae and tmemata on its protonemata. Photo by Kristian Peters through Creative Commons.

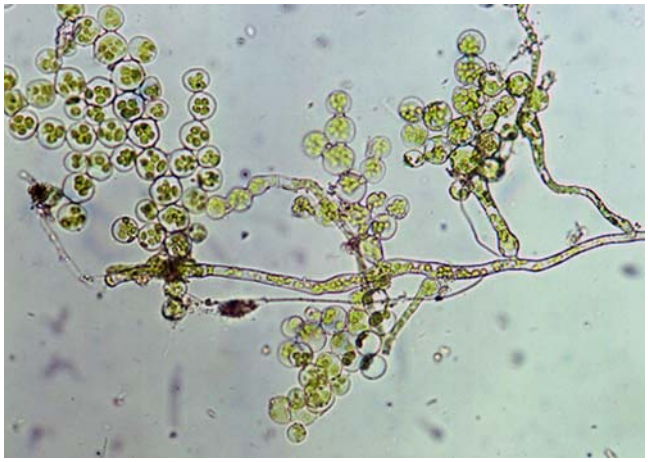

Figure 92. Protonema of ***Schistostega pennata*** showing filamentous protonema and round refractive cells. Photo by Irene Bisang, with permission.

Figure 93. Protonemal gemma (oblong cell) with short **tmema** at its base on ***Schistostega pennata***. Photo by Misha Ignatov, with permission.

In the copper moss ***Scopelophila cataractae*** (Figure 94-Figure 95), copper concentrations, but not other metals tested, affect the production of protonemal gemmae and associated tmemata (Nomura & Hasezawa 2011). Making the assumption that this moss is able to invade copper-rich substrata because of gemmae, the researchers tested the sensitivity of the protonema. Although the gemmae were suppressed, the copper promoted the growth of the protonema.

Figure 94. ***Scopelophila cataractae*** habitat in India. Photo by Michael Lüth, with permission.

Figure 95. ***Scopelophila cataractae***, a "copper moss" in which copper suppresses production of protonemal gemmae but enhances protonemal growth. Photo by Michael Lüth, with permission.

Tmemata are one means of providing vegetative reproductive structures on the protonema. Various types of protonematal asexual reproductive structures will be discussed in Chapter 5-7 on asexual reproduction. A brief discussion of those associated with protonemata is provided here.

Protonemal Gemmae and Tubers

Production of gemmae on the protonema seems to be affected by a variety of substances and conditions. Chopra and Dhingra-Babbar (1984) found that a variety of substances affect gemma initiation and growth rates of the protonema in ***Trematodon brevicalyx***. These included IAA, GA, ABA, chelates, salicylic acid. In addition, responses were altered by temperature, *p*H, agar, sucrose levels, light levels, and photoperiod.

In ***Hyophila involuta*** (Figure 40), in addition to promoting growth, the protonemal diffusate (from gemma-producing protonemata) + kinetin acted synergistically to enhance gemma formation. ABA (10-5-10-7 M) + protonemal diffusate inhibited gemma production (Mehta 1990).

Sarla and Chopra (1989) found that in ***Bryum capillare*** (Figure 96), kinetin slowed protonemal growth. **Bryokinin** (a type of cytokinin growth hormone found in mosses) inhibited protonemal growth at all levels. Rather, gemmae were produced in response to kinetin and bryokinin.

Figure 96. ***Bryum capillare***, a species in which kinetin and bryokinin slow protonemal growth and induce gemmae. Photo by Andrew Spink, with permission.

More recently, Ahmed and Lee (2010) explored the induction of protonemal gemmae in ***Palustriella decipiens*** (Figure 97). They found that concentration of IAA and kinetin was important in stimulating production of protonemal gemmae. Low concentrations promoted gemmae and bud induction.

Figure 97. ***Palustriella decipiens***, a species in which concentration of IAA and kinetin is important in stimulating protonemal gemmae. Photo by Michael Lüth, with permission.

Liverworts

Little seems to be written about the protonemata of liverworts to explain the details of their development in any ways that may differ from that of mosses. Liverwort protonemata differ fundamentally from those of mosses in that the liverwort protonema is thalloid (Figure 98-Figure 100). As mentioned above, the rhizoids of the liverworts in **Marchantiopsida** do not branch apically, but those of the **Jungermanniales** do (Pocock & Duckett 1985).

Figure 98. ***Sphaerocarpus texanus*** thalloid protonema with rhizoids. Photo from Plant Actions through Eugenia Ron and Tom Sobota, with permission.

Figure 99. Early stage of the liverwort ***Fossombronia caespitiformis*** protonema. Photo from Plant Actions through Eugenia Ron and Tom Sobota, with permission.

Figure 100. ***Fossombronia caespitiformis*** protonema showing rhizoids on a liverwort in the **Metzgeriidae**. Photo from Plant Actions through Eugenia Ron and Tom Sobota, with permission.

Figure 101. Protonemata of ***Schistostega pennata*** holding particles of soil together by building bridges between them. Photo by Misha Ignatov, with permission.

Figure 102. ***Buxbaumia aphylla*** showing persistent protonemata. Photo by Janice Glime.

Ecological Considerations

We have discussed the ability of the protonema to branch, then form many gametophore buds. This permits it to produce many upright gametophores in close enough proximity to create capillary spaces and reduce air movement, thus reducing drying. Furthermore, this mat of protonemata can provide bridges across the tiny soil particles (Ignatov *et al*. 2012), binding the soil and creating more capillary spaces for water retention. In ***Schistostega pennata*** (Figure 92-Figure 93, Figure 101), the sticky surface of the propaguliferous protonema extends across the soil particles, stabilizing the surface in a way that helps to create its own habitat (Ignatov *et al*. 2012).

Because of this binding ability, and the ability to withstand drought and revive upon rewetting, protonemata of a number of species can contribute significantly to soil binding in disturbed areas. To this end, mosses like ***Atrichum*** spp. (Figure 27-Figure 28, Figure 35) can stabilize soil on broad paths and soil banks. Mosses with persistent protonemata, like ***Pogonatum*** spp. (Figure 29-Figure 30, Figure 56) and ***Buxbaumia aphylla*** (Figure 102) are able to stabilize the otherwise bare soil where they live, often on soil banks. Hence, protonemata can play an important role in stabilized disturbed soil in ecosystems.

Summary

The **filamentous protonema** of Bryophyta can differentiate into two types: **chloronema** and **caulonema**, distinguished by short cells with perpendicular crosswalls, numerous chloroplasts, colorless cell walls, and irregular branching in the former and longer cells, diagonal crosswalls, brownish cell walls, and fewer, scattered, small chloroplasts in the latter. IAA induces the switch to caulonema; cytokinins promote branching. Protonemata of **Sphagnopsida**, **Anthocerotophyta**, and most **Marchantiophyta** are thalloid.

Protonemata can produce a variety of **brood cells**, possibly stimulated by **ABA**, and sometimes disarticulated from the protonema by **tmema** cells. Light quantity, quality, photoperiod, and temperature influence both the rate of development and the form of the protonema. Their direction of growth is influenced by both gravity and light, causing **negative gravitropism** in the dark and **positive phototropism** in the light.

Other organisms, especially bacteria and fungi, may supply **IAA**, **cytokinins**, and **GA** that influence

development, and **Factor H** (a likely **cytokinin**) may be supplied both endogenously and exogenously to control population size. Nutrients can affect the development; the ratio of sucrose:nitrogen determines if they are beneficial or detrimental, and heavy metals generally cause abnormalities or arrested development.

Rhizoids exhibit **positive gravitropism** and **negative phototropism**, but also possess **thigmotropism**, typically expanding, branching, or flattening upon contact with a substrate.

Liverworts have thalloid protonemata and in many the rhizoids do not branch at the tips.

Protonemata are important ecologically as early stabilizers of the soil in disturbed areas. By branching and producing many buds, they quickly create cushions and mats that can support each other in maintaining moisture.

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CHAPTER 5-4

ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOPHORE BUDS

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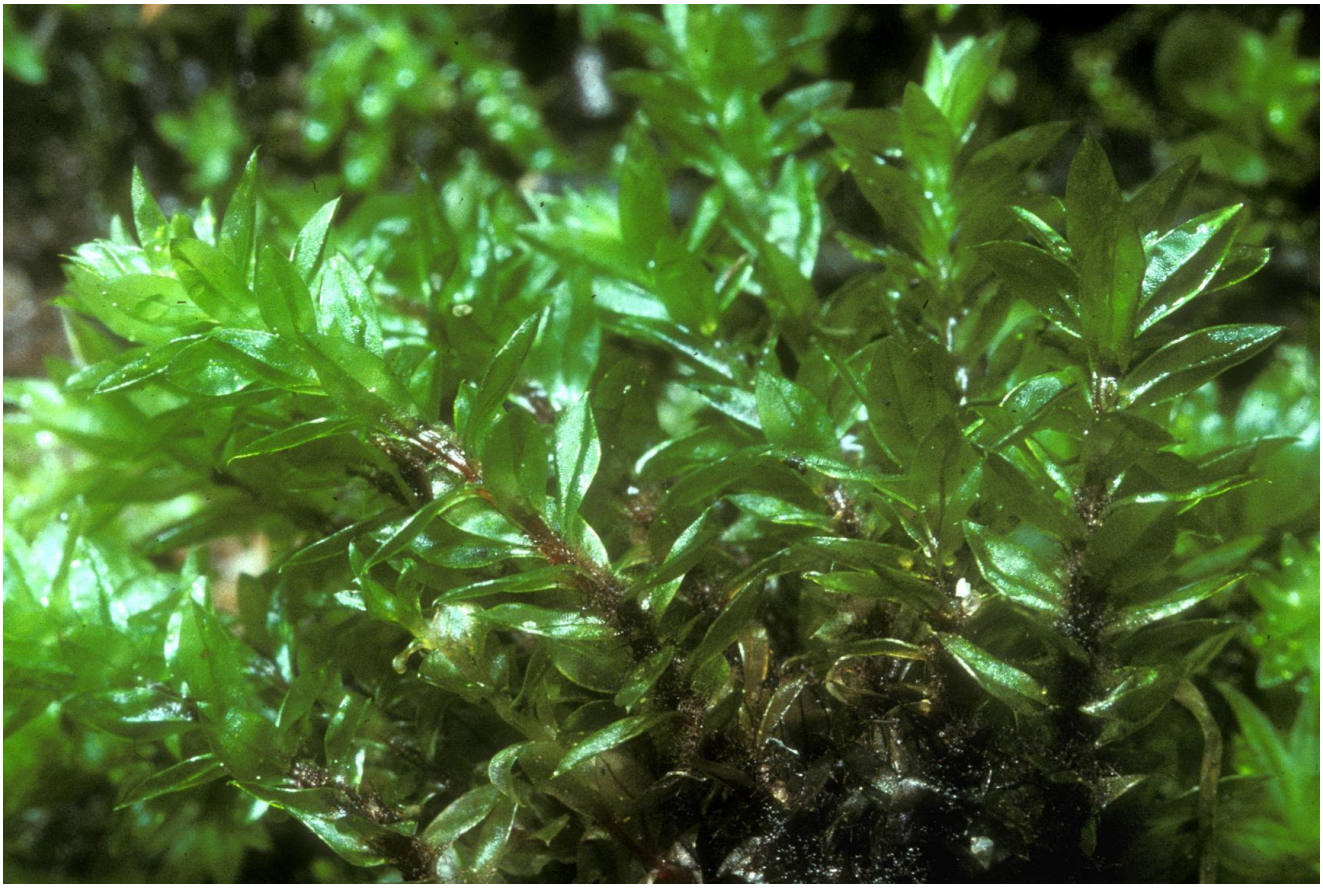

Figure 1. ***Bryum pseudotriquetrum*** gametophores, showing leaves, stems, and rhizoids. Photo by Janice Glime.

Growth

Bryophytes appear to be simple plants, but if one changes perspective, you might agree with Renzaglia *et al*. (2000) that these gametophytes "are the most elaborate of those produced by any land plant." In mosses, it is the apex of branches or stem tips that ultimately develop into reproductive organs. This contrasts with flowering plants that develop their gametophyte without archegonia and antheridia, reducing the male gametophyte to a pollen grain and the female gametophyte to a partitioned embryo sac within the female sporangium (sporophyte tissue).

In mosses and leafy liverworts, gametophore development can be considered a four-part process: stem growth, branch production, leaf development, and rhizoid formation (Figure 1). Since these four processes must compete for energy, it is expected that they are, at least in most cases, distinct events with different environmental stimuli or optima.

Stem Growth

Stem growth in plants occurs primarily as a result of cell elongation, which is sometimes accompanied by cell division (Bidwell 1979). Cell elongation occurs by a loosening of the side walls of the cell to allow expansion. Auxin helps to loosen the wall but exogenous calcium and ethylene inhibit loosening (Ray *et al*. 1983) (probably because Ca forms Ca pectate, which glues cell walls together). Loosening is followed by an uptake of water by the cell, which is an osmotic response to increase of Ca within the cell. The increased turgor then expands the cell. The turgor can be affected by mineral nutrients, photosynthesis, respiration, transpiration, ethylene, water availability, temperature, etc. If **any** of these factors becomes limiting, it can inhibit stem elongation.

When measuring growth, one consideration must be what to measure. When a layperson thinks of growth, it is usually equated with increase in height, but in biological

terms it can include branching and weight gain as well. Measuring extension in height gets complicated by the fact that if light intensity is insufficient, cells will extend with little or no weight gain, and often at a greater than normal rate – the **etiolation** effect (Figure 2). This is especially a problem in laboratory experiments where light intensity is usually considerably below that in nature, even compared to some forested settings. Plants, including bryophytes, become thin, weak, and lose their green color. In this case, false implications of growth occur. This can easily be seen when bryophytes are collected and kept in a sealed plastic bag. Sufficient moisture remains to permit cell extension, and within days (or even hours), one can see thin extensions of the stem with tiny, pale leaves.

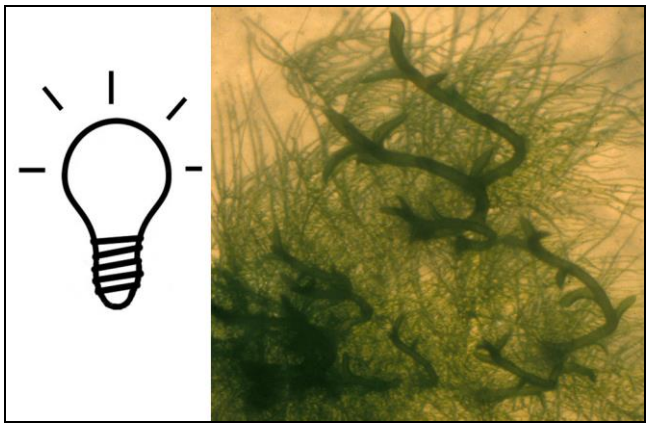

Figure 2. Culture of ***Funaria hygrometrica*** with Petri plate covered on top and the only light source from the side of the plate. Note the etiolated appearance of the shoots in this dim light compared to those in Figure 3. Photo by Janice Glime.

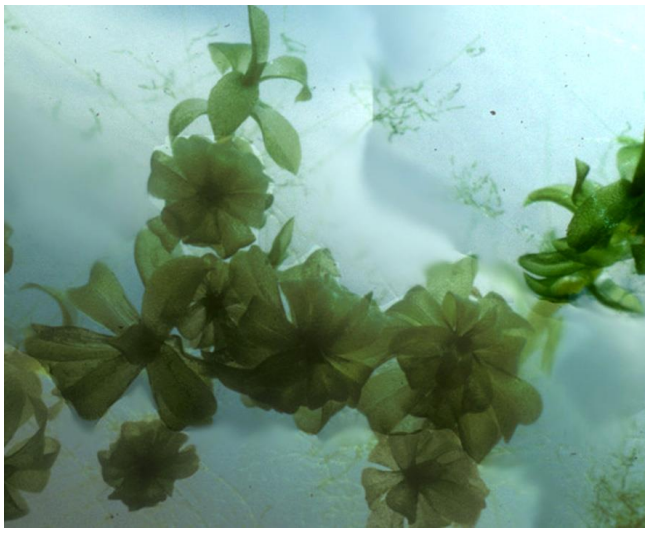

Figure 3. Culture of ***Funaria hygrometrica*** with light from above the plants. Photo by Janice Glime.

Therefore, especially in measuring laboratory growth, one needs to consider weight gain, either alone or in addition to height gain. Furthermore, if the species is pleurocarpous, in particular, and more than a few weeks elapse, length gain of branches and number of branches becomes important. This becomes a non-linear relationship as each branch then starts to grow at a rate similar to that of the main stem.

When growth is promoted, energy is diverted from other events. This diversion can manifest itself as a result of a change in environmental conditions. For example, when grown in red light, ***Ceratodon purpureus*** (Figure 4) exhibited only 20% branching with a weight gain of 16.8 mg per 50 individuals, but when the plants were grown under far-red illumination, there was 100% branching, but only 11.75 mg weight gain per 50 plants (Hoddinott & Bain 1979). This would appear to be counter-intuitive until one recognizes that while the branches were growing, the plants in far-red light were also producing setae, thus diverting energy for another process. Similarly, growth reduction (in length) occurs during archegonia production in ***Fontinalis dalecarlica*** (Figure 5) (Glime 1984). Energy is clearly needed for processes other than branch growth.

Figure 4. ***Ceratodon purpureus*** showing the paucity of branching. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

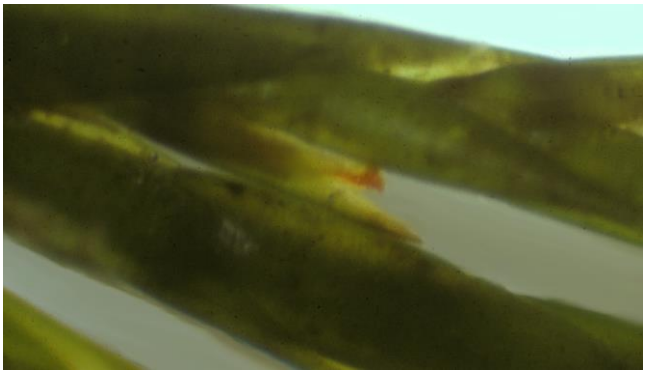

Figure 5. ***Fontinalis dalecarlica*** with archegonium, a phenomenon that coincides with a slowing of vegetative growth. Photo by Janice Glime.

Water

It is certainly nothing new to learn that water is necessary for development of the stem. However, the effect that water availability has on the stem diameter is less well known. In studying ***Sphagnum magellanicum*** (Figure 6) and ***S. papillosum*** (Figure 7), Li *et al*. (1992) found that stem diameter increased in stems with capitula that were farther from the water, and hence drier (Figure 8). This increase in stem diameter resulted from having a greater number of rows of the hyaline cells at the outer part of the stem (Figure 9). This increase in diameter appears to be a tradeoff because at the same time growth rate in stem length decreased.

Figure 6. ***Sphagnum magellanicum***, a species in which stem diameter increases with distance of capitulum from water surface. Photo by Michael Lüth, with permission.

Figure 7. ***Sphagnum papillosum***, a species in which stem diameter increases with distance of the capitulum from the water surface. Photo by David T. Holyoak, with permission.

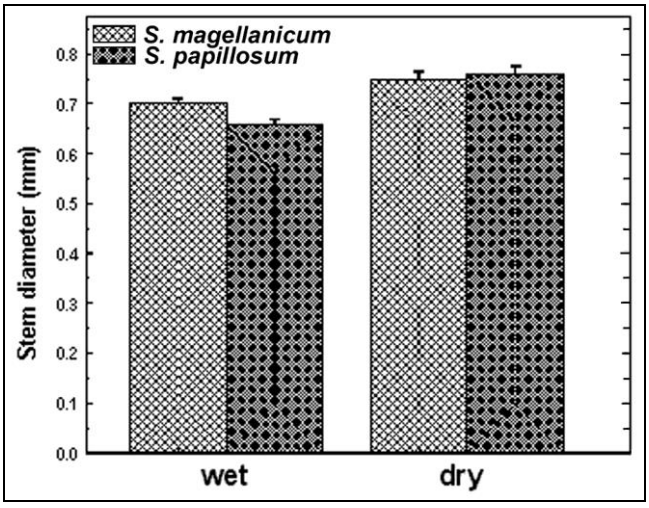

Figure 8. Effect of water level on stem diameter due to number of hyaline cell layers. **Wet** indicates stem tip starting at level 3 (7 cm) above the water; **dry** indicates stem tip starting at level 5 (15 cm) above the water. Based on Li *et al*. 1992.

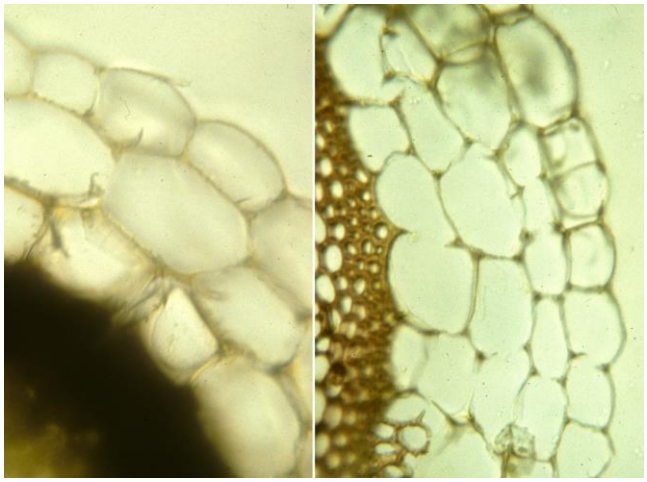

Figure 9. Effect of water level on stem width due to number of hyaline cell layers for ***Sphagnum magellanicum***. **Left:** Stem at level 3 above the water (wet), showing only three rows of hyaline cells. **Right:** Stem at level 5 above the water (dry), showing four rows of hyaline cells. Based on Li *et al*. 1992. Photos courtesy of Yenhung Li.

Light

Too high and too low **light intensity** can control bryophyte growth. At high light intensities, it can be inhibitory, destroying chlorophyll in unprotected leaves, but at suboptimal light intensities, it can cause etiolation, resulting in long, slender stems. For example, the aquatic moss ***Drepanocladus*** (Figure 10) has longer internodes in low light (Lodge 1959), making leaves appear to be sparse.

Figure 10. ***Drepanocladus longifolius***, a species with longer internodes in low light, hence in deep water. Photo by John Game, through Flickr Creative Commons.

Since mosses are shade adapted, optimal light intensity for many is likely to be rather low. ***Riccia frostii*** (Figure 11) females have optimal growth at 3500 lux in continuous light (Vashistha & Chopra 1989), whereas full sunlight is about 70,000 lux. Red light favors their growth (Dagar & Kumra 1988). For ***Marchantia palmata***, optimum intensity for vegetative growth is 4500 lux (Kumra & Chopra 1989), the same intensity needed for maximum number of gametophores in ***Microdus brasiliensis*** (Chopra & Mehta 1987). For ***Fontinalis duriaei*** (Figure 12) photosynthesis attenuated at 5400 lux (Glime & Acton 1979); field intensities where ***Fontinalis duriaei*** grew

ranged up to 6000 lux in spring when leaves were not out yet, diminishing to 4000 lux in summer and 500-1000 lux during much of winter (Glime 1987a).

Figure 11. ***Riccia frostii***, a species in which females have optimal growth in very low light (3500 lux). Photo by Rosemary Taylor, with permission.

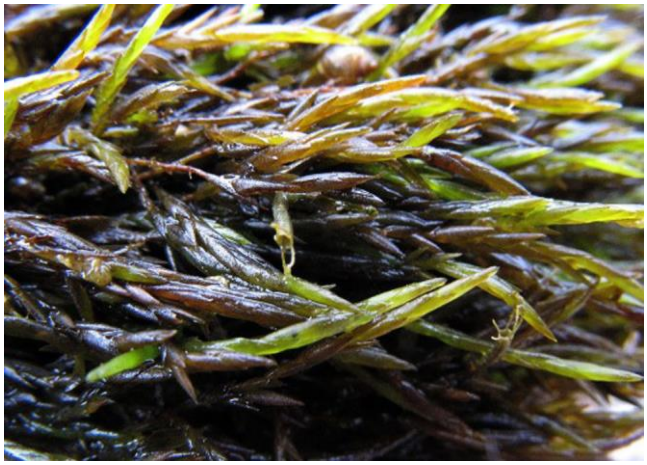

Figure 12. ***Fontinalis duriaei***, an aquatic species where photosynthesis attenuates at low light levels (5400 lux). Photo by Jan-Peter Frahm, with permission.

Stem height can be controlled by light, but some bryophytes respond to different wavelengths from those that affect tracheophytes. In some higher plant species, a five-minute exposure to far-red light at the end of an 8-hour day (with white light) is enough to cause a 400% increase in internode expansion (Morgan & Smith 1981). A flash of red light can stop growth. Stem elongation in etiolated plants can also be stopped by exposing the plant to red light, whereas far-red reverses this effect (Ray *et al*. 1983), suggesting that phytochrome is somehow involved. Incandescent bulbs also cause more stem elongation than fluorescent bulbs because of the higher far-red content of the former (Morgan & Smith 1981, p. 120). On the other hand, moss protonemata bend toward red light. And ***Ceratodon purpureus*** (Figure 4), ***Dicranum polysetum*** (Figure 13), ***Leptobryum pyriforme*** (Figure 14), and ***Polytrichum juniperinum*** (Figure 15) all grew significantly taller in red light than in far-red (Hoddinott & Bain 1979). That may be why these taxa all grow in relatively open areas where full sun is available at least part of the day, providing them with at least some red light.

Figure 13. ***Dicranum polysetum***, a moss that grows taller in red light than in far-red light. Photo by Michael Lüth, with permission.

A comparison of sun and shade forms of these moss species would be interesting. Should we expect moss taxa living under the forest canopy to be more sensitive to far-red light? Or are they necessarily adapted to growing poorly in far-red light in order to prevent growing too tall for their meager support system? Could it be that the chlorophyllous palisade layer of tracheophyte leaves necessitate the response to far-red light in the underlying spongy mesophyll (due to filtering out red light), whereas bryophytes have no such chlorophyllous layer to intervene in the light reaching their primary photosynthetic cells?

Figure 14. ***Leptobryum pyriforme***, a moss that grows taller in red light than in far-red light. Photo by Michael Lüth, with permission.

Branching seems to be under a different set of wave length controls from that of photosynthesis and growth, at least in some bryophytes. The thallose liverwort ***Riccia discolor*** has its maximum apical branching in blue light (Dagar *et al*. 1980). But this type of dichotomous branching is developmentally different from that of mosses and may not be physiologically comparable to the type of side branches produced by mosses.

Figure 15. ***Polytrichum juniperinum***, a moss that grows taller in red light than in far-red light. Photo by Janice Glime.

The chlorophyll *a*/*b* ratios of bryophytes are typical of shade-adapted species (Martin 1980). One must ask how the greater proportion of green light on the forest floor affects development and photosynthesis, and might such shade-adapted plants as most bryophytes be likewise adapted to the wavelengths of light that predominate in the forest. The work of Dagar and coworkers (1980, Dagar & Kumra 1988) on ***Riccia discolor*** may suggest an answer. They found that total chlorophyll content of ***Riccia discolor*** is highest in green light, again attesting to bryophytic adaptation to the low light of shade conditions. But in this species, green light retards growth (Dagar & Kumra 1988), and branches are favored by blue light over yellow or red (Dagar *et al*. 1980). Further discussion on effects of light is in the chapter on light.

Bierfreund *et al*. (2003) found that red light retarded growth of the protonemata in ***Physcomitrella patens*** (Figure 26). On the other hand the leafy gametophytes became elongated, but had shorter and narrower leaves. These effects were more pronounced in far red light.

Bryophytes seem to respond differently to the spectrum than do tracheophytes. Whereas tracheophytes grow best in far-red light, bryophytes seem to respond best to red light. Blue light can cause branching. They experience destruction of chlorophyll at high light intensities and etiolation at low light intensities. Light quality can change the morphology, with red and far red light causing stem elongation and leaf retardation.

Tropisms

It seems that most of the research on tropisms has been done on the protonema. **Phototropism** and **gravitropism** are most likely common for bryophyte stems, but aside from field observations, we know almost nothing about them in mature plants. However, it is clear that stems grow up and rhizoids grow down, just as do stems and roots of tracheophytes. One would expect tropisms in acrocarpous mosses, and surely something is causing their normal upright growth. Yet there seem to be a number of acrocarpous mosses that grow on vertical substrata and do not respond to gravity, and perhaps not to light. Genera such as ***Orthotrichum*** (Figure 16) typically grow outward from their tree trunk habitat and even the sporophyte seems oblivious to gravity. And at least some species of ***Pogonatum*** (Figure 17-Figure 18) and ***Oligotrichum*** (Figure 19) seem to lack a strong gravitropism or phototropism in their gametophytes when growing on a vertical substrate, whereas their sporophytes do bend upward. On the other hand, the stem of the pleurocarpous aquatic moss ***Fontinalis*** exhibits positive phototropism (bends toward light; Figure 20). A strong phototropism is seen for the acrocarpous ***Funaria hygrometrica*** in Figure 3.

Figure 16. ***Orthotrichum sordidum*** growing straight out from its vertical tree trunk substrate. Photo by Janice Glime.

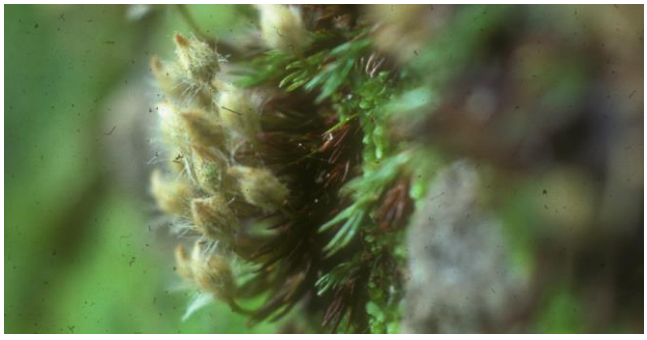

Figure 17. ***Pogonatum sphaerothecium*** showing upward curvature of setae, exhibiting tropisms, while the gametophyte lacks any upward direction. Photo by Janice Glime.

Figure 18. ***Pogonatum tortile*** exhibiting no tropism on stem or seta, but having one at or near seta-capsule junction. Photo by Janice Glime.

Figure 19. ***Oligotrichum hercynicum*** exhibiting a strong geotropism/phototropism in the sporophyte but lacking it in the gametophyte. Photo by Michael Lüth, with permission.

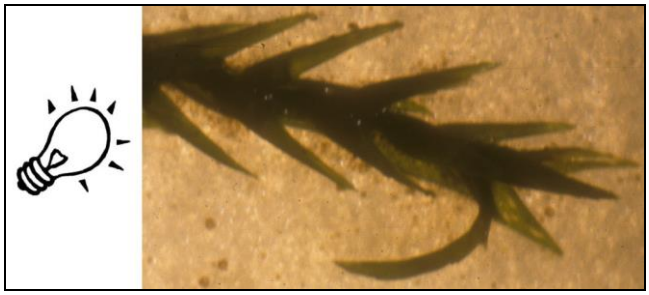

Figure 20. Positive phototropism exhibited by the tip of the moss ***Fontinalis squamosa***. Photo by Janice Glime.

Photoperiod

Not only do light intensity and quality affect bryophytes, but also light duration. Generally, long days result in longer stems along with increased elongation rates in higher plants, but too much light can inhibit elongation. In bryophytes, on the other hand, long days and elevated temperatures often induce dormancy, presumably acting as protection against desiccation during summer (Schwabe 1976). The response in higher plants suggests that increased day length allows more photosynthesis to occur, which in turn increases growth potential. Melstrom *et al*. (1974) suggest that in long days more auxin oxidase inhibitors are produced, allowing auxin levels to increase. Gibberellins also increase in long days. This combination allows growth to continue until hormone levels become too high or building materials are exhausted. Perhaps an inhibitory level may be reached more easily in bryophytes, resulting in earlier dormancy.

On the other hand, in two species of ***Sphagnum*** [***S. magellanicum*** (Figure 6) & ***S. papillosum*** (Figure 7)], there is a high correlation of growth with photoperiod greater than 10 hours; short days induce dormancy (Li & Glime 1991). This perhaps relates to the high light intensity to which these mosses are adapted, and to their higher temperature optimum of 30-35ºC for growth (Li & Glime 1990), compared to an optimum at 25ºC or less in most bryophytes.

But ***Sphagnum*** (Figure 6-Figure 7) is not alone in showing short-day dormancy, and control appears to be unrelated to temperature. In the liverwort ***Reboulia hemisphaerica*** (Figure 21), long days caused archegoniophore elongation at either 15ºC or 25ºC, whereas short days induced no response at any temperature (Koevenig 1973b). Even application of IAA, NAA, VA, and GA_3 could not break the effect of short days. This leaves us to wonder what ultimately controls the response, and is the controlling factor the same in all bryophytes?

Figure 21. Thallus and archegoniophores of ***Reboulia hemisphaerica***. Photo by Michael Lüth, with permission.

In liverworts, it is likely that **lunularic acid**, in response to **phytochrome** activity, plays a role in response to photoperiod (Schwabe 1990). Its ability to induce dormancy would permit it likewise to control growth. Does that mean that ABA controls growth and dormancy in mosses?

Most photoperiod responses in bryophytes have been related to dormancy. While it appears that most bryophytes benefit from cool temperatures of spring and autumn, and are dormant during long, hot days, some taxa such as ***Sphagnum*** are long-day plants and are dormant during short days. Photoperiod plays a role in gametogenesis, with some archegoniophores, like those of ***Reboulia hemisphaerica***, elongating only under long-day conditions.

Temperature

One would expect temperature to play a major role in development of bryophytes, as it does in early spring growth of other plants and a number of **poikilothermic** animals (those, like plants, with their temperatures controlled by the environment). In the aquatic moss ***Leptodictyum riparium*** (Figure 22), elongation increased with temperature until about 23ºC, after which growth declined again (Sanford 1979). This is consistent with the relatively low temperature optimum of most ***Fontinalis*** species, where sustained temperatures above 20ºC are detrimental to growth, and optimal long-term growth is at 10-15ºC (Glime 1987a, b). For the terrestrial ***Microdus brasiliensis***, the optimum is 18ºC (Chopra & Mehta 1987).

Figure 22. ***Leptodictyum riparium***, a species where growth increases with temperature up to about 23°C. Photo by Michael Lüth, with permission.

Schwabe (1976) found that long days and elevated temperatures often induce dormancy in liverworts, putting an end to spring growth. On the other hand, Stevenson *et al*. (1972) found a higher rate of cell division in the moss ***Atrichum undulatum*** (Figure 23) at higher temperatures.

Figure 23. ***Atrichum undulatum***, a moss that has a higher rate of cell division at higher temperatures. Photo by Brian Eversham, with permission.

Growth in ***Tetraphis pellucida*** (Figure 24) seems to be controlled by temperature rather than light (Forman 1964), but in the liverwort ***Reboulia hemisphaerica*** (Figure 25), temperature affected only elongation rate, not length or elongation of the archegoniophore, which was controlled by photoperiod regardless of temperature (Koevenig 1973b). Clearly the growth strategies differ among the bryophytes, but we have little phenological data to demonstrate the periods of growth for most species. We do know that in many spring plants, temperature and photoperiod work together to stimulate growth and elongation. Temperature effects will be discussed more thoroughly in the chapter on temperature.

Growth Regulators

Hormones in plants seem to defy definition (Christianson 1999). In plants, using the terminology of "growth regulators" permits us to define them as substances produced in one place in the organism that acts in small quantities to affect another part. But Christianson contends that this definition does not work well for the "untidy bundle of phenomena in plants." Rather, plant hormones can act locally or be transported and often have numerous roles, interact with other hormones, or are concentration dependent for their functions.

Figure 24. ***Tetraphis pellucida*** with gemmae, a moss in which growth is controlled by temperature rather than light. Photo by Michael Lüth, with permission.

Figure 25. ***Reboulia hemisphaerica*** with archegoniophores, a liverwort that elongates its thallus in response to temperature, but not its archegoniophore. Photo by Michael Lüth, with permission.

Growth and developmental processes are primarily controlled by hormones, particularly the auxin IAA (Sztein *et al*. 1999). In this regard, liverworts differ from mosses and tracheophytes in the way that they regulate their hormone concentrations and activities. Liverworts (and charophytes) regulate free IAA levels by a biosynthesis-degradation strategy, whereas mosses, hornworts, and tracheophytes use conjugation-hydrolysis (Sztein *et al.* 1995, 1999). These lead to differences in total amount of IAA metabolites, proportion of free and conjugated IAA, chemical nature of IAA conjugates, and rates of IAA conjugation. Sztein *et al*. (1999) consider this difference in control mechanisms to have "profound implications for macroevolutionary processes in these plant groups."

Bryophyte hormones operate very much as they do in tracheophytes (Maravolo 1980). In bryophytes, **auxins** are transported directionally, permitting **apical dominance** to occur, and their activity is concentration dependent. The

highest concentrations of auxin occur at the tip and base of the upright gametophore, with distribution throughout the stem, as demonstrated in ***Physcomitrella patens*** (Figure 26) (Bierfreund *et al*. 2003). This species also requires profilin for tip growth (Vidali *et al*. 2007). Profilin is an actin-binding protein and has important regulatory functions, particularly related to the actin cytoskeleton (Wikipedia 2012). Thus it is important in development of organs, wound healing, and identification of "infectious intruders" by the immune system.

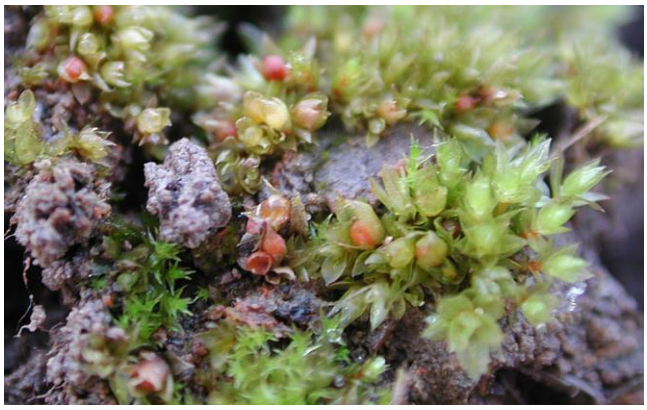

Figure 26. ***Physcomitrella patens*** with capsules, a moss that has demonstrated the concentration of auxin at the tip and base of the upright gametophore, with distribution throughout the stem. Photo by Michael Lüth, with permission.

Chopra and Vashistha (1990) examined the effects of auxins during various stages of the life cycle of ***Bryum atrovirens*** (Figure 27). They found that at lower concentrations of IAA and other auxins the leafy plants developed normally, but at higher levels their forms were not normal.

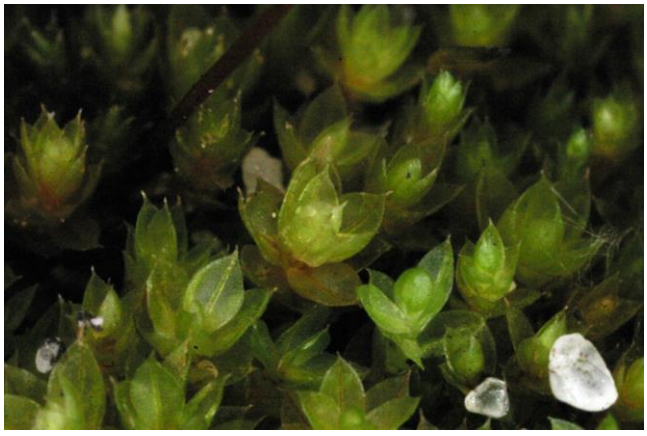

Figure 27. ***Bryum atrovirens***, a species that exhibits abnormal development at higher concentrations of auxins. Photo by Jan-Peter Frahm, with permission.

Gibberellic acid promotes cell enlargement, development of chloroplasts, and degradation of starch, and causes ultrastructural changes in starch granules and **thylakoids** (flattened, membranous vesicle containing chlorophyll; location of photosynthesis), just as in tracheophytes. It influences gravitropic curvature, depending on photoperiod.

While working with *Avena* (wheat) and two liverworts, Kaufman *et al*. (1982) found several basic generalities in hormone-induced cell elongation of plants. During phase one, in which the cellulose fiber matrix of the cell is stretched, rapid growth is due to hormone-induced secretion of H^+, which aids in loosening the cell wall for growth. They discovered that stimulated plants acidified their immediate environment. This rapid response suggests the involvement of H^+ transport (**proton pump**), much like the closing of the Venus flytrap leaf. Ellis and Thomas (1985) demonstrated the same sort of **auxin-stimulated acid efflux** in ***Pellia*** (Figure 28) to create a pH of 4.8 in the medium, in this case as a result of stimulation by light on one side of the seta.

Phase two consists of long-term growth that occurs as new proteins are synthesized. This response occurs much later than phase one, which is basically instantaneous. Hormones and other plant growth regulators can affect both of these steps in a variety of ways.

Bryophytes seem to respond to different concentrations and respond at different rates from those exhibited by tracheophytes. While working with *Avena* (wheat), Kaufman and coworkers (1982) discovered that a tenfold increase in the growth rate of *Avena* internodes appeared about three hours after application of 10^{-5} M **GA_3**, but that 10^{-5} M **IAA** had no effect. On the other hand, when working with the liverworts ***Pellia epiphylla*** (Figure 28) and ***Conocephalum conicum*** (Figure 29), they found that the setae and archegoniophore stalks responded to 10^{-5} M IAA with a two-fold increase in growth rate within 10-15 minutes. Many higher plants also show this rapid response to IAA, but this depends again on the concentration (Osborne 1974; Muir 1974). The rapid response in the liverworts suggested to Kaufman and coworkers (1982) that IAA had a direct effect on the cell membrane, allowing expansion by drawing water into the cell, since growth of the cytoplasm would require slow protein synthesis. We now know that IAA probably works on the cell wall (Goodwin & Mercer 1983), most likely by facilitating the breakdown of **calcium pectate** so the fibers can slide and expand, and this most likely involves an acid efflux via the proton pump from the cells, hence the H^+ observed by Kaufman *et al*. (1982). The freed **Ca^{++}** is then available to enter the cell, most likely accounting for the observed increase in Ca^{++} there.

Figure 28. ***Pellia epiphylla***, a species that responds within 10-15 minutes of an application of 10^{-5} M IAA by rapidly increasing archegoniophore growth. Photo by David Holyoak, with permission.

Figure 29. ***Conocephalum conicum***, a species that responds within 10-15 minutes of an application of 10^{-5} M IAA by rapidly increasing archegoniophore growth. Photo by Jan-Peter Frahm, with permission.

Movement of auxin within the plant is directed and may follow the vascular tissue. In ***Marchantia polymorpha*** (Figure 30), it is transported in the midrib (Maravolo 1976) and movement occurs in both directions at equal velocity. However, the basipetal (away from apical bud) transport is much greater in intensity. Transport can be inhibited by cinnamic acid and ethylene.

Figure 30. ***Marchantia polymorpha*** males with gemmae cups, demonstrating the midribs. Note the notches at the end of each and the dominance of one of them. Photo by Nancy Leonard, with permission.

As is typical with hormone responses, not all bryophytes respond the same way. ***Marchantia palmata*** growth was inhibited by most levels and kinds of auxins (Kumra & Chopra 1989). Furthermore, many chemicals can stop action of IAA (Muir 1974), including other growth hormones. These may actively compete for a binding site on the wall or plasma membrane. Could other plants outcompete bryophytes with a hormonal chemical warfare?

Ethylene is likely to have an early role in gametophore development. We know that seedlings produce ethylene in response to physical contact (Abeles 1973). Thus, if an emerging seedling encounters dense soil or rock, ethylene production inhibits mitosis, thus halting meristematic activity, and the cells respond by less elongation and by growing wider and thicker, giving the stem greater strength. This greater strength, coupled with continuing but reduced cell elongation, can dislodge small obstructions or push through dense soil. If the obstruction is a rock, ethylene production on the side of contact slows elongation on that side, resulting in plant curvature around the rock.

If we apply this principle to a developing or buried moss gametophore, ethylene could respond to particles of dirt and redirect gametophore growth. We have no studies on this aspect of ethylene in mosses, but I have grown ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54) cultures where spores were germinated **under** the cellophane sheet on top of agar. An accumulation of ethylene is to be expected in this confined space. Here the normal vertical growth of the moss was prevented and a very etiolated-looking horizontal growth occurred. The leaves were short and the stem was long.

In ***Fontinalis squamosa*** (Figure 31), ethylene causes crumpled branches and stem tips (Figure 32; Glime & Rohwer 1983). G. Mogensen (pers. comm.) has seen similar crumpled branches as a common phenomenon in the Arctic. The crumpling follows a period of late spring or early autumn snow that results in an ice layer on the moss. Because the ice is thin, light is still available, but growth is obstructed. As the moss pushes against the ice, ethylene might be produced as a stress response. If ice surrounds the plant, only a slight space exists between the moss and the ice, permitting an ethylene build up.

Figure 31. ***Fontinalis squamosa*** in alpine water. Photo from <www.aphotofauna.com>, with permission.

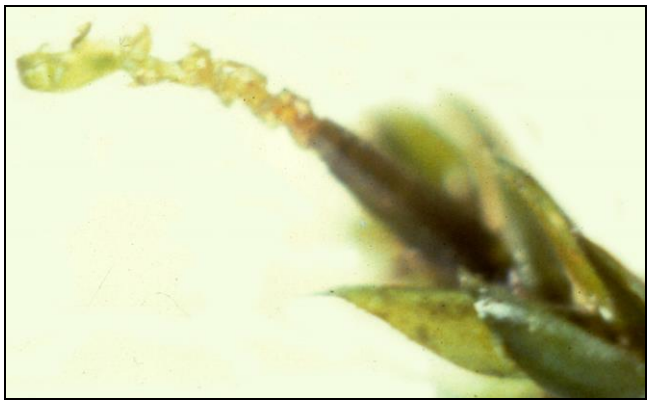

Figure 32. Effects of ACC (and presumably ethylene) on apical leaves of ***Fontinalis squamosa***. Photo by Janice Glime.

Submersed mosses [***Fontinalis*** (Figure 31), ***Drepanocladus*** (Figure 33-Figure 34)] often possess widely spaced leaves and thin stems, whereas the same species in shallow water will have thick stems and overlapping leaves. Fuchsig (1926) observed that this gives the shallow water individuals a greater resistance to desiccation with weight loss during desiccation being greatest in the deep water form. Two factors would implicate ethylene and IAA as the controlling factors here. In deep water, light is dim and no light inhibition of IAA should occur since UV light in particular is filtered out. Therefore an etiolation response is expected. At the surface, two factors known to enhance ethylene production occur: (1) stress due to wave action and alternate wetting and drying; (2) a high ratio of O_2:CO_2 relative to deep water. Endogenous ethylene could easily account for thicker cells and greater stem strength at the water surface.

Bryophytes seem to respond to many of the same hormones as do tracheophytes, but generally they respond at lower concentrations and may be inhibited at the concentrations that are effective for tracheophytes. Little is known of **ethylene** effects, but it may account for the contorted growth of bryophytes that have been encased in ice. **GA** is important in cell elongation and **IAA** is important in growth, most likely being the initiator of the rapid acid growth phase. It appears that IAA may provide the signal that initiates the **proton pump**. The **H^+ flux** into the cell wall spaces causes the **calcium pectate** bonds to break, freeing **Ca^{++}** that then enters the cell, replacing the positive H^+ ions that were just lost. **Anions** that come with the Ca^{++} create a salt within the cell, causing an osmotic gradient. Water follows by **osmosis**.

Figure 33. ***Drepanocladus aduncus*** in an emergent population with leaves close together. Photo by Michael Lüth, with permission.

Figure 34. ***Drepanocladus aduncus*** branch showing leaves close together. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

As with other processes in plants, the production of ethylene requires energy, as demonstrated by De Greef and coworkers (1979) in the thallose liverwort ***Marchantia polymorpha*** (Figure 30). We can therefore assume that when it enters into the development process there will be a tradeoff of energy that might otherwise be used elsewhere in the plant.

As already noted, the thallose liverwort ***Marchantia polymorpha*** (Figure 30) exhibits apical dominance. The thallus produces its own auxin, creating a **basipetal** (toward the base) gradient (Binns & Maravolo 1972). The auxin accumulates in the midribs and the **acropetal** (outward toward shoot apices) regions of excised thallus discs. Binns and Maravolo concluded that maintaining this gradient is essential for normal growth and regeneration. High concentrations of cytokinin in the tissues destroy the polarity by causing an increase in the auxin-synthesizing capacity of the affected tissues.

External application of auxins had no influence on the growth of the thallus, with no growth acceleration or inhibition of regeneration of the thallus (Binns & Maravolo 1972). Transcinnamic acid and dinitrophenol inhibited regeneration, but auxin reversed the inhibition.

Branches and Apical Dominance

Like tracheophytes, bryophytes exhibit a variety of branching types, ranging from total lack of appearance of apical dominance to strong apical dominance (Figure 35). A spruce tree with its strong central trunk and its secondary side branches is the epitome of apical dominance in tracheophytes. Yet, if the tip is broken, one of the side branches becomes a new leader, taking over the dominance that retards development of other secondary branches. In bryophytes, the acrocarpous mosses realize this type of apical dominance. In some cases, the dominance persists even if the tip is lost and the ability for branches to overtake the damaged central stem seems to be absent. But in others, such severance of the controlling tip results in increased growth of side branches, as in ***Fontinalis*** (Figure 36). Nevertheless, the ability of a single side branch to dominate the others after such a decapitation of the apex seems to be absent in the bryophytes. Rather, multiple side branches develop as **innovations**. This is not unlike the response of many herbaceous taxa of tracheophytes. For example, in snapdragons (*Antirrhinum*) the loss of the apex results in the development of a more bushy plant, and for any number of herbaceous garden flowers, pinching off the apex is a common technique for developing a more robust plant with multiple flowering apices.

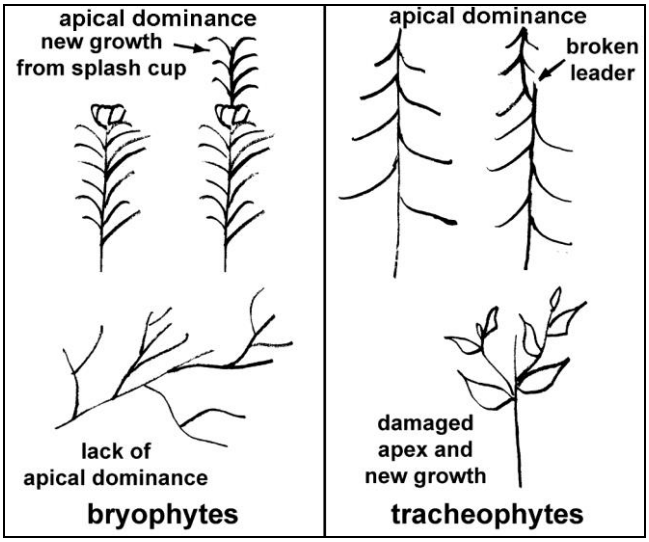

Figure 35. Effects of apical dominance on growth forms of bryophytes and tracheophytes. Drawings by Janice Glime.

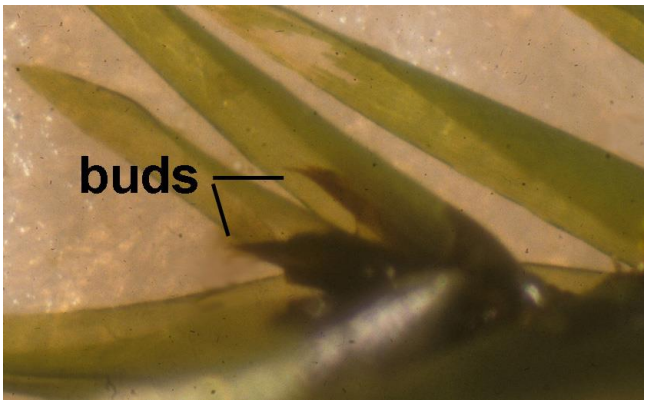

Figure 36. Branch buds developing near the broken tip of ***Fontinalis squamosa***. Photo by Janice Glime.

In acrocarpous mosses, the production of sexual structures terminates the apical growth, particularly the production of antheridial splash cups or capsules. But in some taxa, such as many **Polytrichaceae** (Figure 37), once the splash cup ceases to function in production of sperm, a new stem growth may develop, rendering a series of markers on the stem where remnants of the old splash cups remain (Figure 37). Certainly no flower accomplishes such a strange phenomenon, but cones of the European larch can develop new branches from the ends of the female cones!

Bryophyte branching differs from that of typical tracheophytes in other ways as well. Bryophytes branch below the leaf insertion, whereas tracheophytes produce branch buds in the leaf axil (Figure 38; Schofield 1985). For the tracheophytes, this altered arrangement could provide protection of the developing bud cradled in the leaf base. Furthermore, in tracheophytes, the buds have a meristematic region of dividing cells, whereas in the bryophytes, it is an outer cell of the stem that becomes specialized to form a branch, subsequently forming the apical cell of this branch (Figure 39-Figure 40).

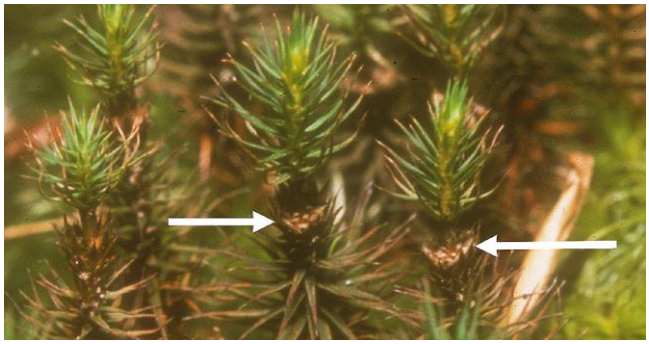

Figure 37. New growth from a senescent antheridial splash cup of ***Polytrichum ohioense***. Photo by Janice Glime.

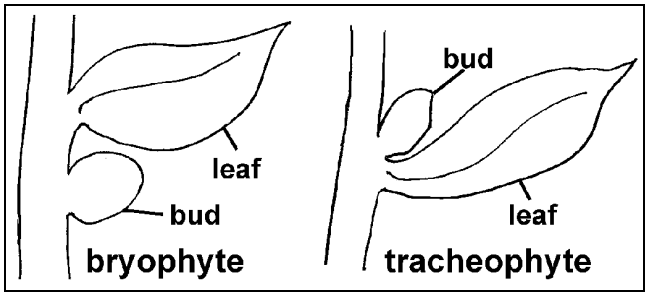

Figure 38. Position of branch buds in bryophytes vs. tracheophytes. Drawing by Janice Glime.

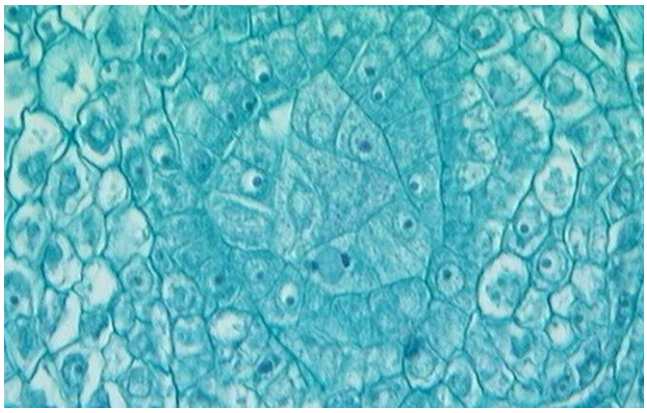

Figure 39. ***Polytrichum*** stem apex cross section showing three cutting faces. Photo by Magda Turzańska, with permission.

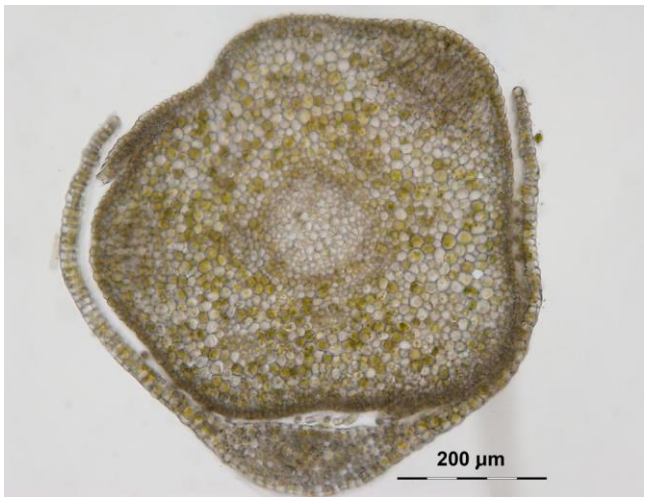

Figure 40. Mature ***Polytrichum*** stem cross section. Photo by Magda Turzańska, with permission.

Despite the differences in their apical development that uses apical cell cutting faces instead of a meristematic region, many bryophytes have apical dominance. In these taxa, removal of the apex promotes the development of branch buds, with those nearest the cut apex developing the most, as one sees in tracheophytes. Once these buds begin development, they re-establish the inhibition of the lateral buds beneath them.

We have already discussed the energy tradeoffs inherent in growth. One thing that is common among the species of mosses studied is the growth of either the main stem or the lateral branches to the exclusion of the other. ***Racomitrium lanuginosum*** (Figure 68) has two periods of main stem growth, one in spring and the other in autumn, whereas the lateral branches are initiated and elongate in the first part of summer (Tallis 1959). ***Hylocomium splendens*** (Figure 41) appears to have one period of elongation during which the bud for the next year of growth is initiated. This bud will not develop further until the present stem section has completed its growth (Busby *et al*. 1978). Sanford (1979), in his studies with the aquatic moss ***Leptodictyum riparium*** (Figure 22), also found that increased branch growth was correlated with decreased main axis growth. With this kind of tradeoff, we should expect an environmental role in determining when the plant elongates shoots and when it elongates branches.

Figure 41. ***Hylocomium splendens*** showing buds for next years growth. Photo from website of the Botany Department, University of British Columbia, Canada, with permission.

Environmental Factors

In his work with ***Racomitrium lanuginosum*** (Figure 68), Tallis (1959) observed that low main stem growth and favorable growth conditions such as temperatures between 12 and 15°C best favored shoot growth. Furthermore, in a cold, humid environment, his plants had few branches and these were small, but in a warm, moist environment, his plants had several long lateral branches. He also found that high humidity and shading may inhibit branching for up to a full year. He suggests that lateral branching might be induced by high light in combination with alternate wetting and drying at a mean temperature that is above the minimum threshold.

Chopra and Rashid (1969) likewise found that increased light intensity promoted lateral bud formation in mosses. This apparent action by light intensity is supported by the fact that in many plant species, bud expansion is initiated in the spring when light intensity increases and tree canopy closure is incomplete. Low light and low temperatures also delay budding in mosses (Bopp 1968).

But when light intensity increases in the spring, the temperature also increases. However, Pitkin (1975) states that the direct effect of temperature on bryophyte growth is small, except at low temperatures, but that temperature has a strong indirect effect through its effect on humidity and **evapotranspiration** (loss of water through evaporation from among plants and from plants themselves). However, temperature may be more direct through control by growth regulators.

Alghamdi (2003) found that the type of available N can greatly influence the production of branches. In solutions containing only amino acids as the N source, the Java moss (***Taxiphyllum barbieri***; Figure 42), an aquatic moss, produced more branches as concentrations increased with four different amino acid sources (but not **methionine** – amino acid that is relatively insoluble in water), while producing many fewer branches in ammonium or nitrate at the same concentrations of N (Figure 43). Could seasonal pulses of leaf litter decomposition, providing pulses of amino acids, play a role in the seasonal timing of branching vs stem elongation for forest bryophytes? What else can play a role?

Figure 42. ***Taxiphyllum barbieri***, an aquatic moss that produces more branches when supplemented with some amino acids than when supplemented with ammonium or nitrate. Photo by Buchling, through Creative Commons.

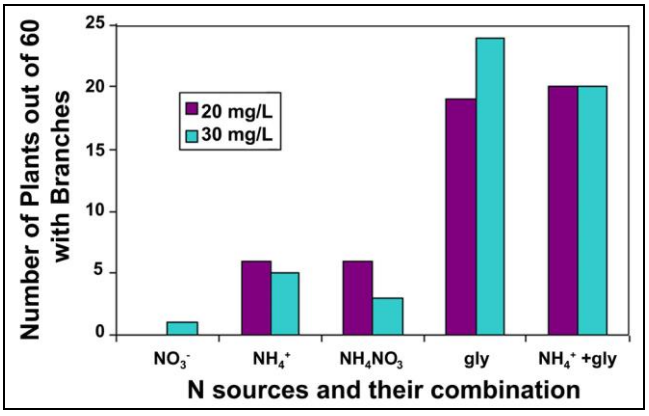

Figure 43. Effects of different types of nitrogen source on branch production in the Java moss, ***Taxiphyllum barbieri***. **gly** = glycine. Graph from Alghamdi 2003.

As discussed in the chapter on Nutrients, deficiencies can alter morphology and color of the bryophytes. Shaw (1991) suggested that for ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54) growing on a mine site, differences in morphology might have been caused by heavy metal toxicity. But coupled with these metal-caused malformations, he suggested that **somatic** (cellular level) mutations could also contribute to the extensive **phenotypic** (form) variability.

Growth Regulators

Apical dominance is indicative of hormone actions. In tracheophytes, IAA produced in the tip of the plant and interacting with cytokinins inhibits the development of branches below the tip, permitting the main stem to be the leader. In bryophytes, we have indications that the same sort of action is present.

Bryophyte apical dominance appears to work the same way as in the meristematic tracheophytes. MacQuarrie and von Maltzahn (1959) linked apical dominance with IAA in the acrocarpous moss ***Splachnum ampullaceum*** (Figure 44). Stange (1964) demonstrated apical dominance in another acrocarpous moss, ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54).

Figure 44. ***Splachnum ampullaceum***, a moss with known apical dominance due to IAA distribution. Photo by Michael Lüth, with permission.

Many acrocarpous mosses lose apical dominance when sporophytes are produced, resulting in innovations such as those in ***Bryum*** (Figure 45) or when antheridia develop as in ***Philonotis*** (Figure 46). This suggests that the sporophyte or archegonium causes the stem apex to cease producing IAA. We have already seen that in ***Polytrichum***, male plants (Figure 37) retain their apical dominance and resume growth from the center of the male splash cup when the succeeding year's growth begins.

Figure 45. Innovation (**arrow**) in ***Bryum versicolor***. Photo by Michael Lüth, with permission.

Figure 46. ***Philonotis fontana*** showing multiple branches just below the antheridial head. Photo by Janice Glime.

The role of apically supplied IAA is indicated in experiments where the gametophore is decapitated and an agar block containing 1mg/ml IAA is placed on the cut tip (Knoop 1984). In this case, stems without the agar block develop buds and branches, but in those with the agar block, the IAA inhibits lateral development in the same manner as an intact apex. Application of kinetin (a cytokinin) induces bud formation in those stems with an apical IAA source. A theoretical relationship to bud development is shown in Figure 47.

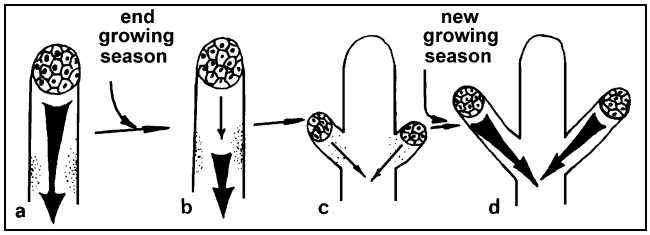

Figure 47. Theoretical relationship of auxin (IAA) and cytokinin in controlling branch production. **a**) Apical region during active growing season shows large production of IAA (**arrow**), inhibiting localized concentrations of cytokinin. **b**) End of growing season slows apical activity and production of IAA. **c**) Increased cytokinin:IAA ratio stimulates bud initiation. **d**) New apices become dominant and begin IAA production with new growing season.

The genus ***Plagiomnium*** exhibits a mix of upright growth that ultimately terminates in gametangia and

horizontal growth (**plagiotropic**). The moss ***Plagiomnium cuspidatum*** (Figure 48) responds to addition of IAA on a decapitated stem by exhibiting varying degrees of lateral bud suppression (Nyman & Cutter 1981). However, for the behavior to mimic that of controls with no decapitation, cytokinin must also be present.

Figure 48. Upright and plagiotropic growth forms of the moss ***Plagiomnium cuspidatum***. Photo by Michael Lüth, with permission.

This relationship of buds with cytokinin does not seem to apply to all mosses. In the moss ***Plagiomnium cuspidatum*** (Figure 48), the cytokinin is synergistic with IAA in inhibiting bud development; IAA alone is unable to inhibit branch buds (Knoop 1984). Because bryophytes have very low concentrations of IAA, they are probably extraordinarily sensitive to it. Thus budding might be inhibited at quite low levels. The apparent synergism may be based on a concentration problem. Furthermore, both cytokinin and IAA can induce production of ethylene, and this could explain the apparent synergism between IAA and cytokinin in ***Plagiomnium***.

Ethylene is known to inhibit development under some circumstances in plants. If ethylene is in fact the effector in branch inhibition, one might look for differences in ethylene production between acrocarpous and pleurocarpous mosses. Inhibition of branches by ethylene suggests that pleurocarpous mosses, or highly branched mosses, must have low endogenous ethylene relative to acrocarpous or unbranched mosses. If this is true, we should expect pleurocarpous mosses to be more sensitive to exogenous ethylene than acrocarpous mosses and that they might be less likely to produce ethylene in response to environmental stimuli; alternatively, they may be highly branched because they are not responsive to it. Whatever the mechanism, we should expect mosses lacking apical dominance to respond differently.

Cytokinins have been shown to enhance IAA-induced ethylene formation (Goodwin & Mercer 1983), which is likely to cause senescence. But in the acrocarpous moss ***Anoectangium thomsonii***, Chopra and Rashid (1969) observed that, at any concentration of added kinetin, there was an increase in the number of buds and the rate of bud initiation. However, further shoot development was inhibited.

We need to further examine the case of ***Plagiomnium cuspidatum*** (Figure 48). Although this moss is acrocarpous, it has lateral (**plagiotropic**) branches in addition to its upright stem (Figure 48). These branches may behave more like branches of pleurocarpous mosses in their response to ethylene, IAA, and cytokinins. Because ethylene is a gas, it is more difficult to work with and quantify.

Pleurocarpous Mosses

Studies on the effects of growth substances on pleurocarpous mosses appear to be rare, probably due to the greater convenience in growing small acrocarpous mosses on agar [*e.g.* ***Physcomitrium*** (Figure 49), ***Funaria*** (Figure 2-Figure 3)]. However, our own studies on ***Fontinalis*** (Figure 50-Figure 51) may offer some insight.

Figure 49. ***Physcomitrium pyriforme*** with capsules, showing its small size. Photo by Jan-Peter Frahm, with permission.

Tremaine and Glime (unpub.) grew ***Fontinalis duriaei*** (Figure 12) in liquid culture with 10^{-6} and 10^{-8} M IAA and found that after two weeks there was significantly more growth at 10^{-8} M than at 10^{-6} M or controls (no IAA), with intermediate growth in the controls (Duncan's New Multiple Range test, $p < 0.05$). This contrasts sharply with the optimum of 10^{-5} M for higher plants (Haney 1978). But effects on branching and apical dominance were inconclusive even after 8 weeks.

In a separate study, Hover and Glime (1983, unpubl) grew ***Fontinalis duriaei*** (Figure 12) with kinetin additions and got rather confusing results. At 0.001 and 0.01 mg L^{-1} added kinetin, the mosses produced fewer branches per stem than did the controls with no kinetin addition, but at 1.0 mg L^{-1} they produced significantly more branches than did controls. They speculated that this may have been due to a competitive action between the exogenous kinetin and the plant's own cytokinin that could have resulted in suppressing production of the natural cytokinin.

Berthier (1966) found that maximum apical dominance in ***Fontinalis*** (Figure 50) occurred at 5% sunlight and that full sunlight caused maximum inhibition of axis growth. Shade inhibited branching. This and the studies mentioned above suggest that shade increases IAA and sun reduces the IAA:cytokinin ratio. This is consistent with events leading

to an etiolation response and the known destruction of IAA by high light intensity, especially UV, in tracheophytes.

Figure 50. ***Fontinalis antipyretica*** with wounded tip that now has grown rhizoids and a new branch. Photo by Janice Glime.

Figure 51. ***Fontinalis antipyretica*** var. ***gigantea***, showing broken branch tip (center) with single new branch that has presumably resulted from loss of apical dominance. Photo by Malcolm Storey, through Creative Commons.

We know that high concentrations of ACC, an ethylene precursor and presumably resulting in ethylene production, inhibit branch development and bud production in ***Fontinalis squamosa*** (Figure 31) and ***F. antipyretica*** (Figure 50) (Glime & Rohwer 1983). Inhibitory effects of high IAA concentrations seem to be due to its effects in increasing ethylene production (Goodwin & Mercer 1983). This relationship implies that it could actually be ethylene that inhibits branch formation. Valadon and Mummery (1971) have shown that abscisic acid (ABA) also has a linear relation to bud reduction in ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54). But abscisic acid is also known to promote ethylene production in some tissues (Craker & Abeles 1969), so it is possible that again ethylene was the actual inhibitor.

Although ***Fontinalis*** (Figure 50) does not appear to have a strong apical dominance, Berthier (1966) demonstrated that removal of its apex resulted in branches on each side of the apex. I (Glime) have observed similar phenomena in explants of ***Fontinalis antipyretica* var. *antipyretica*** (Figure 50, see also Figure 36), but when my student and I removed the apices from ***F. antipyretica* var. *gigantea*** (Figure 51), the removal had no observable effect on branching. Since this variety does little branching normally, it may have been an inappropriate taxon to test.

But why does it appear that ***Fontinalis*** can't grow branches and stems simultaneously? Since both produce leaves that are photosynthetic, where is the tradeoff? Perhaps the experiments of Tremaine and Glime (unpub.) on ***Fontinalis duriaei*** (Figure 12) provide some insight into the relationship. They found the mosses in 10^{-6} M IAA to look healthiest (bright green) at the end of the experiment compared to the controls or those at 10^{-8}M, both of which grew more than those at 10^{-6}M. It appears that the tradeoff may be that the energy used for growth reduces the concentration of chlorophyll in the leaves as it distributes its building materials to new cells and tissues. This will reduce the leaf weight and the magnitude of photosynthesis per leaf area. Hence, it is most likely beneficial to hold one growth type constant while the other expands.

Spiess *et al*. (1972), working with the pleurocarpous ***Pylaisiella selwynii*** (Figure 52), also found that cytokinins increased bud formation but not further development, and thus concluded that the auxin:cytokinin ratio was important. They observed also that the number and morphology of the buds were both concentration dependent.

Figure 52. ***Pylaisiella selwynii*** on bark, where bud formation depends on cytokinin, but not further development. Photo by Janice Glime.

Thallose Liverworts

Even thallose liverworts exhibit apical dominance. In ***Marchantia polymorpha*** (Figure 30), hormones may control the fan shape of the thalli. The apical dominance of these plants is expressed as greater growth of one lobe compared to the other one. When the thallus develops, two apical notches are present. The larger lobe that develops is the one nearest to the midrib. If the two notches are cut at an early growth stage, inhibition of the smaller lob ceases and it grows to equal the size of the dominant lobe. But it is not IAA that causes the new growth, but rather IAA inhibits the growth of the smaller lobe. The larger lobe, on the other hand, is not affected by IAA. This suggests that once a branch of the thallus becomes dominant the two lobes have different sensitivity to IAA as an inhibitor.

Branch buds of bryophytes are known to be sensitive to both cytokinin and auxin concentration. Three cytokinins tested stimulated vegetative growth, as well as archegonial production, in ***Riccia frostii*** (Figure 11),

whereas the auxin NAA only enhanced archegonial induction (Vashistha 1987). In studies on mosses, Chopra and Rashid (1969) found that low concentrations of exogenously applied IAA somewhat increases bud formation. At higher concentrations, IAA is inhibitory (Spiess *et al*. 1973).

Both cytokinins (Chopra & Gupta 1992) and IAA (Tremaine & Glime unpub.) appear to be important in controlling bryophyte growth. Chopra and Gupta (1992) found that of the three cytokinins they tested, 10^{-4}M was optimal for vegetative growth in ***Riccia discolor***.

Nutrients

Koevenig (1973a) suggests that the growth hormones IAA, NAA, **BA** (6-benzyladenine, a cytokinin), and GA_3 may only aid in elongation but not actually induce it, implying that other substances are needed, such as the metals. Many compounds influence plant growth. Sharma *et al*. (1960) reported that ***Haplomitrium*** (Figure 53) gametophytes grew better on media containing various amino acids, indicating that organic material must be present in the substrate. Copper can stimulate growth of some bryophytes at elevated concentrations (0.01 ppm), presumably through greater photosynthesis (Sommer 1931; Glime & Keen 1984), wherein it is needed in plastocyanin, a chloroplast protein. Nevertheless, it soon becomes inhibitory at higher concentrations.

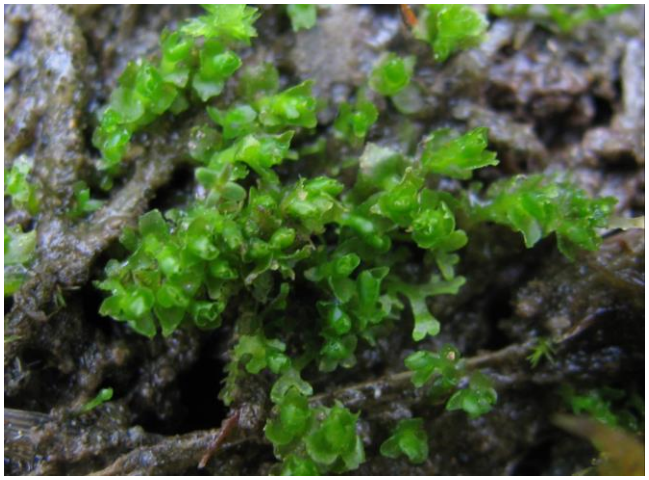

Figure 53. ***Haplomitrium hookeri***, a leafy liverwort that grows best on a medium with amino acids as its nitrogen source. Photo by Janice Glime.

Laboratory cultures are usually much richer in nutrients than are the places where bryophytes normally grow. For example, in ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54), field stem length never reaches that observed in the laboratory. One reason for this might be a deficiency of magnesium in its habitat and ample quantity in the culture medium. Hoffman (1966) found that ***Funaria*** remained small but healthy in a magnesium-deficient medium. Tamm (1953) found that rainwater, the major source of nutrients for ectohydric mosses, contained no magnesium in the open, although it did under spruce trees. Since ***Funaria*** does not grow in the shade of trees, it is likely to be suffering from a magnesium deficiency in the open, and this might account for its shorter stature in nature. However, etiolation due to lower light intensity in the laboratory cannot be ruled out.

Figure 54. ***Funaria hygrometrica*** with archegonia and young sporophytes. Photo by Andrew Spink, with permission.

Leaves

Leaf development occurs when sufficient nutrients are available and temperature and light are adequate for growth. Thus leaf expansion can occur in consort with apical growth and branch growth, or the plant may produce numerous branches and leaves, delaying stem expansion until later, as in the capitula of ***Sphagnum*** (Figure 55). However, controls of these phenomena are different, and the reduced leaves on elongated stems in the ***Funaria*** (Figure 2-Figure 3, Figure 54) cultures under cellophane discussed earlier attest to this fact.

Figure 55. Dense branches in capitula of ***Sphagnum wulfianum***. Photo by Jan-Peter Frahm, with permission.

Moss leaves typically are endowed with pigments and antiherbivore compounds that permit them to survive in their habitats. One of the compounds occurring in some moss cell walls appears to be a phenolic compound, as suggested by its ability to fluoresce under UV light (Figure 56).

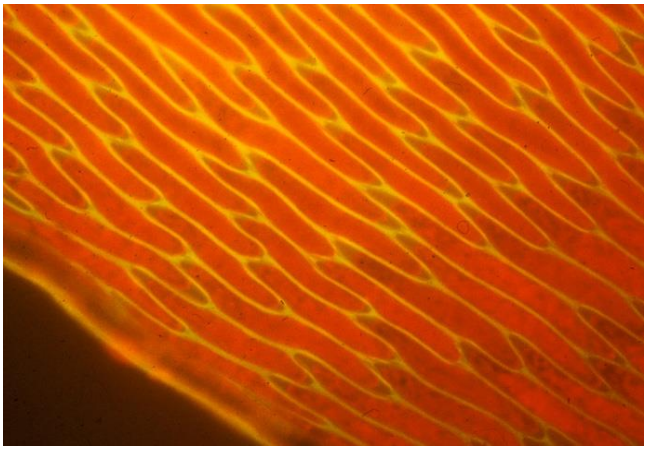

Figure 56. Fluorescence of cell walls under UV light in a leaf of ***Fontinalis antipyretica***. Photo by Janice Glime.

Light

In some species leaf dimensions and leaf shape are highly plastic and dependent on light and moisture conditions. Hoddinott and Bain (1979) found that red vs. far-red light caused significant differences in leaf dimensions. ***Ceratodon purpureus*** (Figure 4) and ***Polytrichum juniperinum*** (Figure 15) had longer leaves in red light, whereas ***Leptobryum pyriforme*** (Figure 14) and ***Pohlia proligera*** (Figure 57) had longer leaves in far-red light. In ***Ceratodon*** and ***Leptobryum***, leaf width was greater in red light, whereas in ***Polytrichum*** it was greater in far-red light. These wave length changes resulted in overall leaf shape changes in ***Leptobryum***, ***Pohlia***, and ***Polytrichum***. ***Dicranum polysetum*** (Figure 13) and ***Funaria hygrometrica*** (Figure 58) leaf shapes were indifferent to red/far-red differences. Hopefully our new molecular techniques will help us sort out some of the environmentally induced differences.

Figure 57. ***Pohlia proligera.*** Some members of this genus has leaves that are longer in far-red light. Photo by Michael Lüth, with permission.

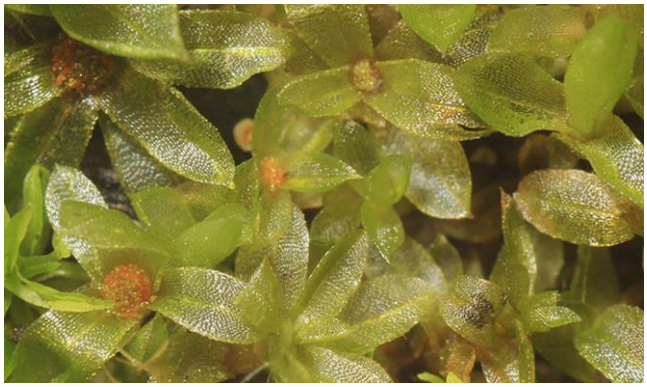

Figure 58. ***Funaria hygrometrica***, a species for which light quality changes did not change leaf shape. Photo by Barry Stewart, with permission.

Water

Water modifies leaf form as well. ***Drepanocladus*** (Figure 59) has longer and proportionally narrower leaves and loses its **falcation** (curved shape; Figure 60-Figure 61) in water (Lodge 1959). Furthermore, the normally straight ***Fontinalis*** leaves (Figure 62) become falcate (Figure 63) when grown in air (pers obs).

Figure 59. ***Drepanocladus fluitans*** growing above water and demonstrating curved leaves. Photo by Michael Lüth, with permission.

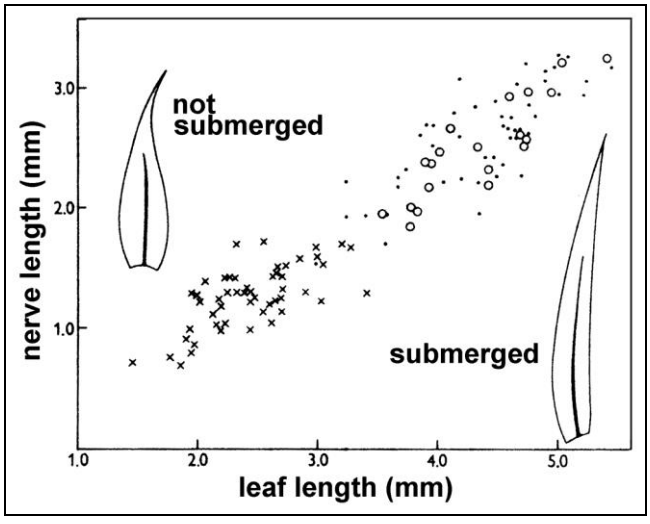

Figure 60. Modifications in leaf morphology of ***Drepanocladus fluitans*** due to submergence, in this case causing elongation. Redrawn from Lodge 1959.

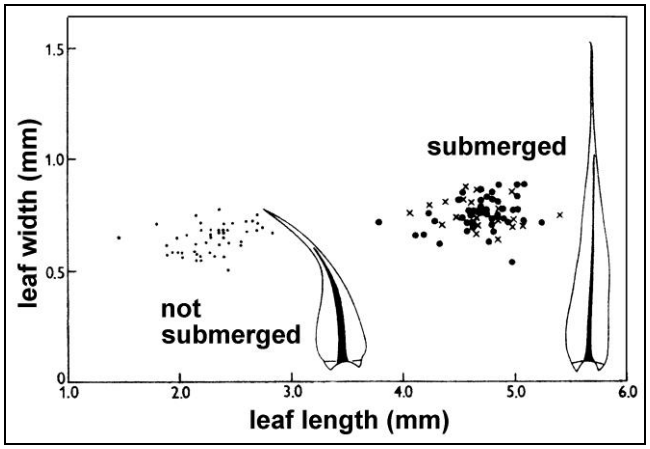

Figure 61. Modifications in leaf morphology of ***Drepanocladus fluitans*** due to submergence, in this case causing loss of falcation. Redrawn from Lodge 1959.

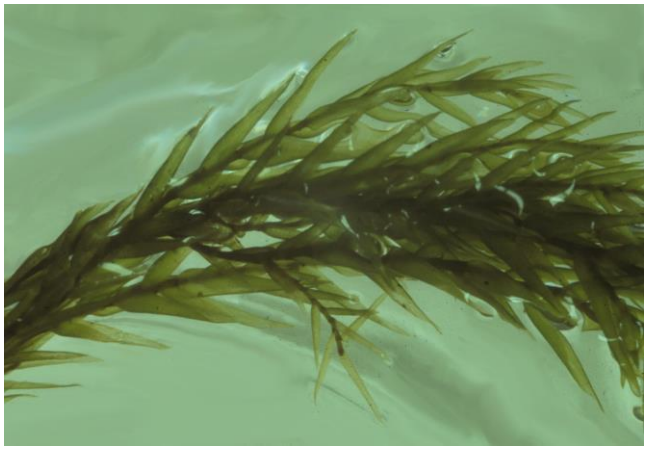

Figure 62. ***Fontinalis novae-angliae*** with normal submerged leaves. Photo by Janice Glime.

Figure 63. Falcate leaves of ***Fontinalis novae-angliae*** grown on moist paper out of water. Compare these to the straight leaves in Figure 62. Photo by Janice Glime.

Salt can cause similar modifications to effects of being above water, suggesting that loss of water from the leaves can trigger these changes. For example, cell length of ***Drepanocladus*** leaves increases as salt concentrations increase (Figure 64; Lodge 1959). On the other hand, Voth (1943) found that ***Marchantia polymorpha*** (Figure 30) had rapid maturity and slightly smaller cells in higher concentrations of salts.

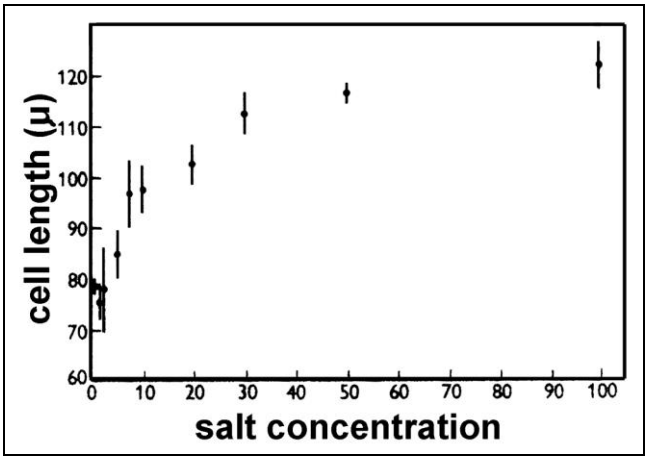

Figure 64. Relationship between leaf cell length and salt concentration in ***Drepanocladus fluitans***. Concentrations are relative percents of highest concentration with individual ions kept in same proportions. Redrawn from Lodge 1959.

In ***Sphagnum***, leaf response differs among species. In ***S. papillosum*** (Figure 7), the leaf becomes significantly longer when the capitulum is farther from water, but in ***S. magellanicum*** (Figure 6), there is little difference (Li *et al.* 1992; Figure 65). ***Sphagnum*** cell dimensions are also altered by water availability, with leaves of these two species grown under drier conditions having longer cells with unaltered width (Figure 66) and more pores per cell (Figure 65 right; Figure 67). Such evidence demonstrates the plasticity of species to respond to the environment and emphasizes the importance for common garden experiments in systematic studies.

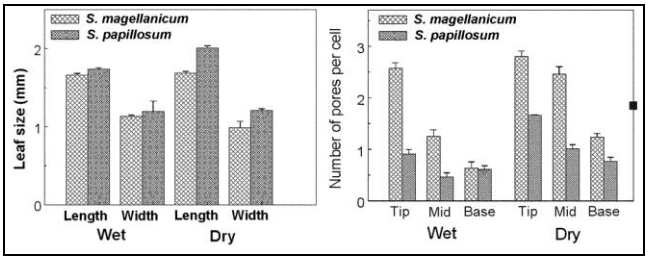

Figure 65. Effect of water level (water availability) on **left:** leaf length and **right:** number of pores per cell in ***Sphagnum magellanicum*** (Figure 6) and ***S. papillosum*** (Figure 7). Wet denotes 0 cm initial distance of capitulum from water; dry denotes 10 cm initial distance. Bars represent standard error. From Li *et al.* 1992.

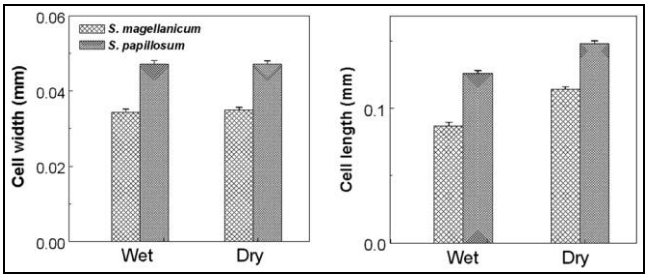

Figure 66. Effect of water level (water availability) on hyaline cell width and length in ***Sphagnum magellanicum*** and ***S. papillosum***. Wet denotes 0 cm initial distance of capitulum from water; dry denotes 10 cm initial distance of capitulum from water. Bars represent standard error. From Li *et al.* 1992.

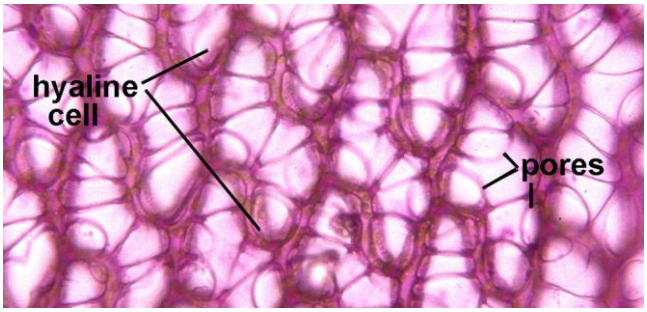

Figure 67. ***Sphagnum papillosum*** leaf showing hyaline cells and pores. Photo courtesy of Yenhung Li.

Hair points (hair-like extensions of leaf tip) in ***Racomitrium lanuginosum*** (Figure 68) are shortened by 50-100% in high humidity or shade (Tallis 1959). Cyclic weather conditions reduce hairs, causing maximal hair length on lateral branch zones but short hairs on in-between zones of the main axis. When the stem apex is removed, leaves have short or no hair points. When branches are produced, hair points arise on their leaves, suggesting that a controlling substance is produced by the stem apex and to a lesser extent by branch apices.

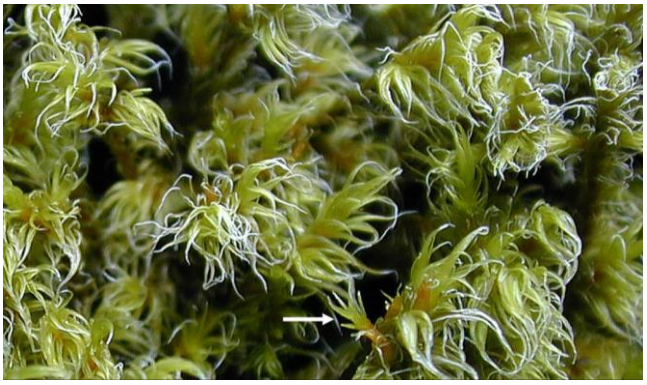

Figure 68. Apical hairs of ***Racomitrium lanuginosum*** showing reduced hairs at arrow. Photo by Michael Lüth, with permission.

The moss ***Schistidium apocarpum*** (Figure 69-Figure 70) varies considerably in the development of hair points, even on the same plant. ***Schistidium rivulare*** (Figure 71), which does not produce hair points, probably differs from ***S. apocarpum*** in its production of some growth-controlling substance.

Figure 69. ***Schistidium apocarpum*** with well-developed hair points. Photo by Michael Lüth, with permission.

Figure 70. ***Schistidium apocarpum*** with no hair points on leaves. Photo by Christophe Quintin, with permission.

Figure 71. ***Schistidium rivulare*** showing the absence of leaf hair points. Photo courtesy of Betsy St. Pierre.

Nutrients

Generally we look at the way nutrients affect whole plants, but they can especially affect development of leaves. For example, the difference between nitrogen as ammonium or organic N rather than nitrates in a low carbohydrate medium caused ***Sphagnum fallax*** (Figure 72) to develop leaves with no hyaline cells (Hintikka 1972). And nutrients can affect color (Glime & Marr unpublished). The role of nutrients on growth and development will be discussed in the chapter on nutrients.

Figure 72. ***Sphagnum fallax***, a species that alters its hyaline cells depending on the form of nitrogen. Photo by David T. Holyoak, with permission.

Growth Regulators

Little seems to be known about the hormonal control of leaf development. Exogenous application of auxin stimulates activity of the GUS-stained GH3 and DR5 genes in leaves of bryophytes, as demonstrated in ***Physcomitrella patens*** (Figure 26), but these genes did not demonstrate activity without the external auxin stimulus (Bierfreund *et al*. 2003).

We do know something about the role of ethylene in creating anomalous effects in leaf development, and these certainly have ecological relevance. As mentioned earlier, when growth of moss leaves and branches in the Arctic is impeded by ice, the result is crumpled leaves and branch ends. Similar crumpling resulted from growing ***Fontinalis squamosa*** (Figure 31-Figure 32) in high concentrations of ACC (resulting in elevated ethylene) and is consistent with effects of ethylene in lignified vascular plants. In some cases, ***F. squamosa*** leaves became wavy, much as the normal form of ***Neckera pennata*** (Figure 74), and in others they were more contorted, like stepping on a wadded up ball of paper (Figure 32; Glime & Rohwer 1983).

In ***Fontinalis antipyretica*** (Figure 73), application of ACC resulted in undulations on both young leaves and old, mature leaves (Figure 74; Glime & Rohwer 1983). Ethylene permits cells that have reached a certain stage to continue elongation, but inhibits it in younger cells. This results in uncoordinated development of the leaf cells and a surface that is not flat. It is very likely that similar hormonal regulation results in the natural waviness of leaves like those of ***Neckera*** (Figure 74). Since ***Fontinalis*** has been considered as closely related to the Neckeraceae, where undulations are characteristic of several species, it suggests that a gene controlling ethylene production or ACC distribution might be responsible for this morphology.

Figure 73. ***Fontinalis antipyretica*** showing normal, smooth leaves. Photo by Kristian Peters, with permission.

In nature, such events are likely to occur in response to leaf litter cover, ice, snow, and other physical barriers. By preventing diffusion of ethylene, unequal concentrations of ethylene result around different parts of plants, and as ethylene buildup occurs, contorted growth can result. An ethylene-induced growth differential between stems and leaves could explain the appearance of reduced leaves on **stolons** (horizontal stems from which upright stems arise) of certain species of ***Fontinalis*** (Glime 1980). If these stolons are a response to burial in a sandy substrate, or even burial among other ***Fontinalis*** branches that impede flow, ethylene production and accumulation could be the biochemical agent.

Figure 74. **Left:** ***Fontinalis antipyretica*** exhibiting undulate leaves induced by 10^{-4}M ACC. **Right:** ***Neckera pennata*** exhibiting genetically undulate leaves. Photos by Janice Glime.

In ***Fontinalis antipyretica*** (Figure 73), the response to ethylene precursor ACC was similar (Glime & Rohwer 1983) to the response of fern gametophytes, where mitosis ceased and cell elongation was enhanced by ethylene (Edwards & Miller 1972). In ***F. antipyretica***, shoot apices appeared truncated because older leaves with yet undeveloped cells had sustained cell elongation, whereas the center of the bud, where cell formation was incomplete, ceased its production of new cells and remained small (Figure 75). In these plants, elongation of outer leaves accounted for all growth of the plant during the 8-week experiment (Glime & Rohwer 1983).

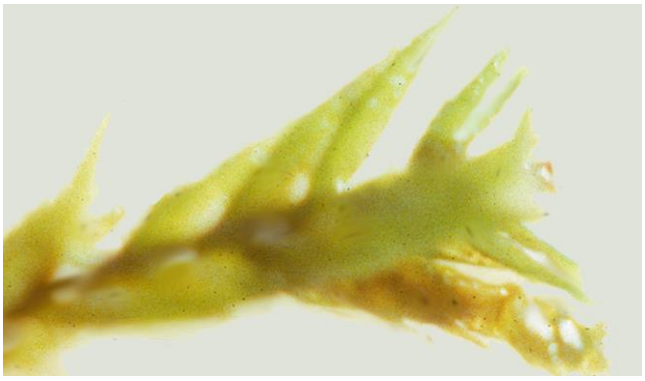

Figure 75. Effects of ACC (and presumably ethylene) on the shoot apex of ***Fontinalis squamosa***. Note truncated tip where leaves did not elongate while nearby leaves continued growth. Photo by Janice Glime.

The modified apex of ***Fontinalis squamosa*** (Figure 31) is usually accompanied by red to brown leaf coloration in elevated ACC (Figure 76). It appears that ethylene (or ACC) stimulates a color change to a reddened color in the cell walls.

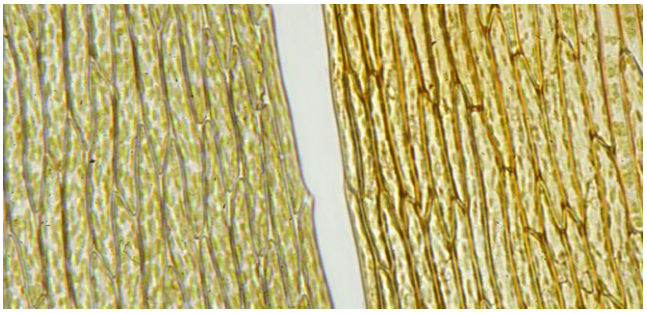

Figure 76. Effect of ACC on leaf cell wall color in ***Fontinalis antipyretica***. **Left:** Normal cells. **Right:** Cells subjected to 10^{-4}M ACC. Photo by Janice Glime.

As noted above, ***Fontinalis*** also can develop a modified leaf shape when grown exposed to air. When it is submersed during growth, leaves are straight, but in our lab cultures where it grew in a thin film of water and continuously received exposure to air while remaining wet, leaves became falcate (curved like a sickle; Figure 63). This may have been another example of ethylene production in the high oxygen, low CO_2 environment of air, as opposed to that in water. It is interesting that the other two genera in the family, ***Brachelyma*** and ***Dichelyma*** (Figure 77), have falcate leaves and grow most of the year out of the water.

Figure 77. ***Dichelyma falcata*** exhibiting falcate leaves. Photo by Michael Lüth, with permission.

Liverwort Leaf Suppression

Something happens as liverwort leaves develop! Something suppresses every third leaf during development. The result is that liverworts have two rows of leaves and a third row that may fail to develop completely or that develops into small leaves called **amphigastria** or **underleaves**.

Ethylene seems to have played a major evolutionary role in these bryophyte leaf arrangements. Basile and Basile (1983a, b, 1984, 1994) have shown that **hydroxyproline** (crystalline amino acid abundant in major glycoprotein of plant primary cell wall) will induce underleaves of liverworts to reach the size of lateral leaves, and in some cases induce development of underleaves when they are unknown in nature. They contend that loss of normal-sized underleaves in bryophytes, such as seen in ***Haplomitrium*** (Figure 78), is an evolutionary result of inhibition by ethylene, because ethylene antagonists such as hydroxyproline can induce these bryophytes to produce normal leaves where small underleaves would normally be. This is consistent with the widespread belief that 3-ranked leafy liverworts (Figure 78) are the primitive form, with 2-ranked ones being derived (and as implied here, derived due to suppression of the third row that results in reduced underleaves typical of many leafy liverworts; Figure 79).

Figure 78. ***Haplomitrium mnioides***, a leafy liverwort with three equal rows of leaves. Photo by Li Zhang, with permission.

Ethylene is known as a **senescence** hormone, *i.e.* it causes aging. In high concentrations it can cause cells to **plasmolyze** (cell membrane & contents pull away from cell wall) and die (Figure 80), as shown by Glime and Rohwer (unpub. data).

Figure 79. Ventral view of ***Calypogeia fissa***, a leafy liverwort with the underneath row of leaves suppressed. Photo by Michael Lüth, with permission.

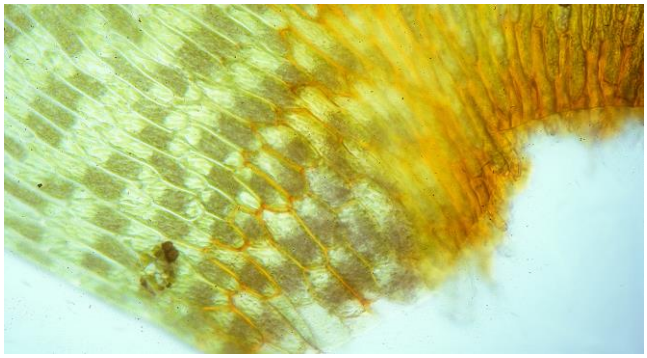

Figure 80. Plasmolyzed basal leaf cells in ***Fontinalis antipyretica*** subjected to 10^{-3}M ACC. Photo by Janice Glime.

Ethylene has a number of potential effects on leaves, but these have rarely been documented. It causes cell walls to become red, makes leaves wavy, and gives stem apices a truncated appearance (due to inability of young cells to elongate while older ones continue to elongate). Its most important role appears to be in the evolution of leafy liverworts with **underleaves** or no underleaves, compared to those with three equal rows.

Cuticle

Bryophytes, for a long time, were considered to lack a cuticle. But in fact, many do have varying degrees of cuticle (Figure 81) (Stránsky *et al*. 1967; Nilsson & Mårtensson 1971; Haas 1982). Cook and Graham (1998) noted the structural similarities between the osmiophilic surface layer on the liverwort ***Monoclea gottschei***, the moss ***Sphagnum fimbriatum***, and the hornwort ***Notothylas orbicularis*** with those of tracheophyte cuticles in that there is an "osmiophilic layer on the outer cell wall that bears some structural resemblance to early developmental stages of vascular plant cuticles." Of 43 moss species tested, Proctor (1979) demonstrated cuticles on 12 that were comparable to those on tracheophyte leaves.

We now know that cuticles in bryophytes can be present in the sporangial epidermis, spiral thickenings of elaters, rhizoids, and leaves (Kroken *et al*. 1996). As time progressed, so did regulation of their deposition. These cuticles initially seemingly had the functions of desiccation resistance and/or microbial resistance, as seen in lower charophytes. They have played a role in embryogenesis in the early land alga/plant ***Coleochaete*** and in embryophytes. Ultimately, they have an important role in decay resistance such as that of rhizoids, sporangial epidermis, and elaters of bryophytes

Salminen *et al.* (2018) noted that as photosynthetic organisms ventured onto land they developed new polymers such as cutin and suberin as a protection against water loss, solar radiation, and other potentially harmful abiotic factors. But we know little about these in bryophytes. Nevertheless, because of the variability of habitats exhibited by bryophytes and their early position in evolution on land, Salminen and coworkers proposed that liverworts and mosses were an attractive model systems for determining the specific functions and activity of lipid transfer proteins (LTPs) associated with cuticle synthesis and evolution of the plant cuticle.

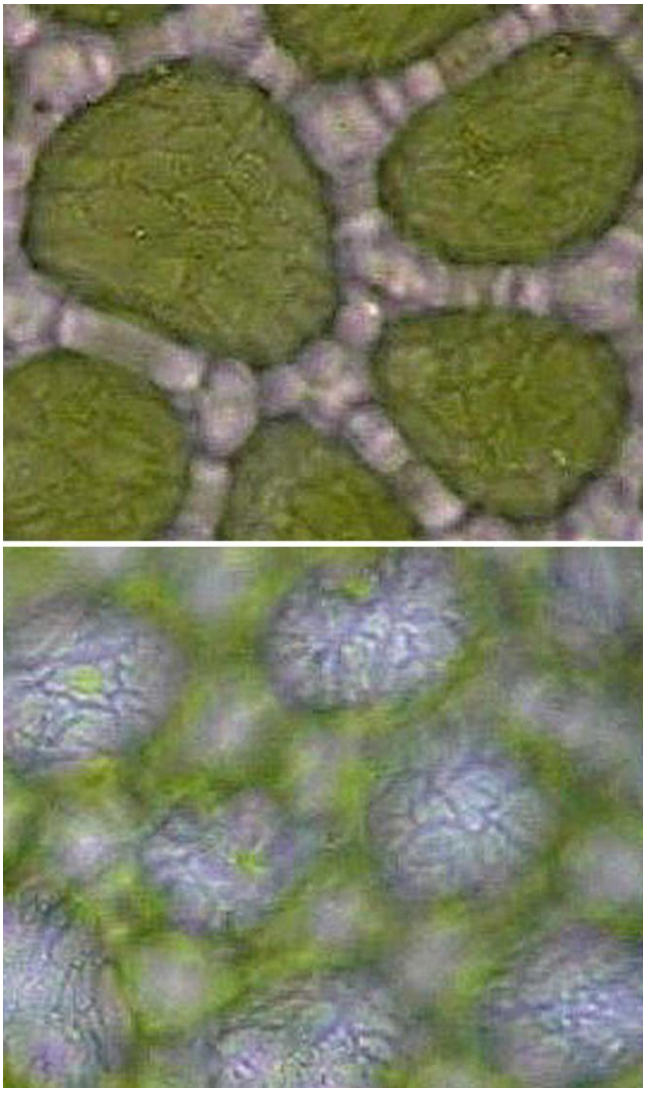

Figure 81. ***Mylia anomala*** showing cuticle. Photo by Paul G. Davison, with permission.

But our ecological knowledge of bryophyte cuticles seems to stop at recognition of their existence. I could find no reports on environmental or physiological control, and thus far there does not even seem to be evidence to support environmental correlation. Nor do we know at what developmental stage the bryophyte leaf or thallus begins production of the cuticle. We know that in tracheophytes, the cuticle can at times serve as a barrier to the entry of fungi and other pathogens (Kolattukudy 1985), but that role seems to be controversial, with many fungi possessing the enzymes needed to gain entry through the cuticle (Köller 1991). Stomata or wounding usually are the points of entry for fungal hyphae.

Do cuticles add to the ability of bryophytes to deter invasion of fungi? Is there any correlation between presence of cuticles similar to those of tracheophytes and the absence of fungal pathogens in bryophytes in nature? Do such pathogens attack the bryophytes only after they have been wounded? And how do the cuticles affect decomposition of dead and dying bryophytes?

Cuticles are **hydrophobic** (repelling water) and thus could facilitate photosynthesis by preventing the water barrier to CO_2 entry in bryophytes. In some cases, this hydrophobia could direct water to the base of the leaf

where there may be no cuticle and water entry is possible, while permitting photosynthesis in the rest of the leaf. This also could facilitate the spreading of the leaf upon hydration by dew or rain. This hydrophobia works on its inner surface as well, reducing water loss through the surface. In trachelphytes, interaction between the plant cell walls and cuticle in the presence of a pathogen on the surface can trigger internal plant chemical defenses (Ziv *et al*. 2018). But do bryophytes benefit from any of these possibilities?

Calyptrae

Since I seem to have neglected the gametophyte role in the protection of the sporophyte, this is perhaps an appropriate place to discuss it because of the role of the cuticle. Further information on the role of the calyptra is discussed in subchapter 5-9 of this volume on the Sporophyte. Budke *et al*. (2011) asked "A hundred-year-old question: Is the moss calyptra covered by a cuticle?"

Using the easily cultured ***Funaria hygrometrica*** as the study object, Budke and coworkers noted the role of the calyptra in protecting the developing sporophyte from desiccation. Using both SEM and TEM, they compared the calyptra, leafy gametophyte, and sporophyte sporangia. These methods revealed a multi-layered cuticle on the calyptra, including layers analogous to the cuticular layer, cell wall projections, electron-lucent, and electron-dense cuticle proper observed in tracheophytes. They hypothesized that the apex of the developing sporophyte in particular would be well protected. They found that the calyptra rostrum has a significantly thicker cuticle than the other tissues examined and differs by specialized thickenings of the cuticular layer (cuticular pegs) at the regions of the anticlinal cell walls – the first report of cuticular pegs in bryophytes.

Budke *et al*. (2013) followed these observations by experiments to verify the role of the cuticle in protecting the developing embryo in ***Funaria hygrometrica***. When the cuticle of the calyptra was removed chemically, they found that under low humidity conditions there is significant negative impact to moss sporophyte fitness, including decreased survival, increased tissue damage, incomplete sporophyte development, more peristome malformations, and decreased reproductive output.

Using four bryophyte species, Budke and Goffinet (2016) subsequently found that shorter sporophytes are associated with smaller calyptrae and thinner calyptra cuticles, whereas taller sporophytes are associated with larger calyptrae and thicker calyptra cuticles. Using sectioning techniques, they found that the cuticle of the sporophyte thickens during later develpment. The calyptrae, on the other hand, have a mature cuticle early in their development, and this persists throughout development. This can become an adaptive strategy in which resources are allocated, or not, to a thickened cuticle. Limited cuticle development can provide resources for other types of development for survival in different developments. Therefore, we should expect differences in cuticle thickness of the calyptra in wet vs dry environments, or at least in the species restricted to each.

Rhizoids

Rhizoids in bryophytes have an important role in anchoring the plants to the substrate and thus helping them adhere under the force of wind, water, or animal activities. It is therefore not surprising that these factors, along with temperature, are influential in the development of rhizoids.

Temperature

Furness and Grime (1982) demonstrated that switching of developmental processes can be due to different temperature optima. In ***Brachythecium rutabulum*** (Figure 82) growth is greatest at 20°C, primary branching at 16°C, and rhizoid production at 12°C. By contrast, in ***Fontinalis hypnoides*** (Figure 83), rhizoids are produced at 15-20°C (Figure 84-Figure 86), whereas the growth optimum is 10-15°C (Glime 1980, 1982; Glime & Raeymaekers 1987), and branching occurs during late winter, spring, and early autumn when the temperature is usually less than 10°C (Figure 86). In ***F. dalecarlica*** rhizoid production is negatively correlated with branch production (Glime 1984). This timing for ***Fontinalis*** permits the rhizoids to grow during warm summer months when the moss is most likely to have a sustained period without disturbance of heavy flow, thus affording it an opportunity to attach.

Figure 82. ***Brachythecium rutabulum***, a moss for which 20°C is optimum for growth. Photo by Michael Lüth, with permission.

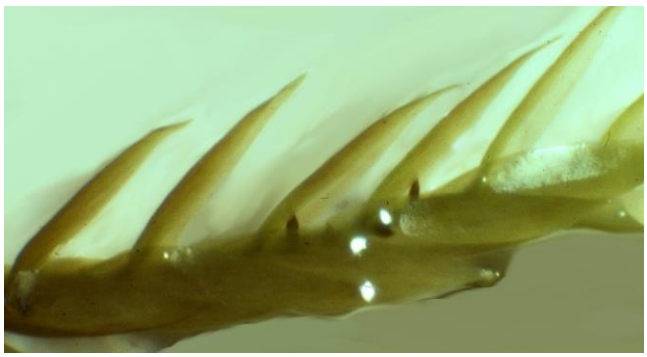

Figure 83. ***Fontinalis hypnoides***, a species that lives in both streams and lakes. Photo by Janice Glime.

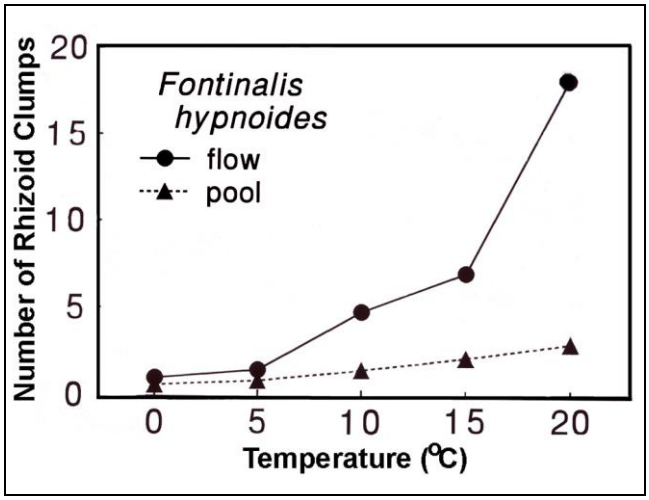

Figure 84. Flow and temperature effects on mean number (n=40 stem tips in each condition) of rhizoid clumps in ***Fontinalis hypnoides*** from the Keweenaw Peninsula of Michigan, USA, after 15 weeks in flowing water and pool conditions in artificial streams. From Glime & Raeymaekers 1987.

Light

Light can influence both form and production of rhizoids in bryophytes. In ***Riccia crystallina*** (Figure 85) red light favors smooth rhizoid production, whereas at high intensities more rhizoids are produced and more are **tuberculate** (having "pegs" or extensions of cell wall protruding into cell; Figure 87) (Chopra & Sood 1973). In 0.5% sucrose, there are 50% more smooth ones than tuberculate ones, but at 2% sucrose there are twice as many tuberculate as smooth ones, suggesting that the role of light in governing morphology may be one of sugar concentration, thus implicating a role for photosynthesis.

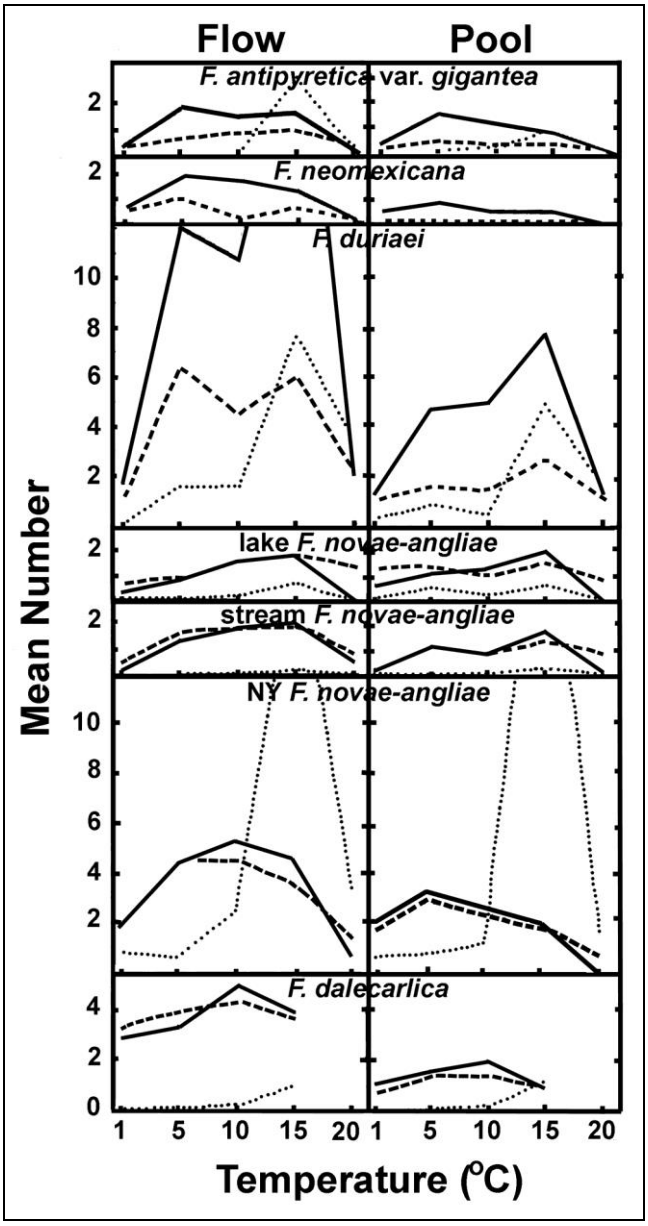

Figure 86. Flow and temperature effects on mean number (40 replicates at each condition) of rhizoid clumps (**dotted line**), branches per cm (**dashed line**), and cm growth of stem + branches (**solid line**) after 15 weeks in flowing water and standing water (pool) conditions in artificial streams. There are no data for ***F. dalecarlica*** at 20ºC. All populations are from the Keweenaw Peninsula of Michigan, USA, except where noted for New York, USA. From Glime & Raeymaekers 1987.

Figure 85. ***Riccia crystallina***, a liverwort in which red light favors production of smooth rhizoids. Photo by Des Callaghan, with permission.

Figure 87. ***Conocephalum conicum*** showing an example of smooth (**upper**) and pegged (**lower**) rhizoids. Photo by Paul Davison, with permission.

On the other hand, **phytochrome** is implicated, not photosynthesis, in controlling rhizoid production, based on research on ***Marchantia polymorpha*** (Figure 88) (Otto & Halbsguth 1976). Production of rhizoids at different wavelengths is subject to the typical red/far-red reversibility that characterizes involvement of phytochrome. Further implication in the role of phytochrome is that application of 10^{-4} M IAA for one hour has the same effect as one hour of red radiation.

Figure 88. ***Marchantia polymorpha*** showing rhizoids. Their production differs depending on wavelength of light and application of IAA. Photo from Botany Website, University of British Columbia, Canada, with permission.

Tropisms

We know a lot about tropisms in protonemata, but that does not seem to be the case for gametophores. As late as 2004, Cove and Quatrano determined that there are no extensive studies on gametophore tropisms. A search in Google Scholar in 2017 confirmed that is still the case, but some genetic studies are helping us to understand tropic responses in bryophytes. We understand that tropisms permit the plant to position its leafy shoot in the best position to obtain the maximum light for photosynthesis (Knight *et al*. 1991).

Early studies by Rawitscher (1932) indicated that ***Marchantia polymorpha*** (Figure 30) exhibits tropic responses to gravity, light and other factors. Miller and Voth (1962) demonstrated negative gravitropism of the thallus of this species. On thalli grown in an inverted position, the gemmae cups curved back toward the thallus. Furthermore, when the thalli were oriented vertically, the gemmae cups curve upward. Position had no effect on rhizoids, internal structure, pores, or position of terminal scales.

Physcomitrella patens (Figure 26) has not escaped tropism studies. Upright stems of this moss exhibit negative gravitropism, with no gravitropic response when the plants are rotated slowly vertically (Jenkins *et al.* 1986). At least three genes appear to be involved in the protonema gravitropism, with mutations in these altering the gravitropic form of the protonema, but none of these mutations affects the gravitropism of the leafy plant.

Genetic knock-out experiments are enabling us to understand many processes in plants, including tropisms in bryophytes. Knight and coworkers (Knight & Cove 1989; Knight *et al*. 1991) used genetic analysis of mutant ***Physcomitrella patens*** (Figure 26) in which the gravitropism was reversed. They found that both protonemata and gametophores respond to re-orientation by growing with negative gravitropism. In the mutant, the protonemata respond, but the gametophores do not, indicating control by mutation of a single gene.

Using ***Physcomitrella patens*** (Figure 26), Bao *et al*. (2015) were able to observe the phototropic response of the gametophore. In this species, the response is slow, taking more than 24 hours after the onset of a directed light source. They attributed the slow response to the slow growth of the moss. They found that red and far-red light were more effective than blue light.

Bennett *et al*. (2014) contributed to the story by experimenting with auxins and auxin transport inhibitors on the gametophytic shoot of ***Physcomitrella patens*** (Figure 26). These disrupt the apical function and leaf development. **PIN-mediated** (a protein) auxin transport regulates apical cell function, leaf initiation, leaf shape, and shoot tropisms in moss gametophytes. PIN mutants sometimes produce sporophytes that are branched, a condition rarely seen among natural moss variants.

In ***Physcomitrella patens*** (Figure 26), we know that cryptochrome signals are important regulators in many stages of moss development (Imaizumi 2002). These include the induction of side branching on protonemata, induction of the leafy gametophyte, and development of the leafy plant. When the cryptochromes are disrupted, auxin responses were altered, including altering the expression of auxin-inducible genes. This study indicates that light signals received by the **cryptochromes** act to repress auxin signals and in that way they control plant development.

In the moss ***Ceratodon purpureus*** (Figure 89), the polarity of the axis from regenerating protoplasts is influenced by the direction of light (Cove & Quatrano 2004). There is a delay in the response when the light direction is changed – a limitation that prevents the stem from tracking the sun as the Earth turns. For example, when protoplasts regenerate in red light at 25°C, there is a delay of about 9 hours before any response is observed. The lag is shorter with far-red light. Their ability to "memorize light direction" indicates use of **phytochrome**. They indicated that the phototropic response "turns off" the gravitropic response in this species and in ***Physcomitrella patens*** (Figure 26).

Figure 89. ***Ceratodon purpureus***, a moss in which polarity is influenced by light. Photo by Michael Lüth, with permission.

Rhizoids locate their substrate by a combination of gravitropism and phototropism, followed by a **thigmotactic** response (contact response) (Glime 1987c). Light can play a strong role in determining the direction of rhizoid growth. In ***Fontinalis squamosa*** (Figure 31), rhizoid growth was strongly photonegative (Figure 90), just as that of roots in tracheophytes. In most cases, this negative phototropism will permit the rhizoids to locate the substrate, which typically occurs in the same direction as the gravitational pull.

Figure 90. Strong negative phototropism of ***Fontinalis squamosa*** rhizoids at broken ends of stems. Photo by Janice Glime.

But in ***Fontinalis squamosa***, direction of light can be overridden by contact. Although the rhizoids were initially negatively phototropic, once they contacted the substrate they continued growing in that direction even when the light was reversed to come through the glass substrate (Glime 1987c).

One might suspect that **gravitropism** (directional growth in response to gravity) could be a cue for direction of growth in ***Fontinalis*** rhizoids, but I have not been able to induce a gravitropic response in ***Fontinalis antipyretica*** or ***F. squamosa*** (Glime 1987c). Instead, a strong negative phototropism occurs, even when it means rhizoids must grow pointed toward the stem apex, as in Figure 90. ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54), on the other hand, has positively gravitropic rhizoids (Figure 91) that are indifferent to light (Kofler 1958). ***Funaria*** does not grow on vertical substrata, so gravitropism would be an adaptive feature for ***Funaria***, whereas in ***Fontinalis*** it could be maladaptive for a plant that tends to grow on vertical faces on downstream sides of rocks. On the other hand, light will always be from above in habitats suitable for ***Funaria***, so absence of phototropism may have no selective disadvantage.

Figure 91. ***Funaria hygrometrica*** showing rhizoids growing downward toward gravity. Photo by Jan-Peter Frahm, with permission.

Schofield (1985) has concluded that in general rhizoids are negatively phototropic and positively gravitropic (Schofield 1985). However, this behavior might be different if we look at taxa that typically grow on vertical rocks, as suggested by ***Fontinalis*** (Figure 92) data (Glime 1987c). Despite all the basic physiological work on plant tropisms in protonemata, we know very little about bryophyte tropisms in other parts of the plants.

Figure 92. ***Fontinalis novae-angliae*** becoming established on a rock. Photo by Janice Glime.

Otto (1976) demonstrated several attributes of the rhizoids of gemmae of ***Marchantia polymorpha*** (Figure 30, Figure 93). They always grow from the **ventral** (lower) side – a response that could be either gravity or light driven. However, in alternating gravity in the darkness they form no rhizoids, but when gravity is constant they produce them with or without light. They also respond to contact, producing more rhizoids when contacting the substrate than when growing free in the air.

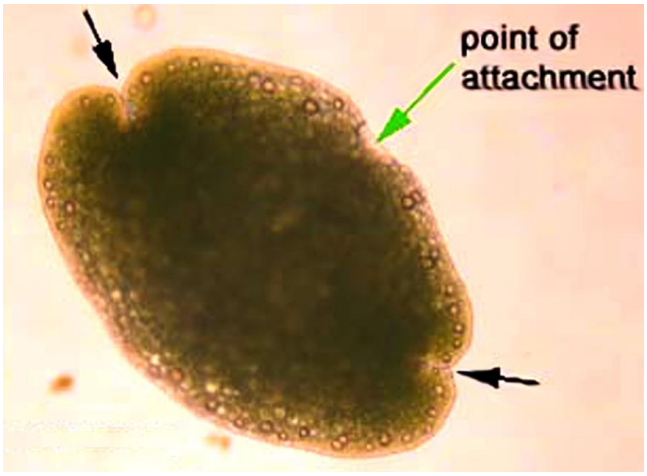

Figure 93. ***Marchantia polymorpha*** gemma. Black arrows indicate apical notches that serve as growing points. Photo by Kavita Uttam, Botany website, UBC, with permission.

Adhesion

Once a bryophyte makes contact with a solid surface, the tips tend to flatten and branch (Figure 94). These branched tips typically produce an adhesive substance that is especially important on vertical surfaces and in streams. Odu (1989) characterized this substance in the leafy liverwort ***Lophocolea cuspidata*** (Figure 95) and determined that it is a sulfated mucopolysaccharide. But attachment to a submersed rock in flowing water is much more challenging. Hence, we might find that this glue is different from that of ***L. cuspidata***.

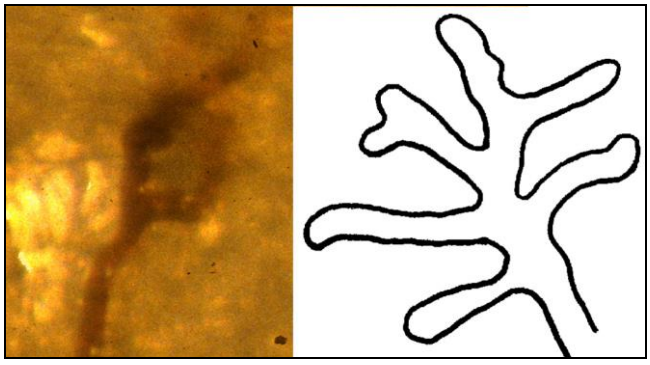

Figure 94. Branched tip of ***Fontinalis squamosa*** rhizoid in response to contact. Photo by Janice Glime; drawing by Margaret Minahan.

It is interesting that the flattened portion of the rhizoid occurs only at the tips in the pleurocarpous mosses, whereas in the acrocarpous mosses it extends far back from the tip (Odu 1989). Yet few acrocarpous mosses occur on vertical surfaces, most likely due to the small area available for adhesion compared to the weight of an outward-growing moss. Pleurocarpous mosses, on the other hand, have abundant surface area in contact with the substrate, and rhizoids typically occur throughout.

Figure 95. ***Lophocolea cuspidata***, a leafy liverwort that produces an adhesive (sulfated mucopolysaccharide). Photo by Jan-Peter Frahm, with permission.

Growth Regulators

Hormones are certainly involved in the differentiation of rhizoids. Maravolo (1980) found that auxins and gibberellic acid both stimulate the formation of rhizoids and cause cell division and elongation. Auxins in tracheophytes are known to stimulate roots and stems differently, so it is not surprising that rhizoids and stems of bryophytes respond differently to the same concentrations. Kumra and Chopra (1987) have shown that in callus cultures, lower concentrations of auxins stimulate differentiation into thalli and rhizoids, but at higher concentrations, only the rhizoids develop. Kaul *et al*. (1962) likewise found that high concentrations of NOA, 2,4−D, TCPA, IBA, and IPA stimulate rhizoid production in ***Marchantia*** (Figure 96). They also found that the responses of rhizoids to growth hormones differed in liquid vs solid culture media. Others have shown that IAA induces rhizoid production in wounded parts of plants (LaRue 1942; Maravolo & Voth 1966).

Figure 96. ***Marchantia polymorpha*** ventral side showing rhizoids. Photo by Botany Website, UBC, with permission.

Contrary to the popular belief that rhizoids function only in anchorage, Rose and Bopp (1983) found that rhizoids actually take up auxins from the environment. They found that the auxins are transported from the tip to the base of the rhizoids, where it accumulates.

Wounding

New growth results in most bryophytes as a result of wounding. In ***Fontinalis*** (Figure 97), this is typically preceded by the production of rhizoids that appear to be highly negatively phototropic. Furthermore, the rhizoids are **thigmotactic**, responding to contact by branching. But to find that surface, they have an interesting growth habit. They grow in a spiral (Figure 97). This spiral permits them to experience a larger area in which to locate a surface to which they need to attach. I am unaware of this behavior in other bryophytes, and it may indeed be peculiar to aquatic bryophytes.

Figure 97. Rhizoids on an explant of ***Fontinalis squamosa***, exhibiting spiral growth from the cut stem. Photo by Janice Glime.

LaRue (1942) has shown that in liverworts wounding induces rhizoids. He also showed that 1% IAA induced rhizoids all over the setae and capsules of ***Amblystegium*** sp. (Figure 98). IAA is produced by the breakdown of tryptophan in dying cells (Sheldrake 1971), and Maravolo and Voth (1966) have shown that IAA stimulates rhizoid production in gametophytes. In ***Fontinalis*** (Figure 100), I have found that my explants always produce rhizoids at or near the broken lower end of a stem piece, as in Figure 97, suggesting a polar substance such as IAA is responsible. However, the ultimate effector could be IAA-induced ethylene. Disintegrating xylem is a major source of IAA, as a result of tryptophan breakdown, so that this may be an important source for some bryophytes that establish primarily on rotting logs.

Numerous experiments show that ethylene levels rise as a result of wounding. In fact, most experiments on plants probably begin with elevated ethylene due to handling by the experimenter. If this is true, what occurs in a moss subjected to continual stress of a fast current? Using artificial streams in the laboratory, Glime and her students (Glime *et al*. 1979) found that rhizoids of several aquatic mosses [***Hygroamblystegium fluviatile*** (Figure 99), ***Fontinalis duriaei*** (Figure 100)] began to adhere to rocks after about 9 weeks and little additional attachment occurred after 14 weeks of contact (Figure 101). In these experiments, pieces of freshly wounded moss were tied to the rocks to insure contact and maintain their location. Odu (1978b) found a much shorter period of rhizoid growth for ***Calliergonella cuspidatum*** (Figure 102), ***Pleurozium schreberi*** (Figure 103), and ***Brachythecium rutabulum*** (Figure 104), species that grow mostly on soil or in standing water. Their rhizoid growth rates leveled off after about 6 weeks, and after 10 weeks there was no further growth.

Figure 98. ***Amblystegium radicale***. Photo by Des Callaghan, with permission.

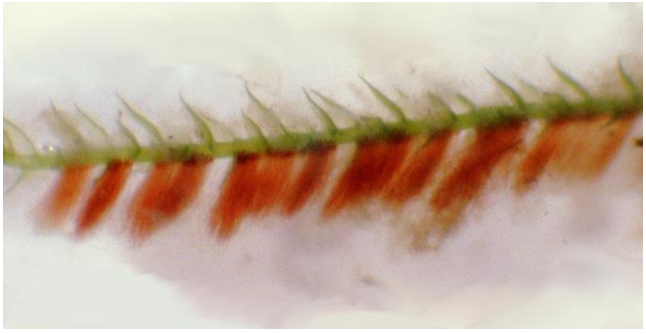

Figure 99. ***Hygroamblystegium fluviatile*** with rhizoids grown in culture. Photo by Janice Glime.

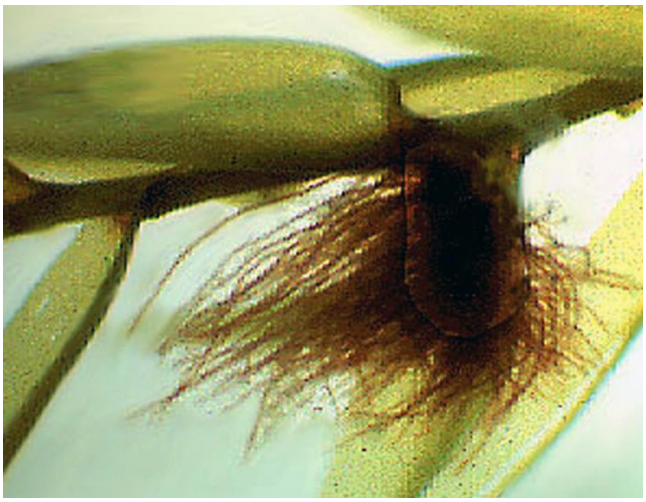

Figure 100. ***Fontinalis hypnoides*** rhizoids produced in culture. Photo by Janice Glime.

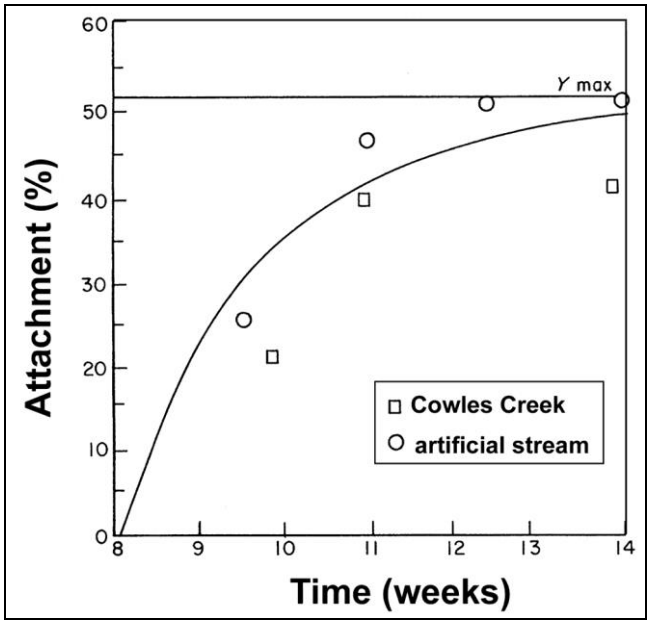

Figure 101. Model for rhizoid attachment to four rock types (shale, granite, basalt, sandstone – data combined) in ***Fontinalis duriaei*** in a natural and an artificial stream. n = 12 for each rock type and each stream. Based on Glime *et al.* 1979.

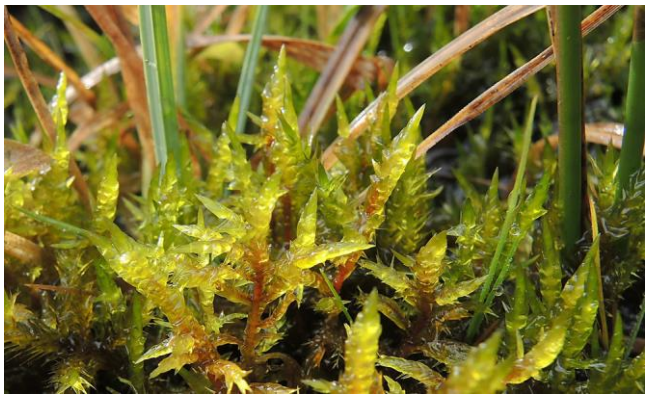

Figure 102. ***Calliergonella cuspidata*** in its typical habitat. Photo by Michael Lüth, with permission.

Figure 103. ***Pleurozium schreberi***, a ground-dwelling species with rapid rhizoid development. Photo by Sture Hermansson, with online permission.

Habitat Conditions

Odu (1978a, 1979) has found that acrocarpous mosses produce rhizoids all the way around the stem, but these are generally restricted to the stem base (Figure 106-Figure 105). These patterns are adaptive to the growth habit since acrocarpous mosses grow outward from a substrate and therefore can utilize only basal attachment. Compare that to the ventral positions in the two pleurocarpous mosses in Figure 99 and Figure 100. But substrate is not the only determining factor in rhizoid form. Acrocarpous moss rhizoids typically are longer, due to longer cells, than those of pleurocarpous mosses, even on vertical substrata (Figure 107; Odu 1978a).

Figure 104. ***Brachythecium rutabulum***, a ground- and rock-dwelling species with rapid rhizoid development. Photo by J. C. Schou, with permission.

Figure 105. ***Bryum*** sp. showing rhizoids that surround the stem at base. Photo by Michael Lüth, with permission.

Figure 106. ***Cyrtomnium hymenophyllum*** demonstrating rhizoids that surround the stem at base. Photo by Michael Lüth, with permission.

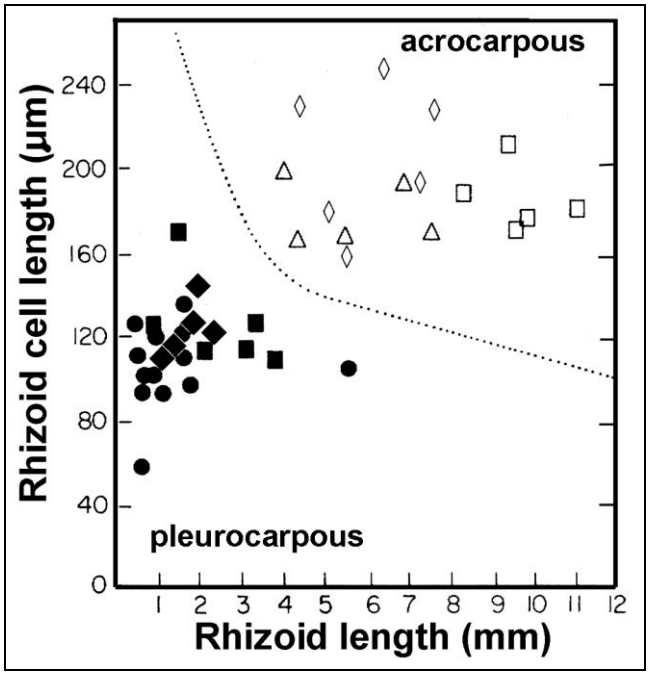

Figure 107. Relationship of cell length to rhizoid length in acrocarpous (◊***Bryum capillare***, Δ***Pohlia nutans***, □***Dicranum scoparium***) and pleurocarpous (●***Hypnum cupressiforme* var. *cupressiforme***, ■***Rhynchostegium confertum***, ♦***Homalothecium sericeum***) mosses, showing the greater length typical of acrocarpous mosses. Means are of 50 cells with 10 rhizoids used per species. Redrawn from Odu 1978a.

Figure 108. ***Hypnum cupressiforme*** on one of its many substrates. Photo by Dick Haaksma, with permission.

Mosses that grow prostrate on hard substrates typically develop rhizoid tufts (Odu 1978a), as seen for ***Fontinalis*** (Figure 100). In some cases these fuse, creating even greater physical strength. Pleurocarpous mosses generally produce rhizoids on only one side of the stem and these can occur throughout the stem (Odu 1979), as they do in most Jungermanniopsida (leafy liverworts; Schuster 1966). They have a **dorsi-ventral** (top-bottom) orientation so that if a pleurocarpous moss is turned upside down, its rhizoids initially grow from its new **dorsal** (upper) surface and then bend downward. However, eventually the stem itself twists so that it once again has the original ventral side next to the ground (Odu 1979). This twisting takes 5-18 days to turn 90º in ***Hypnum cupressiforme*** (Figure 108) and 10-30 days to turn 180º. Rhizoid production increases on the new growth in this twisted position. This twisting indicates that the stem has a top-bottom polarity that controls rhizoid orientation and that the growth of the rhizoids on that side of the stem is not a tropic response. Even in pleurocarpous mosses that initially grow upright, such as ***Pleurozium schreberi*** (Figure 103) and ***Calliergonella cuspidatum*** (Figure 102), rhizoids grow on only one side of that vertical stem. That upright stem eventually becomes the horizontal stem and the rhizoids are on the ventral side. In ***Funaria hygrometrica*** (Figure 2-Figure 3, Figure 54), rhizoids of germinating spores formed toward the positive electrode (Chen & Jaffe 1979), suggesting that this polarity may begin at the spore stage.

Based on Odu's (1978b, 1979) observations, I predicted that the pleurocarpous ***Fontinalis*** (Figure 31) should have rhizoids arising on all sides of the stem, since moving water prevents it from having one side that is always down. That is exactly what I observed in my culture experiments (Figure 109) (Glime 1980). Such an arrangement in stream mosses facilitates attachment in moving water. But how do these rhizoids attach without wasting energy by growing in all the wrong directions? Perhaps the rhizoids release ethylene upon contacting a substrate and the ethylene serves to inhibit further lengthening and instead serves to thicken the cells to provide a more secure attachment. We know, in fact, that once the rhizoids of ***Fontinalis squamosa*** (Figure 94, Figure 97) contact a surface they branch prolifically and attach (Glime 1987c; Figure 94). This is consistent with observations of Odu and Richards (1976) on the leafy liverwort ***Lophocolea cuspidata*** (Figure 95) and the mosses ***Hypnum cupressiforme*** var. ***cupressiforme*** (Figure 108) and ***Platyhypnidium riparioides*** (Figure 110) that respond similarly to contact.

The number of rhizoids produced by gametophores is also related to substrate. Odu (1978a, b) found that mosses that grew on boulders or tree trunks produced more rhizoids than did those on soil. When several species were moved from boulders to soil, they produced fewer rhizoids.

Stream mosses often produce abundant rhizoids (Figure 99-Figure 100), but taxa from other wet habitats often lack them. This absence is typified by such genera as ***Sphagnum*** (Figure 6-Figure 7) and ***Drepanocladus*** *s.l.* (Figure 111). The only species of ***Sphagnum*** known to have rhizoids is an epiphyte. If wet habitat species are grown out of water, will rhizoids develop? I tested this by gathering submersed ***Drepanocladus exannulatus*** (Figure 111) with no rhizoids and placing explants on a Petri plate of inorganic nutrient agar. Rhizoids appeared. Thus rhizoids in ***D. exannulatus*** seem to be under environmental control.

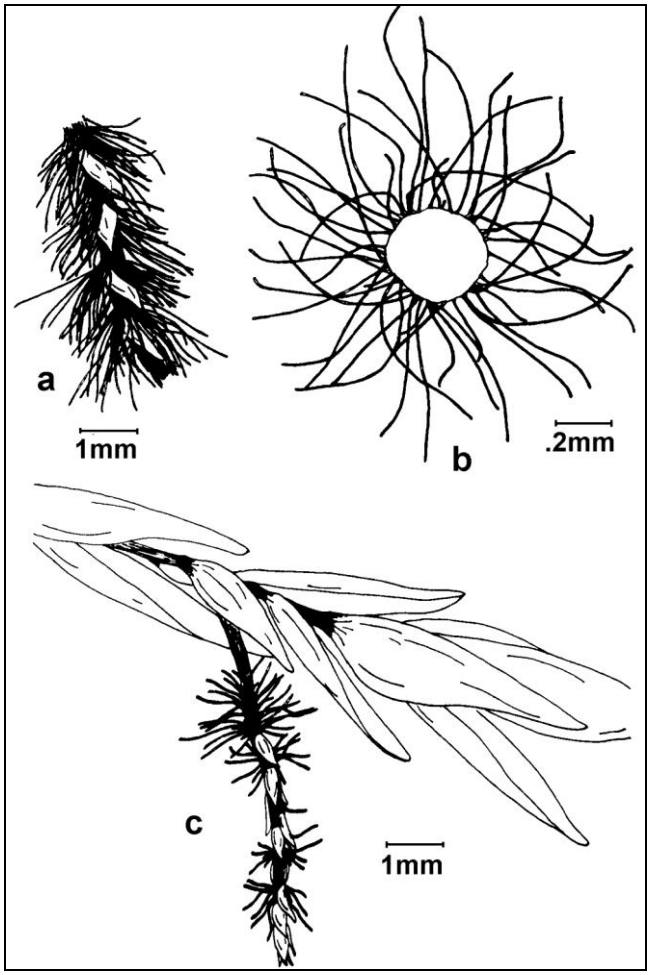

Figure 109. Rhizoids of ***Fontinalis*** on stoloniferous branches. **a**. ***Fontinalis dalecarlica***. **b**. cross section of stoloniferous branch of ***Fontinalis dalecarlica***. **c**. ***Fontinalis novae-angliae***. From Glime 1980.

Figure 110. ***Platyhypnidium riparioides***, a rock-dwelling species that produces rhizoids in response to contact. Photo by Hermann Schachner, through Creative Commons.

My observations on ***Fontinalis hypnoides*** (Figure 100) (Glime 1980) help to explain the control of rhizoid production in the aquatic habitat. The number of rhizoids increased with temperature when cultured at 1, 5, 10, 15, and 20°C. Furthermore, mosses in flowing water produced more rhizoids than those in standing water. The latter observation might be explained by **ethylene** control, since ethylene is known as an inhibitor of rhizoid elongation in ferns (Miller *et al*. 1970). In our experiments on ***F. squamosa*** (Figure 31), **ACC** (ethylene precursor) inhibited rhizoid production with increasing concentrations in cultures on wet filter paper, and the inhibition was more severe in mosses in water (Glime & Rohwer 1983). Since ethylene is not very soluble in water, it could easily accumulate around the moss and be a cause for the retardation of rhizoids in standing water, whereas flowing water would remove the ethylene. On the other hand, this removal action must counteract the increased production of ethylene we might expect to result from the mechanical stress of flowing water. But no one has demonstrated that mechanical stress does indeed induce ethylene production in bryophytes, as it does in tracheophytes. And we can reasonably expect the effective concentrations are different in bryophytes. Just as roots and shoots respond differently in tracheophytes, different parts of bryophytes can respond differently from each other and from parts with similar functions in tracheophytes.

Figure 111. ***Drepanocladus exannulatus***, a species that is devoid of rhizoids under water, but that can produce them when grown on an agar substrate. Photo by Michael Lüth, with permission.

Rhizoids seem to have evolved in adaptive ways to fit the habitats of their owners. Acrocarpous mosses that generally are upright have rhizoids that surround the base of the stem; pleurocarpous mosses that generally grow horizontally produce rhizoids only on their lower sides. The aquatic pleurocarpous moss ***Fontinalis*** produces them all around the stem, enabling it to attach from whatever side makes contact with a substrate. Mosses that grow on vertical substrata produce numerous rhizoids. Many mosses, especially on vertical substrata, have rhizoids that branch upon contact, permitting them to occupy a greater cementing surface. Stream mosses produce many rhizoids, whereas quiet-water species usually lack them, and this can differ within the same species in response to flow. Quiet water species may similarly produce rhizoids when growing out of the water. ACC inhibits the production of rhizoids, suggesting ethylene may be involved in these environmental responses.

Conduction

If ***Dicranella heteromalla*** is in any way typical of mosses, we have been underselling the role of the bryophyte rhizoid. Rather than simply anchoring the mosses, it appears that they may have important roles in nutrient absorption (Duckett & Matcham 1995). Their structure is very similar to that of food-conducting cells in leafy gametophyte stems and sporophytes. From this they suggested that the major role of the rhizoids might be solute uptake.

Bryophyte Senescence

Senescence is the process in which the cell reaches a state wherein it cannot undergo either progressive or regressive development and its only future change will lead toward death of the cell (Giles 1971).

Only in bryophytes can the lower part of the plant be completely dead while the upper part is still very much alive. ***Sphagnum*** is a classic example, exhibiting healthy, reproductive tops and dead bases, decades old (Figure 112). In mosses such as ***Hylocomium splendens*** (Figure 113), one might find 4-7 years of live growth atop several more years of senescent or dead plant.

Figure 113. Living plants of ***Hylocomium splendens*** forming a turf on top of their own senesced branches (**arrow**). Photo by Michael Lüth, with permission.

Figure 112. ***Sphagnum girgensohnii***, showing dying and dead lower parts. Photo by Bernd Haynold through Wikimedia Commons.

Figure 114. Mature capsules that mark the onset of senescence in ***Tetraphis pellucida***. Photo by Janice Glime.

At least in some taxa, the initiation for senescence results from the production of male gametangia or capsules. In many acrocarpous mosses, these structures can effectively prevent further growth of the plant by occupying what would have been the region of apical growth, as shown for ***Tetraphis pellucida*** (Figure 114) (Kimmerer 1991). In this species, high density increases sexual reproduction, which increases capsule production and proportion of males, which in turn initiate senescence for the population. Some mosses overcome this apical growth termination by producing innovations – side branches near the tip that become new tips and continue the growth upward (see chapter on gametophore development).

As in higher plants, it appears that ethylene induces senescence, as shown in ***Marchantia*** (Figure 30) (Stanislaus & Maravalo 1994). Spermine, spermidine, and putrescine can reverse it. If we dare to generalize from this meager example, the story makes sense. As the moss grows and the cushion or mat (or whatever) becomes more dense, there is less and less air movement in the lower part of the growth form (see Figure 115). This permits gases to accumulate, so if ethylene is being produced, this surely is a place for it to reach higher concentrations. Now all we need to do is show that indeed there is ethylene given off here, that it accumulates, that it reaches high enough concentration, and that it indeed induces senescence in most (all?) bryophytes!

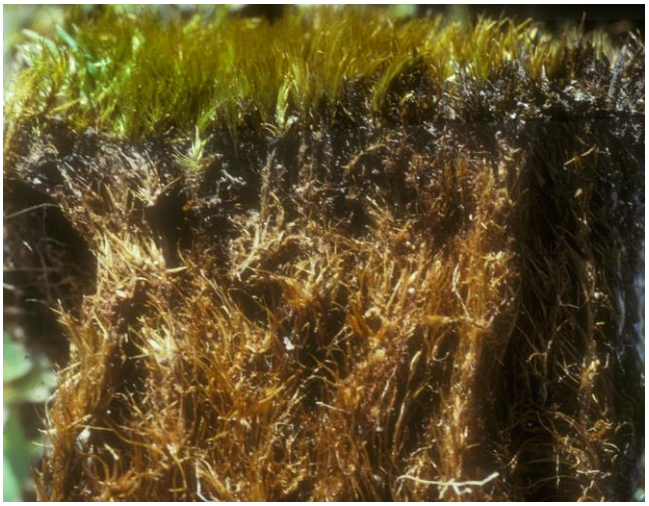

Figure 115. Senescence in lower, brown portion of ***Dicranum scoparium***. Photo by Janice Glime.

Ecological Interaction

External factors may control differentiation and growth of gametophores in bryophytes. The physical effects of accompanying plants are widely recognized. However, with sensitivities at such microlevels as affect bryophytes, exudates from other organisms also have the potential to effect changes in developmental patterns. This might be especially true if dying plants leak substances that collect on the surfaces of the bryophytes, dissolved only in the adhering humidity and readily absorbed by the mosses in what would, under these circumstances, be relatively high concentrations. Nevertheless, although the potential seems relatively high, few studies have addressed these potentials.

The presence of other plants will naturally affect moisture and light availability. In general, other plants help to maintain a more humid environment than would be available if the bryophyte were directly exposed to air. This seems to be accomplished mostly by maintaining a small space in which air movement is reduced, thus reducing the evaporation rate from the bryophyte. In ***Brachythecium*** (Figure 104) populations, litter of the stinging nettle (*Urtica*) stimulates growth (Willis 1978). Willis attributes this added growth to moisture and nutrient release, but we cannot rule out the possibility of hormonal interaction as well.

The reduction in light caused by accompanying plants may provide an advantage by reducing the destructive effect of UV light when the bryophyte is dry. However, when the surrounding plants become too dense, they can effectively block the light and also prevent the bryophyte from occupying the substrate, thus crowding it out. Deciduous trees are very effective at this by losing their leaves and completely covering the bryophytes, thus preventing them from getting any light. They may further inhibit bryophyte growth during decay by releasing humic acids that can inhibit growth (see discussion under spore germination), or possibly even releasing growth regulating substances. Whatever their action, leaves seem to be destructive to my moss garden if I leave them there over winter, even if I remove them as soon as the snow melts. Considerable decay occurs during that snow-covered period.

Leaf litter seems to be the major cause for the paucity of bryophytes on the forest floor in a deciduous forest. Bryophytes there are restricted to elevated areas such as rocks or slopes where leaves do not collect. In one set of experiments to determine what species of plants would grow following a disturbance similar to a tip-up hole (from a tree falling over), researchers dug holes in the forest floor. Bryophytes invaded the holes, but only on the sides. Litter collected on the bottoms of the holes, and although tracheophytes germinated there, no bryophytes succeeded.

Sheldrake (1971) has suggested that natural exogenous hormones could be important in bryophyte distribution. He found **IAA** in many substrates inhabited by bryophytes, and he concluded the IAA was not produced by the bryophyte because the same concentrations occurred without bryophytes. Garjeane (1932) noted that contact with soil and decaying vegetation stimulated rhizoids in liverworts, and Maravolo and Voth (1966) showed that liverwort rhizoid length and rhizoid formation are stimulated by IAA. Therefore, bryophytes might grow better in microhabitats where these hormones collect. Disintegrating xylem is a major source of IAA, so this may be a contributing factor to the luxuriant growths of liverworts on logs in moist woods.

Odu (1978b) found that living tracheophytes had just the opposite effect on moss rhizoids. Mosses transplanted from grassland to bare soil increased their number of rhizoids and those transplanted from boulders to bare soil produced more rhizoids than those transplanted to grasslands. It would seem that IAA was not the inhibitor involved since we have already seen that it stimulates rhizoids, but perhaps concentration is a factor. Furthermore, bare soil may have more available IAA as a result of bacterial breakdown of organic matter (Sheldrake 1973), with a cover of grass depriving the mosses of access (Odu 1978b). On the other hand, an easily diffusible substance such as **ethylene** could account for the ability of living plants to inhibit the rhizoids, since no inhibition occurred on soil with plants removed but with the litter remaining.

Neighboring plants can affect bryophyte growth by altering the available light and level of humidity. They can serve as a filter, protecting the bryophytes from damaging UV rays. The environment experiences a wide range of exudates from the plants that live there, undoubtedly influencing development of some bryophyte taxa. Litter provides humic acids that are known to inhibit bryophyte growth, and decaying xylem releases IAA that can stimulate rhizoid production. Crowding is likely to create patches of elevated ethylene that could be inhibitory to bryophyte development.

Summary

Growth in bryophytes is both stem and branch growth, making it non-linear, but can also be a weight gain without any elongation. Growth in very low light causes etiolation. Water and light are necessary for growth, with a wide range of light being optimal among

the various taxa. A common optimum seems to be around 3500-5500 lux for shade-adapted taxa.

Stems usually exhibit a strong positive phototropism and negative gravitropism, whereas rhizoids exhibit the opposite. Short or long photoperiods may induce dormancy, depending on the habitat and species.

Bryophytes respond to most of the same hormones as tracheophytes but at different, usually lower, concentration levels. Among other things, IAA enhances growth, cytokinins stimulate buds, gibberellins affect rhizoid growth and form, and ethylene causes senescence and in leafy liverworts inhibits dorsal leaf development. These hormones furthermore affect each other's actions. Many bryophytes exhibit apical dominance, facilitated by IAA. In addition, the form in which N is available can alter the growth form, branching, and growth rate.

Apical sexual structures usually terminate growth of that stem, but innovations (new branches near the tip) can cause the plant to continue growth and may facilitate lateral spread.

Humidity, light, salt concentration, and nutrients all influence the leaf shape, hairs, and color, and can cause the species to appear to be a different one in a different habitat.

Rhizoids respond to contact with a substrate by flattening and widening their tips, branching, and halting growth in other directions. Wounding causes the production of rhizoids and/or protonemal growth at the site of the wound.

Leaf litter inhibits the growth of bryophytes, in part by blocking light, but apparently also by depositing humic substances that are inhibitory or even lethal. In other cases, other plants, fungi, or bacteria in association with the bryophytes provide them with needed hormones.

Bryophytes are the only plants where the lower portion of the plant can be senescent or dead and still maintain a healthy upper portion.

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CHAPTER 5-6

ECOPHYSIOLOGY OF DEVELOPMENT: FRAGMENTS

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ECOPHYSIOLOGY OF DEVELOPMENT: FRAGMENTS

Figure 1. ***Dicranum viride***, a moss that fragments regularly by a row of abscission cells across the upper half of the leaf. Note the broken leaf tips. Photo by Michael Lüth, with permission.

Fragmentation

Fragmentation may be random pieces that break due to abrasion, decay, or animal severance, or they may be programmed genetically by means of an abscission layer such as demonstrated in ***Dicranum viride*** (Figure 1). In certain habitats, fragmentation may be a regular phenomenon, accounting for nearly all the reproduction.

Even fossil evidence supports the importance of fragments in the dispersal and reproduction of bryophytes (Miller 1985). And buried fragments often retain viability, providing the source for the flora when a disturbance returns an area to previous conditions (Wasley 2004).

Yet, when we diagram life cycles, fragmentation is usually ignored, and certainly for many flowering plants it is unimportant. However, in bryophytes it is often the fragments that perpetuate the species. Likewise, Giordana and coworkers (1996) found that regeneration from the detached leaves was the major form of regeneration in moss ***Pleurochaete squarrosa*** (Figure 2). Other bryophytes, such as ***Hyophila crenulata***, share their successful regeneration from fragmentation with other means such as gemmae (Olarinmoye 1981).

Mishler and Newton (1988) contend that in perennial mosses reproduction and spreading is almost entirely by means other than spores. Many populations exist for which capsules are unknown, particularly for dioicous taxa (having males and females on separate plants; unisexual). Even when all individuals in the population can produce both sexes (monoicous; bisexual), water is needed at the right time for sperm and egg to meet, so success rate will vary with habitat and with weather in a given year. Newton and Mishler (1994) suggest that vegetative reproduction, including specialized propagules, can occur

under more stressful conditions. Whereas spores germinate best on previously uncolonized substrates, vegetative reproductive units can do well even in contact with existing colonies. However, they suggest that such vegetative units cannot travel as far as spores – tradeoffs again.

Figure 2. ***Pleurochaete squarrosa***, a moss that relies on detached leaves for regeneration. Photo by Michael Lüth, with permission.

Some mosses even provide special means to accomplish fragmentation. ***Dicranum viride*** (Figure 1), ***D. fragilifolium*** (Figure 3), and ***Tortella fragilis*** (Figure 4) have a weakened area of cells that break easily, releasing the upper portion of the leaf. This is so typical that these species can be identified by their chopped off appearance. Other species have **caducous** leaves (leaves that normally detach).

Figure 3. ***Dicranum fragilifolium*** on rock, showing broken leaves. Photo by Janice Glime.

Figure 4. Broken tips on leaves of ***Tortella fragilis***. Photo by Michael Lüth, with permission.

The success of fragments within short range (Newton & Mishler 1994) is supported by experiments by Nehira and Nakagoshi (1987). They removed a community of bryophytes and found that the community became re-established within 1-2 years. Most of the growth occurred in spring and autumn despite little seasonal variation in propagule dispersal. Thallose liverworts and pleurocarpous mosses were able to regenerate more quickly than the acrocarpous mosses. Yet these same fragments may have been eaten or decayed before ever growing if the researchers had not removed the parent colony. Newton and Mishler (1994) found that at least for the dry habitat mosses they studied, the parent plants seemed to inhibit growth of the fragments, with growth commencing once they were separated.

Fragmentation is likely to determine success of the species in some environments. Miles and Longton (1990) found that ***Funaria hygrometrica*** (Figure 5) reproduced and spread easily by spores, whereas ***Atrichum undulatum*** (Figure 6) and ***Bryum argenteum*** (Figure 7) were likely to experience failure before sporelings produced gametophores. On the other hand, these latter two species freely accomplished regeneration from shoot fragments. This ability of ***Atrichum*** to regenerate easily from leaf fragments permitted it to dominate the ground cover rapidly after the construction of a parking lot on the Michigan Technological University campus (Glime 1982). ***Funaria hygrometrica***, on the other hand, apparently manages to arrive, presumably by spores, and colonize charred ground within a year after a fire, as occurred after the big Yellowstone fire (Glime pers. obs.).

Figure 5. ***Funaria hygrometrica***, demonstrating the prolific production of capsules. Photo by Niels Klazenga, with permission.

Even on rocks, where one might expect a small spore and protonema to have more success than a large fragment, it seems that fragments dominate the reproductive success. Keever (1957) did find that spores germinated on granite, but colonization through fragmentation was more rapid. One such rock-dwelling (and bark-dwelling) species is ***Orthodicranum montanum*** (Figure 8). Chrobak and Sharp (1955) established that this species grew well from leaf fragments. The proximal (basal) half of the broken leaf was more successful than whole leaves or the distal portion of the leaf (Figure 9).

Figure 6. ***Atrichum undulatum*** with drying plants that can break more easily than hydrated plants. Photo by Michael Lüth, with permission.

Figure 7. ***Bryum argenteum***, a moss that easily loses its tips as dispersal units. Photo by Janice Glime.

Figure 8. ***Orthodicranum montanum*** on bark. Photo by Janice Glime.

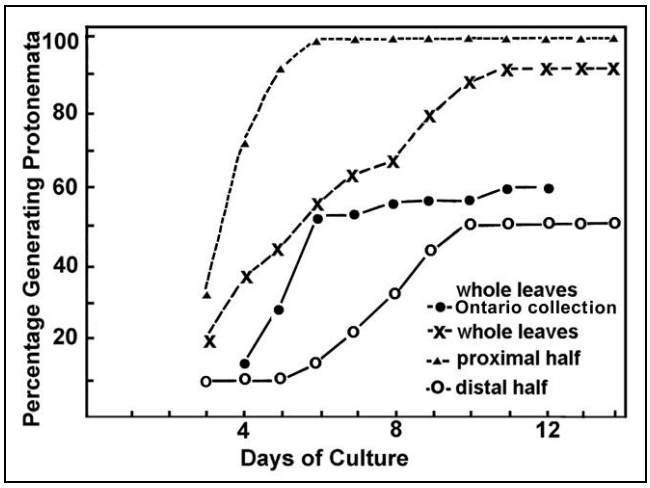

Figure 9. Success of producing protonemata from various leaf parts of ***Orthodicranum montanum*** from Michigan, USA, and Ontario, Canada. Redrawn from Chrobak & Sharp 1955.

Arctic and Alpine

Mogensen (1986) found that ***Platydictya*** (Figure 10) was dispersed in Greenland primarily by vegetative propagules and Bonde (1959) found viable ***Polytrichum piliferum*** (Figure 11) fragments among the wind-blown debris of a Colorado glacier. Lindskog and Eriksen (1995) found that the fragments of mosses, in particular, that were on the glacier reflected accurately the composition of the surrounding vegetation.

Figure 10. ***Platydictya jungermannioides***. Photo by Des Callaghan, with permission.

Figure 11. ***Polytrichum piliferum***, a moss that reproduces by fragments on the Colorado Glacier. Photo by David T. Holyoak, with permission.

McDaniel and Miller (2000) demonstrated the importance of fragments in alpine areas of the Adirondack Mountains of New York, USA, and suggested that fragments dispersed in winter might be a significant means of establishing new populations following spring snowmelt. It would certainly much easier for fragments to glide across a snow pack than to travel amid ground vegetation.

In the Arctic, fragments on the ice are common, and are easily moved around over the smooth surface, permitting rapid transport over considerable distances. Miller and Howe Ambrose (1976) found that fragments of mosses were distributed across the snow by wind on Bathurst Island in the Canadian high Arctic. They were able to grow these fragments in culture, with only 12% of the fragments producing evidence of viability by growth of protonemata, shoots, or rhizoids. The leaf-bearing tips of leafy shoots were the most likely to produce new growth. Nevertheless, this yielded an estimate of more than 4000 viable fragments per cubic meter of snow! Liverworts, however, did not fare as well, with only one fragment producing new growth. They surmised that such moss fragments may be "routine" in Arctic climates.

The importance of fragments may reach its climax in the Antarctic. In colonizing a new Antarctic volcanic island, fragments of ***Campylopus*** (Figure 12), ***Marchantia***, (Figure 13) and ***Bryum*** (Figure 7) species seemed to be the most important means of arrival (Smith 1984).

Figure 12. ***Campylopus pilifer*** showing fragments formed by tips of plants. Photo by Michael Lüth, with permission.

Figure 13. ***Marchantia polymorpha*** with dead portions that can create fragments. Photo by Michael Lüth, with permission.

In Antarctica on Mt. Rittmann, ***Pohlia nutans*** (Figure 14-Figure 17) only establishes on geothermally heated ground (Skotnicki *et al*. 2002). The geothermal heat (17-35°C) permits the moss to survive. It is apparently dispersed only by fragments (Figure 16-Figure 17) from elsewhere in Antarctica.

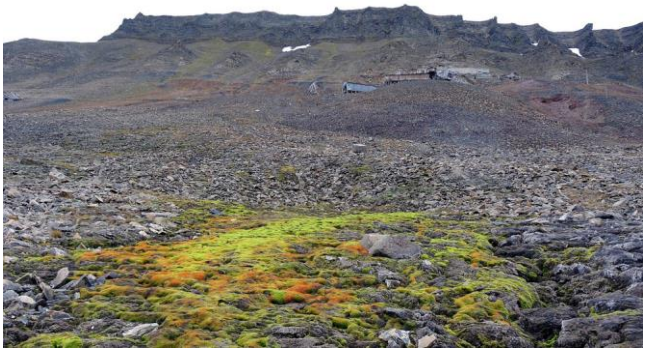

Figure 14. ***Pohlia nutans*** below old mine on Svalbard (Arctic). Photo by Michael Lüth, with permission.

Figure 15. ***Pohlia nutans*** on Svalbard (Arctic), a species often spread by fragments. Photo by Michael Lüth, with permission.

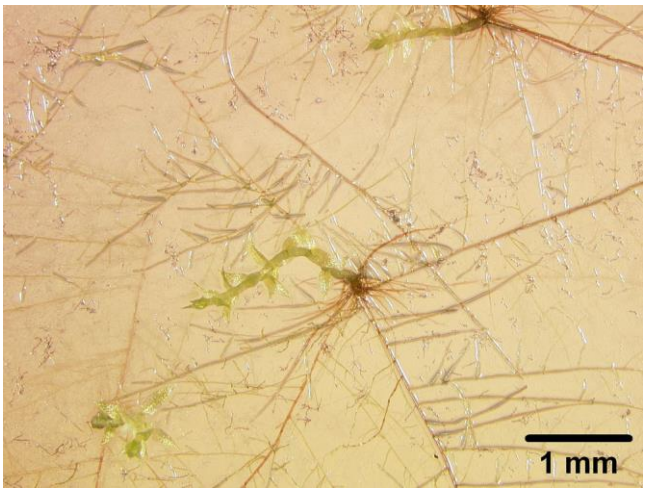

Figure 16. ***Pohlia nutans*** fragment and protonemata with buds and developing gametophores. Photo by Sean Robinson, with permission.

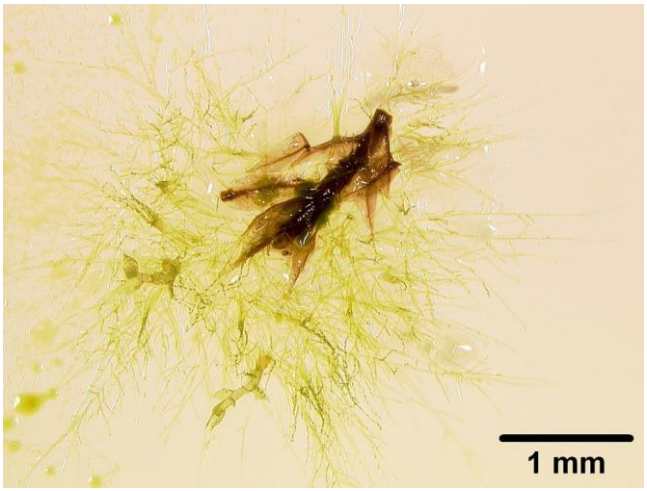

Figure 17. ***Pohlia nutans*** fragment and protonemata with buds and developing gametophores. Photo by Sean Robinson, with permission.

Figure 18. Fragments of ***Fontinalis dalecarlica*** caught in ice of a stream. Fragmentation appears to be its primary form of dispersal and new establishment. Photo by Janice Glime.

Streams and Other Aquatic Habitats

In flowing streams, sporophytes seem rare, and asexual propagules seem to be unimportant. However, significant dispersal can occur from fragments during spring runoff, and entire clumps as well as branches and smaller fragments become impinged on rocks and roots in the stream. The larger surface area of fragments makes it more likely that they will become lodged than will the small spores and asexual propagules. Glime *et al*. (1979) demonstrated that for ***Fontinalis duriaei*** these actually do become established in nature, occasionally even achieving upstream movement (by feet of bears?).

For aquatic mosses and liverworts, fragmentation may be the only means of reproduction for many years before appropriate conditions exist for completing sexual reproduction. In dioicous mosses such as ***Fontinalis***, sexual reproduction seems to be so infrequent as to be totally ineffective as a means of providing dispersal units (spores), whereas fragments are numerous during times of ice melt and high water (Figure 18; Conboy & Glime 1971, Glime *et al.* 1979, Glime & Knoop 1986). Even when spores are produced in this genus, the spore faces numerous challenges in becoming located where its subsequent protonema will neither be washed away nor desiccated, and sufficient light will be available for development. Since there is no documentation of the occurrence of any protonema of any ***Fontinalis*** species in the field, we can only conjecture about the success of reproduction by spores in this genus.

Dedifferentiation

Dedifferentiation is the process involved in the return of a cell to its embryonic state (Figure 19). It is necessary before a mature cell can form into a different kind of cell, or into a protonema, permitting the development of new plants from fragments. In bryophytes, virtually all cells seem to have the ability to undergo dedifferentiation once they have been isolated from the intact plant (Giles 1971). This is not the case for cells such as xylem elements of tracheophytes, which no longer have protoplasm and hence are non-living.

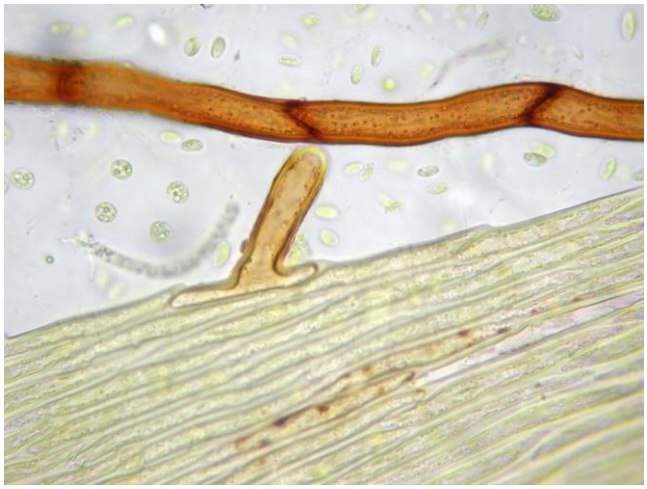

Figure 19. ***Warnstorfia fluitans*** leaf fragment with rhizoid that has dedifferentiated and redifferentiated into a different kind of cell. Photo by Heike Hofmann © swissbryophytes <swissbryophytes.ch>, with permission.

Moss fragments seem to retain their polarity, resulting in protonemata at the apical end and rhizoids at the basal end, but inverting them causes the base to act as the apex and vice versa (Westerdijk 1907), suggesting a gravimetric response by some growth factor. Mosses tend to have more regenerative ability at the base of the gametophyte than at the apex. Their sporophytes, however, are strongly polar in regeneration (von Wettstein 1924). Liverworts, on the other hand, seem to be much more strongly polar, and new growth is nearly restricted to the apical end of the gametophytes, but the sporophyte seems to lack polarity (Giles 1971). This strong polarity of the liverwort gametophyte regeneration, however, decreases with tissue age (Kreh 1909).

Earliest known reports on regeneration from bryophyte fragments come from Necker in 1774 (Giles 1971). Kreh (1909) showed that for liverworts, every part of the plant except the antheridia could regenerate. Nevertheless, few reports of liverwort regeneration from fragments are known. In mosses, even the seta will regenerate into a protonema, forming diploid gametophytes (von Wettstein 1924).

It is common for the nuclei to increase in size in dedifferentiating cells (Giles 1971). The dedifferentiation process involves a sort of "budding" of the chloroplasts and mitochondria, producing more of these organelles. At the same time, nucleolar volume increases only in regenerating cells. We now understand that the nucleolus is not an organelle in its own right, but rather that it is the site of extensive protein synthesis, hence staining more densely. This is an indication of building activity in the regenerating cell.

In ***Campylopus pyriformis*** (Figure 20) fragments, it is the chloronema that gives rise to buds, with no caulonema forming. By contrast, and unlike the growth from a spore, the caulonema of ***Plagiomnium affine*** (Figure 21) grows nearest the plant fragment and the chloronema is the farthest and youngest tissue (Sironval 1947; Bopp 1959a,b; Giles 1971). The ensuing buds develop, therefore, nearest the leaf fragment from the caulonema. Up to 100 secondary protonemata may originate from the dedifferentiated leaf cells of a single leaf in this species.

Figure 20. ***Campylopus pyriformis*** showing fragments of branch tips. Photo by Michael Lüth, with permission.

Figure 21. ***Plagiomnium affine***, a moss that develops protonemata from fragments. Photo by Janice Glime.

Secondary Protonemata from Fragments

Secondary protonemata are those produced from mature tissues that have been damaged or cut. Hence, these protonemata develop on fragments. At first thought, one might expect that these would behave in the same way as primary protonemata (produced from a spore), but further consideration should remind us that fragments provide a large store of nutrients, including energy sources, from the plant fragment.

Like primary protonemata, the secondary protonemata of the moss ***Tortula modica*** is negatively gravitropic in the dark (Ripetskyj *et al*. 1999). When placed in the light, the apical parts of the protonemata begin to branch and apical cells of side branches and main protonemal filaments frequently differentiate as buds. One might consider this event as being possible because of the energy sources available from the fragment. When the fragments were illuminated from below, an intensity of at least 4.5 μmol m-2 s-1 was necessary to induce phototropism and light-directed development of branch buds and directed growth of side branches. In lower light intensities the apical cells grew away from the light (*i.e.*, away from gravity as well).

To further understand the role of spore grown vs secondary protonemata, Wagner and Sack (1998) grew the moss ***Ceratodon purpureus*** from protoplasts. In these protoplasts, the emerging filament was mostly gravimorphic, with more than 66% of the filaments emerging above the horizontal. The tip-growing cells of these filaments began to exhibit a gravitropic response within 1-2 cell divisions. But in these filaments, plastid sedimentation did not occur, contrasting with dark-grown filaments.

Gravity Effects

As we might expect, based on studies on protonemata, secondary protonemata also respond to gravity. In ***Tortula modica***, the secondary protonemata are negatively gravitropic in the dark (Ripetskyj *et al*. 1999). In the light, these protonemata branch near the apical cells and these branch tips typically differentiate as buds. A light intensity of at least 4.5 μmol m^{-2} s^{-1} was required to induce phototropism in apical cells, cause light-directed initiation of branch primordia, and direct development of side branches and bud initiation. At lower light intensities, the apical cells grew away from light (*i.e.* negatively gravitropic).

Callose Formation

Scherp *et al*. (2001) documented the formation of callose in tissue fragments in all groups of multicellular photosynthetic organisms, including bryophytes. They found that in bryophytes and other multicellular green plants, callose is a regular component of the developing septa in juvenile cells during cytokinesis. Wound callose did not occur in cells that already had callose in the newly formed septa.

Establishment

It appears that fragments may survive better in water than spores, thus providing an additional means of long-distance dispersal. Dalen and Söderström (1999) tested

five species of mostly terrestrial mosses and found that in all five taxa, regeneration frequency of fragments was lower than that of spores, but that fragments survived as well in water as they did dry, whereas spores did not.

Light quality and intensity may be influential in success of fragments. Dagar and coworkers (1980) found that for the thallose liverwort ***Riccia discolor*** regeneration is best in diffused light. Red light can induce regeneration; far-red inhibits it (Giles & von Maltzahn 1967, 1968). There is evidence the red/far-red system may affect the "budding" or division of the chloroplasts (Hahn & Miller 1966), and its reversibility suggests that phytochrome may be active during the process. Little else seems to be known about light effects specifically on fragments, so these phenomena may be restricted to certain taxa or habitats.

When dispersal occurs over long distances, it is quite likely that only one gender will arrive, making its survival dependent on asexual means. As discussed elsewhere, fragments seem to provide the easiest means by which bryophytes can be propagated for gardens, so one should expect that nature makes widespread use of this ability as well. When a plant is damaged, the damaged surface will often produce protonemata and/or rhizoids (LaRue 1942) and subsequently develop a new leafy gametophore. In other cases, the new plant may develop directly with no protonemal intermediary, as in the leafy liverwort ***Scapania undulata*** (Figure 22) that developed from a leaf fragment (Figure 23; Glime 1970).

Figure 22. ***Scapania undulata*** growing in its streamside, wet habitat. Photo by Michael Lüth, with permission.

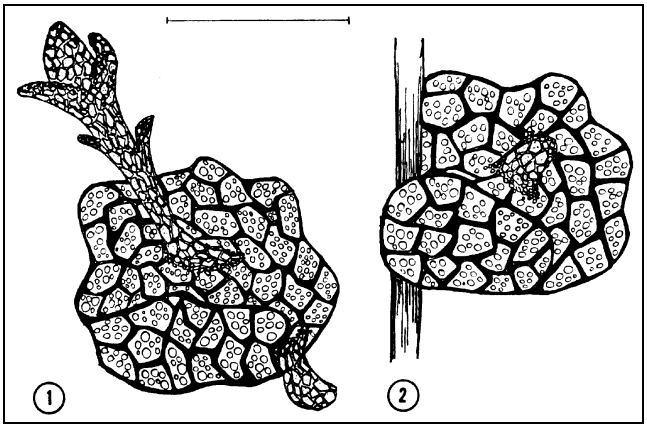

Figure 23. ***Scapania undulata***, leafy liverwort known to reproduce from fragments. **1:** Young plant growing from detached leaf. **2.** Bud of young plant growing on leaf still attached to living stem. Drawings by Flora Mace.

It is the parent plant that determines which of these will develop – chloronema, caulonema, or rhizoids. Knoop (1984) tells us that small explants result in reversion to an early developmental stage wherein single leaf cells behave like spores and form chloronemata. On the other hand, large fragments revert back only to caulonemata, or go directly to buds and gametophore plants. Furthermore, apical leaves regenerate more easily than basal leaves (Gay 1971). It is puzzling that in ***Plagiomnium undulatum*** (Figure 24), basal leaves regenerate only from the lamina, whereas apical ones regenerate only from the costa (Schröder *et al.* 1970).

Figure 24. ***Plagiomnium undulatum***; basal leaves regenerate from the lamina, apical cells from the costa. Photo by Janice Glime.

Mishler and Newton (1988) demonstrated that fragments can require conditions opposite to those required for spores. In their study, ***Syntrichia princeps*** (Figure 25) fragments were slightly more successful when they experienced periodic drying, whereas the spores required continuously hydrated conditions. With such requirements, it is easy to understand why fragments are more successful on rocks and sand than are spores. Mishler and Newton attribute this success to the ability of fragments to produce a protonemal mat and new shoots much more rapidly than could spores. Furthermore, as mentioned earlier, the existing plants exhibited a much stronger inhibitory effect on the spores than on the fragments.

Figure 25. ***Syntrichia princeps***, a moss whose fragments fare better with periodic drying. Photo by Jan-Peter Frahm, with permission.

On the other hand, both spores and fragments can be inhibited by the presence of mature plants (Mishler & Newton 1988). ***Dicranum*** (Figure 1, Figure 3) seemed to be more inhibitory that ***Syntrichia princeps*** (Figure 25), perhaps relating to the dry habitat of the latter. They considered that at least some of this inhibition was due to chemical exudates.

In an aquatic habitat, Florschütz and coworkers (1972) found that fragments of ***Fissidens crassipes*** (Figure 26) produced caulonemata, this time on moist bricks. This ability permitted them to spread rapidly in response to a rise in water temperature.

Figure 26. ***Fissidens crassipes*** growing on rock. Photo by Michael Lüth, with permission.

Regeneration often occurs from small leaf fragments that have begun to decay. This could be an indication that an inhibitor has been lost, or some colonizing microorganism could be providing a hormonal signal that starts the development. When growing ***Leucolejeunea clypeata*** on Ca-free media, Geldreich (1948) discovered that only contaminated leaves of Ca-deficient plants produced regenerants. It was only mature or old and necrotic leaves that regenerated, and these Ca-deficient leaves had oil bodies that were characteristic of old, senescent leaves. Since the contaminating microorganisms were typical of soil flora, and regenerants of this species are known in nature (Fulford 1947), perhaps the microorganisms do indeed play a role in providing the necessary stimulus.

Liverworts rarely regenerate from fragments. Occasionally a leaf may produce a new plant, as for example that of ***Scapania undulata*** (Figure 22-Figure 23), an aquatic leafy liverwort mentioned earlier (Glime 1970). Could it be that liverworts dry out too rapidly and cells lose their viability before new plants can arise? Would this explain the accomplishment of this aquatic species?

Growth Regulators

Like all other developmental processes, hormones and other growth regulators influence the developmental pathway of fragments. Patidar and coworkers (1987) found that 0.03 ppm gibberellin can stimulate regeneration in the thallose liverwort ***Targionia hypophylla*** (Figure 27). Concentration is of course important; at 0.1 ppm gibberellin is inhibitory to ***T. hypophylla***.

Figure 27. ***Targionia hypophylla***. Photo by Michael Lüth, with permission.

Few studies seem to have centered specifically on growth regulators of fragments, yet many in vitro studies are actually studies of fragments, particularly those of pleurocarpous mosses. Presumably, the same growth regulation applies to fragments as to the intact plants covered earlier. Yet, literature on the wound response seems to be lacking, as is literature on the remarkable ability of some fragments to persist under extremely stressful conditions. For example, we have grown ***Fontinalis flaccida*** from specimens dried for three months under herbarium conditions (ca. 30% relative humidity). In another case, ***Fontinalis novae-angliae*** that had been boiled for about 12 hours daily for two weeks developed new leaves on one portion of the remaining stem when it was returned to its native stream (Glime & Carr 1974). And what permits a partially decayed stem to suddenly spring forth a new plant after it has been uncovered from many years of burial (During *et al*. 1987)?

Using the aquatic moss ***Palustriella decipiens*** (Figure 28-Figure 29), Ahmed and Lee (2010) experimented with a wide range of IAA and kinetin concentrations on fragments. They found that protonemal gemma production varied with concentration, but was best at 10^{-8} M IAA and kinetin. Higher concentrations caused the gemmae to become brown. Low concentrations of IAA and kinetin induced bud formation.

Figure 28. ***Palustriella decipiens***, an aquatic moss that regenerates from fragments and protonemata of those fragments respond to applications of IAA + kinetin to produce buds. Photo by Michael Lüth, with permission.

Figure 29. ***Palustriella decipiens*** protonemata with gemmae, produced at 10^{-8} M kinetin. Photo by Ahmed and Lee, with permission.

Animal Dispersal

Dispersal by animals is scarcely known in the bryophytes. Yet, we must suppose that the various activities of animals contribute to bryophyte movement. Various aquatic insects, especially Trichoptera (caddis flies), use mosses or liverworts in their cases, so the insect will carry the bits around wherever it goes. When drift carries the insect downstream, the moss goes too, and if the insect crawls upstream in the quiet interface at the bottom, the moss comes along. Lacewings [***Leucochrysa*** (***Nodita***) ***pavida***] carry viable bryophytes (and lichens) on their backs as camouflage (Slocum & Lawrey 1976).

Bears, beaver, and other animals can get mosses tangled among their toes and carry them for miles. Birds carry them off to build nests. I have even concluded that the turtle in my garden room was responsible for the distribution of ***Conocephalum conicum*** (Figure 30) all over the room from the single spot where it had been planted. When the turtle died, the spread of the liverwort stopped. In a field experiment, I found fragments of tagged ***Fontinalis duriaei*** (Figure 31-Figure 32) upstream from their initial location, a movement that could only have been effected by animals such as bears or humans.

Figure 30. ***Conocephalum conicum*** showing evidence of herbivory (arrows) that could lead to dispersal of fragments. Photo by Janice Glime.

Figure 31. ***Fontinalis duriaei*** held by Janice Glime, demonstrating how easily mosses might be dispersed by flowing water and trapped by branches and roots in the water. Photo by Zen Iwatsuki, with permission.

Figure 32. ***Fontinalis duriaei*** fragment. Photo by Janice Glime.

It is likely that rodents contribute to dispersal, although they may do more harm than good. I have watched chipmunks run across my moss garden and kick up clumps as they ran. Nancy Ironsides (Bryonet 10 June 2011) found rhizoids on the apical leaves of ***Leucobryum glaucum*** (Figure 33) and attributed these to disturbance by animals. Pénzes Kónya (2003) considered "big wild animals" to be major dispersers of ***Leucobryum juniperoideum*** (Figure 34) during dry periods. The caducous leaves function as gemmae by producing rhizoids (Figure 35) and forming new plants, especially during the rainy spring, but the disturbance of dry mosses seems to outpace the regeneration from disturbed plants.

Others may spread bryophytes as they eat them (Slack 1936, Mutch & Pritchard 1984), particularly if they only digest the surface organisms and return the moss fragments with their feces. Suren and Winterbourn (1991) found that 14 aquatic invertebrate taxa had bryophyte fragments in their guts, and two tipulid larvae regularly consumed bryophytes. I tested the hypothesis that rainbow trout, known to strike at anything, could serve as dispersal agents by eating the aquatic ***Fontinalis duriaei*** (Figure 31-Figure 32). However, the fish could not be tempted to strike at or eat the moss, even when it housed numerous aquatic insects. Finally, we force fed the fish. The moss was

delivered back as feces in a neat, cylindrical package with bright green moss (Figure 36). At last it seemed we had demonstrated a potential upstream dispersal mechanism! But, alas, we were surprised the following day to find that the moss had lost all its color, even though it was maintained in a gallon jar of its own stream water at a cool temperature. It does not appear that rainbow trout are likely dispersal vectors after all!

Figure 36. Feces of rainbow trout consisting primarily of ***Fontinalis duriaei*** as a result of force-feeding. Photo by Janice Glime.

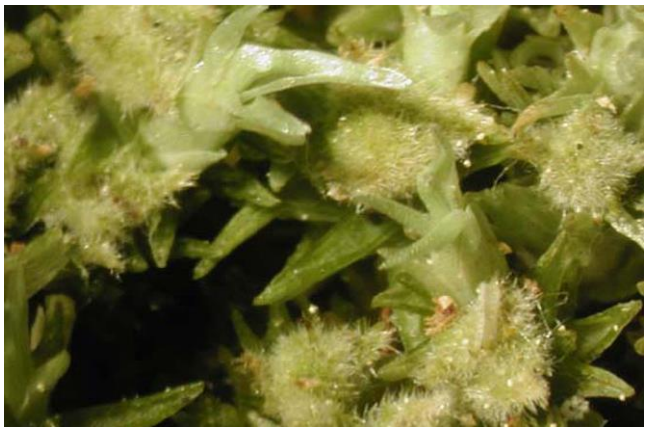

Figure 33. ***Leucobryum glaucum*** with apical rhizoids on leaves. Photo by Nancy Ironsides, with permission.

I have watched larvae of the **Rhyphidae** dipteran eat wet, dirty (most likely with diatoms) mosses and observed fragments of green moss come out the other end, clean. These fragments would be ideal propagules, although not dispersed very far, but I did not culture them to see if they met the same fate as the trout package.

Further discussion of bryophyte fragment dispersal is in the adaptations subchapter on dispersal.

Figure 34. ***Leucobryum juniperoideum*** cushions. Photo by Michael Lüth, with permission.

Figure 35. ***Leucobryum juniperoideum*** with leaf rhizoids after overturn by cattle. Photo courtesy of Erika Pénzes-Kónya.

Summary

Fragmentation results from random breakage or from genetically programmed cleavage areas on leaves, buds, or stems. For perennial mosses, especially pleurocarpous mosses, it is typically the primary means of spreading. Arctic/alpine and aquatic habitats may rely primarily on this type of reproduction. Fragments are more likely to become established than spores and survive better in water than do spores. Their establishment can be inhibited by the presence of mature plants, but they have a greater competitive ability than spores.

Regeneration from mature cells requires **dedifferentiation** and may begin as protonemata, rhizoids, or both. Light quality and intensity may play a role in early development. Little is known about growth regulation, but gibberellin can stimulate regeneration in at least some bryophytes. Dispersal can be accomplished by wind, water, and animals, sometimes because the animal transports the bryophyte for use in a nest or house.

Acknowledgments

Inspiration for this chapter evolved from discussions with Dr. Martin Bopp and especially with Dr. Gert Steen Mogensen. I appreciate the many suggestions from a student's perspective by Medora Burke-Scoll and KT McConnell. Nancy Ironsides introduced me to the rhizoids on *Leucobryum* leaves and provided images; Noris Salazar Allen and Erika Pérez-Kónya helped me to piece together the story.

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CHAPTER 5-7
ECOPHYSIOLOGY OF DEVELOPMENT: BROOD BODIES

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CHAPTER 5-7

ECOPHYSIOLOGY OF DEVELOPMENT: BROOD BODIES

Figure 1. ***Syntrichia laevipila*** (=*Tortula pagorum*), an acrocarpous moss with terminal gemmae. Photo by Michael Lüth, with permission.

Introduction

Ecology is a field of interconnections. Hence, writing any chapter brings with it many choices about where to include information. This chapter is in part redundant with the chapters on dispersal because an understanding of propagules was necessary to complete the dispersal story. That chapter emphasized travelling about and the environmental factors that influenced the success of that travel. This chapter emphasizes the physiology, but for clarity there is considerable overlap in what one must understand. The chapter is written to be independent so that one can read it without having to read the earlier chapter in order for it to make sense.

Definitions

Imura and Iwatsuki (1990) defined **propagules** as vegetative **diaspores** that have an apical cell and can grow directly into leafy shoots. However, most diaspores produce a protonema. **Gemmae**, by their definition, are vegetative diaspores that lack an apical cell and in which a protonema precedes development of a leafy shoot (Figure 2, Figure 37). While this is a clean separation, it is not always practical to determine the germination pattern, and multicellular gemmae may be construed as propagules. In the multilingual glossary for bryology (Magill 1990), **propagule** (Figure 3-Figure 4) is defined in a more practical way as a reduced bud, branch, or leaf serving in reproduction. This does not imply absence of a protonema, and indeed, there often is one. **Diaspore** is given as a synonym. **Gemmae** (Figure 2) are distinguished as uni- or multicellular, filamentous, globose, ellipsoidal, cylindrical, stellate, or discoid brood bodies, **relatively undifferentiated**, serving in vegetative reproduction. In other words, they are specialized structures. **Brood body** is the more inclusive category, including both propagules and gemmae. These are genetically identical to their parents, thus producing clones (Laaka-Lindberg 2000). Bryophytes are the only group of plants with any sort of gametophytic brood body (Wyatt 1994).

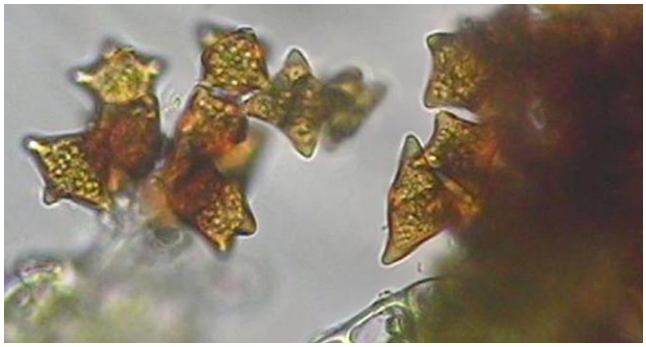
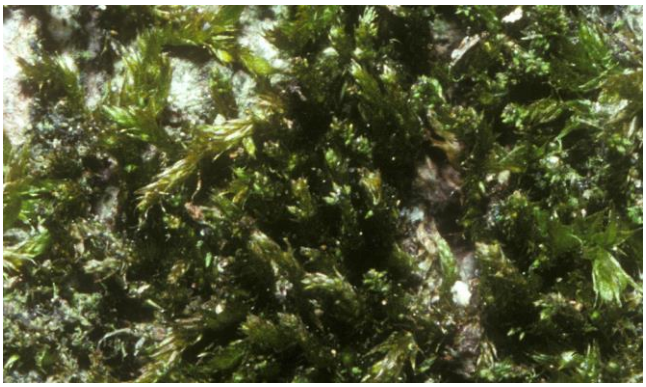


Figure 2. Leaf gemmae of ***Lophozia bicrenata***, a leafy liverwort. Photo by Paul Davison, University of North Alabama, with permission.

Figure 3. ***Leskeella nervosa*** with bulbils at leaf bases. Photo by Michael Lüth, with permission.

Figure 4. ***Bryum gemmilucens*** showing axillary propagules. Photo by Michael Lüth, with permission.

The evolutionary pathway has capitalized on success of fragments by selecting more and more specialized fragments. Mosses such as ***Leskeella nervosa*** (Figure 3), ***Platygyrium repens*** (Figure 5), ***Dicranum flagellare*** (Figure 6), and ***Bryum argenteum*** (Figure 7-Figure 8), to name a few, have special shoots that easily break off and disperse. This explains why ***Bryum argenteum*** is so common along paths in open areas such as cemeteries and roadsides. Each step of a boot carries tiny branches from the parent plants to a new location. To demonstrate its remarkable dispersal success, Clare and Terry (1960) prepared bare soil, then used a matchbook to "walk" on ***Bryum argenteum*** (Figure 7-Figure 8). They then "walked" on the bare soil with the same matchbook. As a control, they "walked" on a different part of the prepared soil with a different matchbook. True to its natural success, the ***Bryum argenteum*** grew well where the matchbook had previously walked on the moss, but did not appear on the control area.



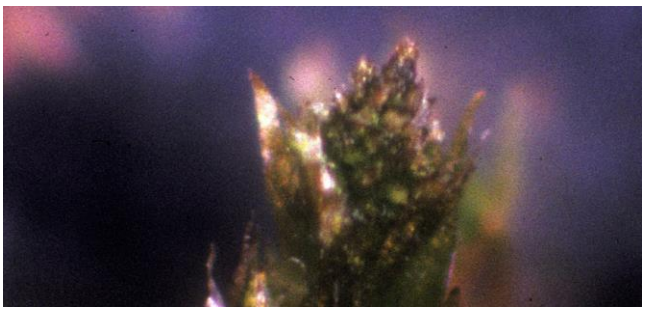


Figure 5. ***Platygyrium repens Platygyrium repens*** with bulbils crowded at branch tips. Photos by Janice Glime.

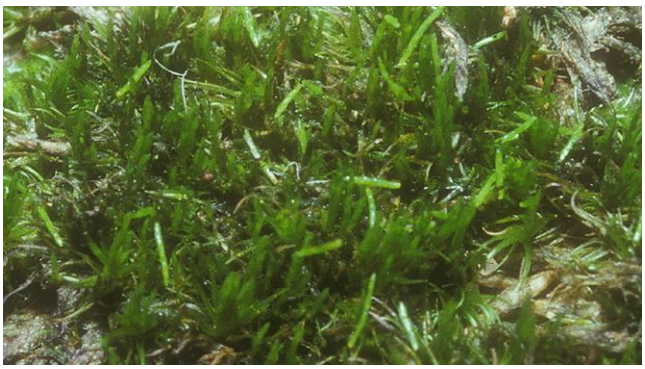


Figure 6. ***Dicranum flagellare*** with deciduous flagelliform branches. Photo by Janice Glime.

Figure 7. ***Bryum argenteum*** showing bulbous tips that break off easily to form new plants. Photo by Michael Lüth.

Figure 8. ***Bryum argenteum*** showing bulbous tips that break off easily to form new plants. Photo by Michael Lüth, with permission.

Imura (1994) recognized specialized vegetative reproductive structures in 186 species (15.7%) of Japanese mosses, including deciduous shoot apices, caducous branchlets, bulbils, flagella, rhizoidal tubers, gemmae, caducous leaves, and endogenous gemmae. He considered these to be adaptations to the dioicous habit (*e.g.* ***Syntrichia laevipila***, Figure 1) and unstable habitat conditions.

Brood Bodies

Brood bodies are a specialized means of asexual reproduction that permit plants to propagate and disperse, often when conditions are unfavorable in the present location. Perhaps this is why, among dioicous mosses, they are more common on upright mosses (Figure 4), where there is some hope of falling away from the parent plant, rather than landing within a mat that keeps them where they started. Herben (1994) claims that reproductive processes, including brood bodies, are crucial for between-habitat dispersal. Those mosses in the British flora that inhabit small patches and unstable habitats are more likely to have vegetative brood bodies. But shoot density also can determine the number of brood bodies. Kimmerer (1991a) found that low-density populations of ***Tetraphis pellucida*** (Figure 9) were more likely to reproduce asexually by gemmae, whereas greater density increased incidence of sexual reproduction and subsequent spores. She (1991b) found that most **gemmae** landed within 10 cm of the colony, whereas spores travelled as far as 2 m. [Brodie (1951) considered that ***T. pellucida*** was too delicate to benefit much from splashing by raindrops, perhaps accounting for the much shorter dispersal distance compared to that of sperm in ***Polytrichum*** of up to 60 cm.] The asexual strategy permits mosses to colonize an area rapidly by gemmae, then move on by spores when space is saturated. Kimmerer (1991a) felt this was of particular importance in unstable environments such as rotting stumps where ***T. pellucida*** commonly occurs. On the other hand, ability to "move" by gemmae provides an opportunity to seek a mate when stranded in a single-sex clone.

Chrobak and Sharp (1955) showed that scales from the deciduous flagelliform branches of ***Dicranum flagellare*** (Figure 6) were more likely to form protonemata than whole leaves or their proximal or distal halves (Figure 10).

Figure 9. ***Tetraphis pellucida*** with terminal gemma cups, the only moss with gemma splash cups. Upper photo by Janice Glime, lower by Paul Davison, University of North Alabama, with permission.

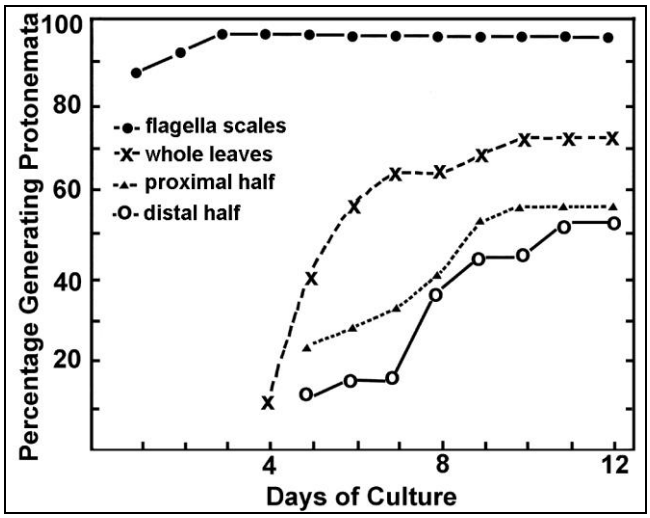


Figure 10. Success of producing protonemata from various leaf parts of ***Dicranum flagellare*** compared to that of the scales on the flagelliform brood branches. Redrawn from Chrobak & Sharp (1955).

Even in the ***Sphagnum***-dominated peatlands, dispersal by gemmae is an advantage in regeneration. While ***Sphagnum*** must wait for recolonization by spores that often have poor success on the acid peatland substrate with its low nutrient quality, ***Aulacomnium palustre*** (Figure 11) can colonize rapidly from gemmae that have survived the disturbance (Li & Vitt 1994). Furthermore, perhaps again due to the more advanced state of the propagula, *A. palustre* had a much wider tolerance range for nutrient concentrations, being the only species not inhibited by N inputs. ***Sphagnum angustifolium*** (Figure 12), ***S.***

magellanicum (Figure 13), and ***Polytrichum strictum*** (Figure 14) all had poor regenerative ability.

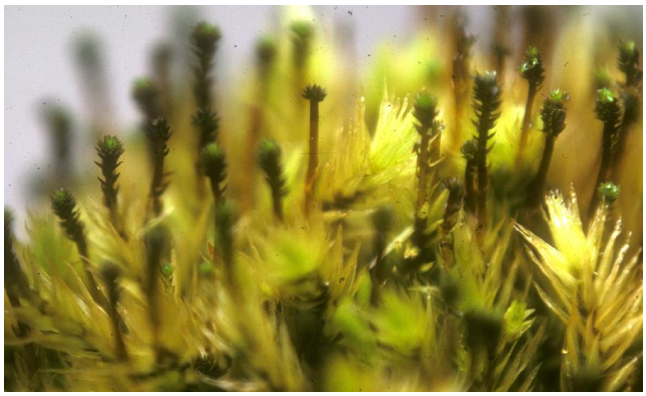


Figure 11. ***Aulacomnium palustre*** showing special extension of the stem with gemmae. Photo by Zen Iwatsuki, with permission.

Figure 12. ***Sphagnum angustifolium***, a species that does not regenerate well. Photo by Michael Lüth, with permission.

Figure 13. ***Sphagnum magellanicum***, a species that has poor regenerative ability. Photo by Michael Lüth, with permission.

Figure 14. ***Polytrichum strictum***, a species with poor regenerative ability. Photo by Des Callaghan, with permission.

It is reasonable then, that certain habitat conditions might favor the **apogamous** (condition of producing sporophytes without union of gametes) or **aposporous** (producing gametophyte from sporophyte tissue without meiosis) reproduction of bryophytes. Chopra (1988) was able to increase apogamy by reducing water or light levels and by raising the sugar concentrations in the growth medium. Likewise, low hormone concentrations favored apogamy. Not surprisingly, this plasticity was correlated with a high chromosome number (suggesting polyploidy) and genetic variation. Apospory, on the other hand, was favored by the opposite conditions: suitable temperature and light, sufficient humidity, and lack of sugar in the medium. It was furthermore stimulated by wounding and the removal of apical dominance.

In the leafy liverwort ***Odontoschisma denudatum*** (Figure 15-Figure 16), gemmae are produced in branched chains on the leaf margins (Duckett & Ligrone 1995). The initial cells of these gemmae are distinguished by forming a protrusion that contains a large central nucleus, small vacuoles, starch-free chloroplasts, and scattered cytoplasmic lipid droplets. Unlike other leaf cells, they lack oil bodies. However, as the gemmiferous filaments develop, oil bodies arise. These are closely associated with the cytoplasmic lipid bodies. These bodies swell rapidly, quickly reaching their final diameter. As the gemmae mature, the walls become dense and may account for their extreme water repellence. This repellant surface could permit them to be dispersed on the surface of a water film or in the air.

Figure 15. ***Odontoschisma denudatum***, a species with apical gemmae. Photo by Michael Lüth, with permission.

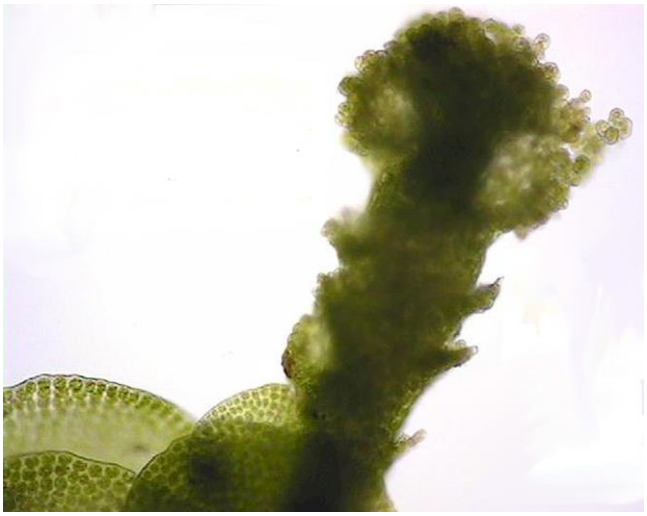


Figure 16. ***Odontoschisma denudatum*** showing apical gemmae. Photo by Paul Davison, with permission.

The germination and development of gemmae in the tropical moss ***Calymperes*** have been described (Egunyomi & Olarinmoye 1983; Duckett & Ligrone 1991).

Tubers

Tubers are defined very differently in mosses and liverworts (Magill 1990). In liverworts, they are extensions from the growing apex, growing downward gravitropically, and serving as perennating structures during conditions unfavorable for growth. In mosses, they are gemmae formed on the rhizoids (Figure 17).

Figure 17. ***Bryum radiculosum*** rhizoids with tubers. Photo by Michael Lüth, with permission.

A number of moss species form tubers on their rhizoids (Arts 1987a; Table 1). Risse (1987) described these rhizoidal gemmae in 82 species of European mosses. They serve as asexual means of reproduction, although one must question just how they get dispersed. Perhaps earthworms and other forms of disturbance accomplish the task. However, in their study of plant diaspores from earthworm guts, van Tooren and During (1988) found few bryophytes that regenerated from tubers so obtained, although bryophytes emerged frequently from some samples by other means. They interpreted this as a low survival rate of vegetative diaspores in the earthworm digestive tract. Risse (1987) reported that mites disperse protonemal gemmae in ***Schistostega pennata*** (Figure 18).

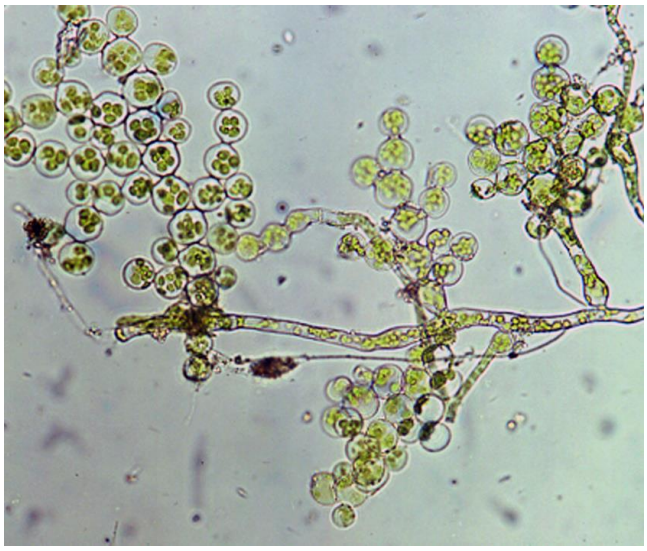


Figure 18. ***Schistostega pennata*** showing pinched off gemmae on the protonema. Photo by Irene Bisang, with permission.

These tubers are densely packed with lipid droplets or starch grains (Duckett & Pressel 2003). In ***Phaeoceros laevis*** (hornwort; Figure 19), tuber cells deposit protein into the cell vacuoles as the cells differentiate, forming abundant starch in their plastids and lipid droplets in the cytoplasm (Ligrone & Lopes 1989). Such rich storage contents suggest that they should germinate rapidly and produce new plants quickly, using their abundant food reserves. Using ***Haplodontium notarisii*** (Figure 20), Arts (1988a) showed that this moss did just that, germinating in two weeks, and several weeks later producing numerous upright gametophores to form a colony.

Figure 19. ***Phaeoceros laevis*** with sporophytes. Photo by Bob Klips, with permission.

Figure 20. ***Haplodontium notarisii***, a moss that stores dense starch in its tubers, permitting them to germinate and grow rapidly. Photo by Jan-Peter Frahm, with permission.

Such tubers provide a diaspore bank that can help to revegetate disturbed ground (During *et al*. 1987) and benefit from extended longevity. Arts (1989) has demonstrated that even in a state of desiccation in a herbarium, such tubers can survive and germinate after 10 years. Such a strategy is common among colonist species (During *et al*. 1987; Arts 1990a; Table 1), and seems to be confined among the mosses to acrocarpous species. This colonist connection suggests that perhaps they do not have to arrive, but are already there, much like buried seeds awaiting the day they once more arrive at the surface and receive light. During (1995) suggests that such colonist populations are maintained completely through occasional recruitment. He suggests that within extant populations there must be a density-dependent tuber mortality to regulate the population.

Development

There are more developmental pathways for propagules than there are kinds of propagules. Even within the same genus, Ligrone and coworkers (1996) found differences in the origins of the gemmae. In ***Tortula latifolia*** (Figure 21), gemmae develop on the upper leaf surface from single initial cells of both the lamina and the costa, whereas in ***Syntrichia*** (=***Tortula***) ***papillosa*** (Figure 22) they develop only on the costa. In both cases the old wall and cuticle of the cell initial rupture and a new, highly extensible wall replaces it. Subsequent divisions of this gemma **primordium** produce a 6-8-celled gemma. Mucilage develops around these gemmae and eventually the plasmodesmatal connections are severed, leaving only the mucilage to connect the gemmae to the leaf. Multiple gemmae may form in this way from the same initial and remain in a chain until the leaf becomes fully hydrated. Despite their disconnection from the parent leaf, these gemmae accumulate lipids, indicating that they are functionally photosynthetic.

Table 1. Examples of bryophytes with tubers reported in the literature.

Species	Reference
Archidium alternifolium	Arts 1990b
Archidium globiferum	Arts 1998
Atrichum crispum	Arts 1987d
Atrichum tenellum	Arts 1987d
Barbula cylindrica	Ellis & Smith 1983
Didymodon tophaceus	Side 1983
Bryum barnesii	Wilczek & Demaret 1980
Bryum bicolor	El-Saadawi & Zanaty 1990
Bryum bicolor	Risse 1993
Bryum cruegeri	Whitehouse 1978
Bryum dunense	Cortini Pedrotti & Aleffi 2001
Bryum veronense	Cortini Pedrotti & Aleffi 2001
Campylopus pyriformis	Arts 1986c
Chrysoblastella chilensis	Matteri 1984
Conocephalum conicum	Paton 1993
Cynodontium bruntonii	Arts 1990a
Didymodon nicholsonii	Arts 1987b
Discelium nudum	Side & Whitehouse 1987
Ditrichum difficile	Arts 1998
Ditrichum heteromallum	Deguchi & Matsui 1986
Ditrichum heteromallum	Risse 1985b
Ditrichum lineare	Matsui *et al*. 1985
Fissidens beckettii	Arts 1998
Fissidens cristatus	Arts 1986a
Funaria hygrometrica	El-Saadawi & Zanaty 1990
Haplodontium notarisii	Arts 1988a
Leptobryum pyriforme	Imura *et al*. 1992
Pleuridium acuminatum	Arts & Risse 1988
Pleuridium ecklonii	Arts 1998
Pleuridium nervosum	Arts 1998
Pohlia lutescens	Hart & Whitehouse 1978
Pohlia molanodon	Arts 1986b
Pottia bryoides	Arts 1987c
Pottia intermedia	Risse 1985a
Pottia lanceolata	Arts 1987c
Pottia truncata	Arts 1987c
Pseudocrossidium revolutum	Arts 1988b
Scopelophila cataractae	Arts 1988b

Figure 21. ***Tortula latifolia*** showing gemmae on costa and lamina. Photo by Michael Lüth, with permission.

Figure 22. ***Syntrichia*** (=***Tortula***) ***papillosa*** showing gemmae restricted to costa. Photos by Michael Lüth, with permission.

Lipids are commonly stored in brood bodies of mosses, including ***Aloina aloides*** var. ***ambigua*** (Figure 23), ***Pohlia annotina*** (Figure 24), ***Ephemerum serratum*** (Figure 25), ***Leptodictyum riparium*** (Figure 26), ***Weissia controversa*** (Figure 27) (Goode *et al*. 1993), and ***Splachnum ampullaceum*** (Figure 28) (Mallón *et al*. 2006). Due to the hydrophobic properties of lipids, large amounts can be stored, permitting these brood bodies to survive when the protonema or plant is damaged by desiccation. Such lipids are most common in long-lived propagules.

Figure 23. ***Aloina aloides***, a species with brood bodies that store lipids that help them survive desiccation. Photo from Proyecto Musgo, through Creative Commons.

Figure 24. ***Pohlia annotina*** with bulbils, a species that stores lipids in its brood bodies, permitting them to survive desiccation. Photo by Dick Haaksma, with permission.

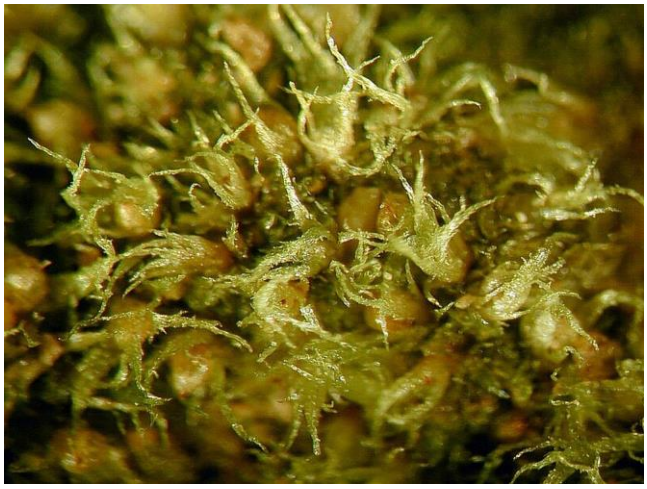


Figure 25. ***Ephemerum serratum*** with capsules. This species produces brood bodies that store lipids, a protection against desiccation. Photo by Michael Lüth, with permission.

Figure 26. ***Leptodictyum riparium***, a species that produces brood bodies that store lipids and survive when the moss dies from disturbance or desiccation. Photo by Tan Sze Wei, Aquamoss website <www.aquamoss.net>, with permission.

Figure 27. ***Weissia controversa*** with capsules. This species stores lipids in its brood bodies, permitting the to survive when the plants die of desiccation or disturbance. Photo by Michael Lüth, with permission.

Figure 28. ***Splachnum ampullaceum*** with capsules. This species stores lipids in its brood bodies, permitting them to survive desiccation. Photo by Michael Lüth, with permission.

Some gemmae can even produce more gemmae. In ***Bryoerythrophyllum campylocarpum*** (=***Hyophila crenulata***), the still-attached gemmae can germinate to produce more gemmae (Olarinmoye 1981).

Hormonal Effects

Hormones control every stage of development, but their role in gemma production and germination is not clear, or at the very least, differs among species.

Rawat and Chopra (1976) found that secondary protonemata of ***Bryum klinggraeffii*** (Figure 29) produce a diffusable substance when gemmae are produced. This induces gemma production on young protonemata that have not yet reached the critical size. Such a mechanism could insure maximum gemma production and greater survival if the initial stimulus for gemma production was indeed an unfavorable environment. The biggest advantage may be that it creates a colony that can reduce water loss.

Figure 29. ***Bryum klingraeffii***, a species in which protonemata produce a diffusable substance that stimulates gemma production on young protonemata. Photo by Des Callaghan, with permission.

Auxins

Stange (1971, 1977, 1983) suggested that gemmae require **auxin** transport from the parent plant, based on disruption of gemma differentiation in ***Riella helicophylla*** (Figure 30) when treated with an auxin antagonist. Contrasting with the auxin requirement suggested by Stange (1983) for ***Riella helicophylla*** gemmae, external auxins inhibit production of gemma cups in ***Marchantia palmata*** (Kumra & Chopra 1989). In ***Lunularia cruciata*** (Figure 31), auxins produced in the apical buds of the thalli inhibit the germination of the gemmae on the thallus (LaRue & Narayanaswami 1957).

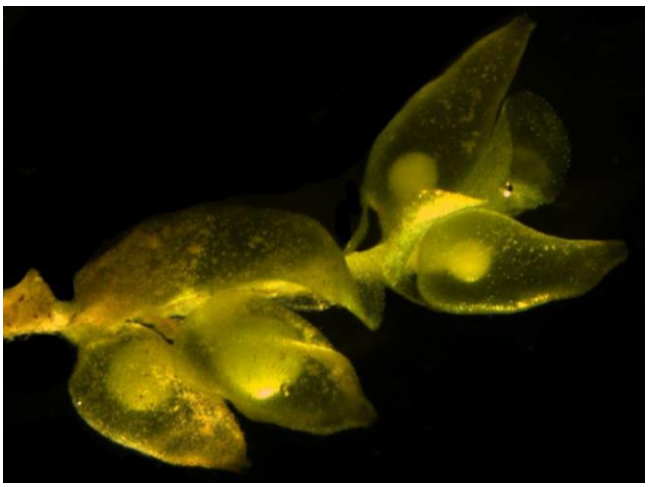


Figure 30. ***Riella helicophylla***, a liverwort that seems to require external auxins for gemma differentiation. Photo from NACICCA, through Creative Commons.

Figure 31. ***Lunularia cruciata*** showing ungerminated gemmae on the thallus. Photo by Martin Hutten, with permission.

However, when ethylene and IAA are applied together in cultures of ***Riella helicophylla*** (Figure 30), the combination has positive, additive effects on cell elongation of gemmae (Stange & Osborne 1988). On the other hand, gemmae generally fail to germinate while still on the parent thallus of ***Marchantia*** (Figure 44-Figure 45) species, suggesting that these two genera might, like roots and stems of tracheophytes, respond differently to the same hormones. Botanists have assumed that the inhibition of gemmae on the parent thallus is due to an inhibitory substance diffused from the parent. That inhibition can carry over to germination in the vicinity of the parent as well. Schneider and Sharp (1962) found that when gemmae of ***Tetraphis pellucida*** (Figure 32) were grown on culture media that previously had had mature plants, the germination was inhibited. This suggests some sort of hormone leakage, but probably not the gaseous ethylene.

Figure 32. ***Tetraphis pellucida*** with gemmae, a species where the gemmae are inhibited by the parents. Photo by Michael Lüth, with permission.

Marchantia polymorpha (Figure 44) exhibits apical dominance, resulting from polarity (Binns & Maravolo 1972). This can be attributed to the behavior of auxins. Binns and Maravolo found evidence that there is an endogenous, basipetal auxin gradient that is vital to normal growth. Interestingly, cytokinins can destroy the polarity by causing the auxin-synthesizing capacity to increase.

Since gemmae are such diverse structures, arising from protonemata, thallus, apical branches, leaf axils, and leaves, one might expect a variety of environmental and hormonal controls over their production. Naming the hormones would be pure speculation, but we know that IAA moves basipetally, hence accumulating downward. We also know that more ethylene is likely to be produced in the older part of the stem, and there is less air movement, resulting in more accumulation. Perhaps it is some interaction of these two hormones that results in the basal propagules, but why in some taxa and not others? Bulbils are apical in some taxa, such as ***Platygyrium repens*** (Figure 3), and gemma cups are apical in ***Tetraphis pellucida*** (Figure 32).

Cytokinins

We know that cytokinins are needed to stimulate bud production on protonemata, so early researchers experimented with cytokinin effects on gemma production on the protonema. Logic would suggest that if cytokinins stimulate buds, they might inhibit protonemal gemma production.

Rahbar and Chopra (1982) found that the usual substances did not induce buds in the moss ***Hyophila involuta*** (Figure 33). In fact, when the protonemata were grown on basal Knop's medium, auxins, gibberellic acid, abscisic acid, chelates, vitamin B_{12}, activated charcoal, coconut milk, and altered hydration, *p*H, temperature, and light intensity and duration all failed to induce buds. Rather, they found that added cytokinins could initiate multicellular protonemal gemmae. Chopra and Dhingra-Babbar (1984) found similar responses in the moss ***Trematodon brevicalyx***. Demonstrating the complexity of the bryophyte developmental system, Rahbar and Chopra (1982) demonstrated that for bud induction ***H. involuta*** required the interaction of IAA with kinetin or DMAAP.

Figure 33. ***Hyophila involuta***, a moss in which cytokinins can induce gemma production. Photo by Niels Klazenga, with permission.

Mehta (1990) further explored the role of kinetin on ***H. involuta*** (Figure 33) and was able to isolate a protonemal diffusate from those protonemata that had gemmae. These protonemata served as "nurse protonemata" by promoting the growth of nearby protonemata. He found that kinetin (10^{-5}-10^{-8} M) plus the protonemal diffusate acted synergistically on gemma formation. ABA (abscisic acid, 10^{-5}-10^{-7} M), on the other hand, was inhibitory, resulting in no gemma formation.

Unlike ***Hyophila involuta*** (Figure 33) in Knop's plus Nitsch's medium, ***Ptychostomum*** (=***Bryum***) ***capillare*** (Figure 34) produced gemmae in both solid and liquid Nitsch's basal medium (Sarla & Chopra 1989). When the medium was supplemented with kinetin or 2iP (bryokinin), the protonemata produced gemmae, whereas the cytokinin 6-benzylaminopurine (BAP) caused the formation of buds instead, while the 2iP inhibited the growth of the protonemata. Gemmae on media with kinetin or BAP regenerated, producing secondary protonemata, but these failed to produce gemmae or buds in response to kinetin. Hence, not all cytokinins are created equal – they may cause opposite responses.

Figure 34. ***Bryum capillare***, a moss that responds differently to different cytokinins, in some cases producing protonemal gemmae whereas in others they are inhibited. Photo by David T. Holyoak, with permission.

More recent work by Ahmed and Lee (2010) demonstrated that production of protonemal gemmae can vary with the concentration of IAA and kinetin in the moss ***Palustriella*** (=***Cratoneuron***) ***decipiens*** (Figure 35-Figure

36). In this species, kinetin influenced both gemma formation and gametophyte regeneration. Only low concentrations of IAA and kinetin (10^{-8}M) caused production of green, oval, mostly intercalary gemmae. Higher concentrations resulted in brown gemmae.

Figure 35. ***Palustriella decipiens***. Photo by Michael Lüth, with permission.

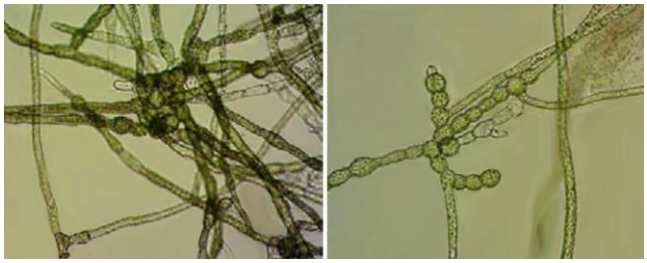


Figure 36. Effects of 10^{-8}M IAA (**left**) and 10^{-8}M kinetin (**right**) on gemma formation on protonemata of ***Palustriella decipiens***. Photos modified from Ahmed & Lee 2010.

But the role of cytokinin not only interacts to control production of gemmae, in ***Marchantia polymorpha*** (Figure 44) it inhibits the germination of the gemmae (Binns & Maravolo 1972). Could production of exogenous cytokinins be the factor that prevents germination of gemmae on the parent thallus?

Environmental Effects

For any plant system to be effective, it must be tuned to its environment. Propagules are no exception, being finely tuned to kick in when conditions favor their growth and development.

Temperature

For plants living outside the tropical regions, cold can inhibit growth and freezing may actually kill the tissues. Therefore, it is reasonable to expect that those species that survive have developed means to sense temperature conditions in both the production and germination of gemmae and to maximize these when conditions are best suited to continued growth.

In Arctic populations of ***Tetraphis pellucida*** (Figure 32), gemmae (Figure 37) have a broad range of germination conditions similar to those of the spores (Forman 1964). The broad 18-30°C range for gemma production sharply contrasts to sporophyte maturation requirements of -0.2 to 7.3°C, or 0-5°C in dark cultures. Such low temperature requirements account for the capsule maturation in spring. Gemmae, as for example gemmae of ***Aulacomnium heterostichum*** (Figure 38), which germinated after two years of storage in a freezer, seem to be able to persist as well as spores in cold conditions, and certainly better than some (Imura *et al*. 1991).

Light

Chopra and Rawat (1977) found that the response to temperature can be light dependent. In ***Bryum klinggraeffii*** (Figure 29) the initiation of secondary protonemata is correlated with protonemal age and growth. Although the gemmae of ***B. klinggraeffii*** are formed at or above 20°C in both light and dark, at 10-15°C in the light this species forms larger, lobed green structures and stunted gametophores. The addition of 1.0 ppm kinetin causes moruloid buds to differentiate on the protonemata, but at lower concentrations of kinetin, these protonemata produce gemma-like structures. This 1ppm concentration even inhibits previously formed gemmae from developing into gametophores, instead resulting in stunted gametophores. But in a sister species, ***Bryum coronatum*** (Figure 39), temperatures of 30°C in both light and dark induce the formation of protonemal gemmae that resemble the rhizoidal gemmae. In ***Leptobryum pyriforme*** (Figure 49), the gemmae develop on both the protonemata and gametophores in the dark. The short story is that for these species low temperatures and sufficient light results in energy being shifted to the development of gametophores. The conditions that favor gemma formation do not favor bud formation.

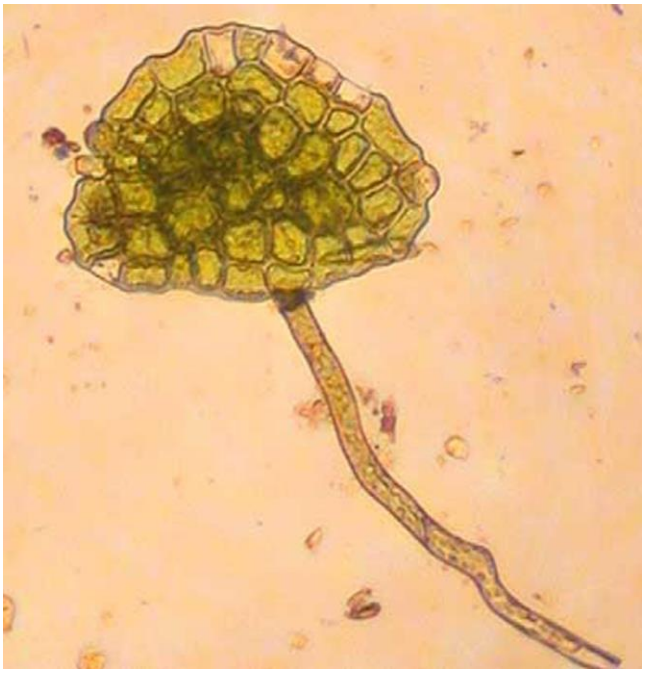


Figure 37. ***Tetraphis pellucida*** gemma showing germination and development of rhizoid. Photo with permission from Biology 321 Course Website at the University of British Columbia, Canada, with permission.

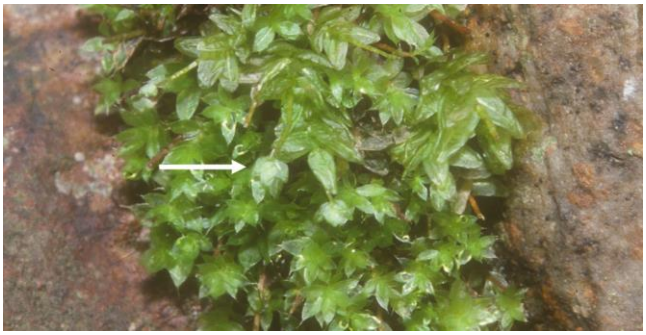


Figure 38. ***Aulacomnium heterostichum*** showing terminal gemmae (arrow). Photo by Janice Glime.

Figure 39. ***Bryum coronatum***, a moss that produces protonemal gemmae when the temperatures reach 30°C. Photo by Michael Lüth, with permission.

Whitehouse (1980) found that ***Schistostega pennata*** (Figure 18), ***Eucladium verticillatum*** (Figure 40), ***Gyroweisia tenuis*** (Figure 41), and ***Saelania glaucescens*** (as ***Didymodon trifarius***; Figure 42) all produce protonemal gemmae at low light intensities, but not at higher ones. These species can all grow in rock crevices, and such a mechanism might permit them to "try again" by dispersing if they germinate in a crevice that is too dark to complete the life cycle. A similar low-light response causes many protonemata to produce aerial shoots that break off and presumably serve as propagules (Whitehouse 1980). Similarly, in ***Marchantia palmata***, maximum production of gemma cups is attained when the plants receive continuous light at 4500 lux (Kumra & Chopra 1989). Full sunlight is about 70,000 lux. In ***Marchantia nepalensis***, having only 50-100 lux inhibits the production of gemma cups (Chopra & Sood 1970).

Figure 40. ***Eucladium verticillatum*** (Whorled Tufa-moss) with mite. This moss responds to low light intensities by producing protonemal gemmae. Photo by Barry Stewart, with permission.

Figure 41. ***Gyroweisia tenuis***, a moss in which the protonemata produce protonemal gemmae in low light. Photo by Michael Lüth, with permission.

Figure 42. ***Saelania glaucescens***, a moss that responds to low light by producing protonemal gemmae. Photo by Michael Lüth, with permission.

Hence, we might surmise that photoperiod plays a role in gemma production. Lockwood (1975), working with the leafy liverwort ***Cephalozia media***, found that the magnitude of the normal reproductive response could be significantly stimulated or inhibited by low concentrations of certain amino acids or kinetin. Certain metabolites (10^{-6}M arginine, cysteine, tryptophan plus kinetin) could overcome photoperiodic control of the reproductive response. Generally, organic compounds which stimulated asexual reproductivity under short photoperiod inhibited sexual reproductivity under long photoperiod.

Germination of gemmae and other propagula is likewise affected by light. In ***Philonotis hastata*** (Figure 43), the greatest germination rate for brood branches was around 750 lux, with percentage germinating decreasing in both directions from that figure (Egunyumi 1981). Such a low optimum would permit these propagula to germinate in the presence of tracheophyte ground flora where light is often minimal. In ***P. hastata***, elongation of the propagules occurs on older, basal parts of the stem, and these are the most mature, becoming partially detached. However, both young and old brood branches will form new plants from any part. These are able to germinate in both low and high light, but in high light they typically fail to complete development of gametophores.

Figure 43. ***Philonotis hastata***. This wet habitat moss has its greatest gemma germination at around 750 lux. Photo by Jan-Peter Frahm, with permission.

Otto and Halbsguth (1976) found that rhizoid induction on gemmae of ***Marchantia polymorpha*** (Figure 44) was dependent on wavelength of light. The most effective wavelength was 350 nm, whereas no rhizoids were produced at less than 550 or more than 670 nm. They attributed this response to phytochrome and showed that an application of 10^{-4}M IAA for one hour had the same effect as the red-far red reversibility known for phytochrome.

Figure 44. ***Marchantia polymorpha*** thallus with gemma cups. Photo by Michael Lüth, with permission.

Water Relations

No growth can occur in the absence of water, but water can also affect the production of gemmae as an adaptive strategy to take advantage of flooding. In ***Bryoerythrophyllum campylocarpum*** (=***Hyophila crenulata***), gemmae occur on the protonema and are sensitive to humidity, with greater humidity causing greater gemmae production (Olarinmoye 1981). Flooding results in abundant basal protonematal gemmae. In its habitats of gutters, drainage areas, and other periodically flooded areas, these abundant gemmae facilitate spreading. The location of gemmae on protonemata provides them with the longest conditions of sufficient humidity compared to those on the stem or leaves.

In ***Marchantia***, which is not typically a flood plain species, Kaul *et al*. (1962) found that gemmae did not produce rhizoids when grown in liquid culture, but did in solid media.

Gender

It appears that gender can also play a role in timing of gemmae production. This is expected, since the energy required by production of antheridia and sperm is considerably less than that needed for the development of the sporophyte following fertilization. Thus, we might expect a delay in gemma production in females of a species, providing a longer span of energy to be diverted to the young sporophyte. Fuselier and McLetchie (2002) addressed this relationship in the dioicous ***Marchantia inflexa*** (Figure 45). In a low-light environment, the onset of gemma production and plant size early in development were under sex-specific selection. Furthermore, females paid a higher price for plasticity in the onset of gemma production under high light. Selection for asexual fitness shifted the offspring toward monomorphism rather than sexual dimorphism. However, there were negative tradeoffs between the asexual and sexual fitness, at least in females, under some light conditions. Fuselier and McLetchie suggest that the opposing selection forces of these two reproductive strategies (sexual and asexual) might explain the persistence of sexual dimorphism of mature plants, while selection favored immature plants in which gender was indistinguishable.

Figure 45. ***Marchantia inflexa***, a species where the sexes respond differently to light intensity. Photo by Scott Zona, through Creative Commons.

Mallón *et al*. (2006) experimented with vegetative propagules in the dung moss ***Splachnum ampullaceum*** (Figure 46) and suggested that ABA might be important in the ability of the protonema to produce brood cells and survive desiccation. This added production of brood cells would also permit the colony to spread, perhaps accounting for the very dense populations that are typical (Figure 46).

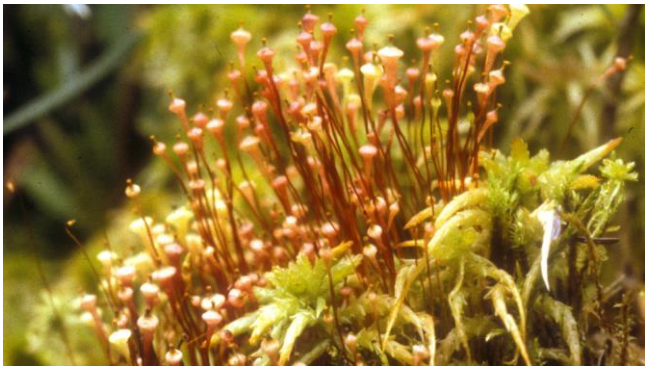


Figure 46. ***Splachnum ampullaceum*** growing on dung in a cow pasture. Photo by Janice Glime.

Nutrients and Inhibitors

We know that sucrose can cause germination of gemmae in ***Marchantia nepalensis***, suggesting that a photosynthetic response is needed to provide a continuous energy supply (Chopra & Sood 1970). This is supported by the increased germination with increased light intensity.

One factor we know to be important in any cell growth is calcium. Grotha (1983) found evidence in ***Riella helicophylla*** (Figure 30) suggesting that the distal lobe of the gemma and the non-dividing cells of the rhizoid initials of the gemma have zones that facilitate Ca^{+2} absorption.

Other plants can have an effect on the success of gemmalings. This is manifest not only in competition for light, but in chemical warfare as well. The epiphytic leafy liverwort ***Radula flaccida*** is affected by leachates and extracts of the supporting tree upon which it grows (Olarinmoye 1982). Although these seem to have no effect on the germination of the gemmae, they are important in the later establishment of the gemmaling, affecting cell length, leaf size, and rhizoid development. These effects seem to be dependent on the species of tree leaf involved and could account for differences in the colonization success on different species of trees.

Dormancy

One control of gemmae survival under conditions of cold or dehydration lies in their ability to maintain dormancy. We know that ***Marchantia*** gemmae (Figure 44) are unable to germinate while remaining on the parent plant, a condition in which we assume the parent to be responsible for inhibiting the germination and thus attaining gemma dormancy. But some dormancy seems to be under environmental control in ways that protect the young gemmalings from unfavorable environmental conditions. For example, the leafy liverwort ***Lophozia ventricosa*** var. ***silvicola*** (Figure 47) produces gemmae that are able to grow and replace dead shoots of the parent colonies. But these gemmae can be deposited throughout the growing season, some of them arriving upon favorable substrata when winter is imminent. Laaka-Lindberg and Heino (2001) propose that some gemmae are destined to become non-germinating gemmae, entering a "season-specific" dormancy. They suggest that only the dormant gemmae are able to survive winter. this is a good "bet hedging" strategy that permits some gemmae to get an early start on the competition while the season is still favorable, but permits some gemmae to safely overwinter while some of the germinated gemmalings might not make it through.

Figure 47. ***Lophozia ventricosa*** with gemmae that can replace dead shoots. Photo by Jan-Peter Frahm, with permission.

Dormancy is an adaptive strategy of utmost importance to organisms inhabiting unpredictable environments. Laaka-Lindberg (2000) considered it a way to spread the risk and enhance survival by making more effective use of resources. By remaining dormant when conditions are less favorable, resources are not lost to competition (Rees 1996; Hyatt & Evans 1998). Dormancy has been viewed by some as an alternative to dispersal, creating a facultative response in patchy environments where some patches are suitable and others are not (Cohen & Levin 1991; McPeek & Kalisz 1998). It is also a way to survive over winter in the leafy liverwort ***Lophozia ventricosa*** var. ***silvicola*** (Figure 47), with summer-produced gemmae germinating immediately and late-season gemmae becoming dormant for the winter (Laaka-Lindberg 2000).

Like spores, gemmae are typically under the control of light for germination, failing to germinate in the dark (Risse 1987). Schwabe (1972) reported that ***Lunularia cruciata*** (Figure 48) could survive dormant for months in total darkness. In ***L. cruciata***, long days induce dormancy. Nevertheless, it is a complex interaction of photoperiod, temperature, and phytochrome response that determines dormancy or germination. Furthermore, lunularic acid within the gemma cup promotes dormancy. The presence of other plants of their own or other species also provides an inhibitory function, as discussed earlier. The ability of lunularic acid to inhibit algal and fungal growth and to delay seed germination in some species suggests it may be allelopathic not only to its own offspring, but to other groups of taxa as well, thus potentially making the environment more friendly toward the success of the gemmalings once conditions are suitable for them.

Figure 48. ***Lunularia cruciata***, a liverwort where dormancy is induced by a variety of environmental conditions. Photo by David Holyoak, with permission.

This dormancy in ***Lunularia cruciata*** (Figure 48) permits gemmae to remain dormant underground in soil banks (Schwabe 1972). However, it is not that simple. If they are wet, they will not survive more than 10 days without germinating, and their fat reserves are depleted in 15 days if they are unable to replace it through photosynthesis. Furthermore, once they have imbibed water and begun to germinate, in as few as 12 hours, they are sensitive to desiccation and will not survive if dried at that stage.

Many have observed the dormancy of gemmae while still in the cups on the thallus of ***Marchantia polymorpha*** (Figure 44). Yet, when these gemmae get splashed onto the soil or the thallus dies around them, they seem able to germinate immediately. Schwabe (1976) has shown that it is lunularic acid from the parent thallus, serving as an inhibitor, that is responsible for this dormancy. Kumra and Chopra (1989) have shown that application of exogenous auxins inhibit growth of both gemma cups and vegetative plants of ***Marchantia palmata***. The auxin IAA is likewise known to inhibit germination of gemmae of ***Lunularia cruciata*** (Figure 48) in the lab (LaRue & Narayanaswamy 1957).

Lunularic acid occurs in the soluble fraction of the cell (as well as in association with the cell wall; Schwabe 1990). Therefore, inhibitors such as lunularic acid can be leached from the plant (Schwabe & Nachmony-Bascomb 1963), especially older parts of the thallus (Schwabe 1990), therefore potentially having an effect on neighbors of the same or even different species. Since leaching is likely to be greater during dry periods or immediately following them, this could cause a seasonal or weather-related response.

Germination Time

Germination times vary with type of propagule, size, age, and available water. And light seems to be required for most (all?). Propagula can germinate in 2-4 days in ***Bryum*** and ***Syntrichia*** (Llo Stark, pers. comm. 3 February 2015).

Tradeoffs

There are tradeoffs in using energy to produce brood bodies instead of spores. Whereas spores require a prior fertilization, which requires abundant water for sperm to swim, spores disperse farther than brood bodies and are able to germinate maximally on previously uncolonized substrates; brood bodies do not require fertilization, hence negating the need for excessive water, but can only disperse locally, yet, at least in some cases, are more successful amid other plants than are spores (Newton & Mishler 1994). Egunyomi (1978) found that the protonemata of gemmae grow faster, a factor likely to be true for most bryophytes, but that spores produce more gametophytes. However, one must be cautious in transferring these laboratory results to field generalizations. In the field, protonemata from spores may be less successful than gemmae just because they take longer to develop and therefore are more likely to encounter unfavorable conditions, including competition. In a later study on ***Bryum coronatum*** (Figure 39) in Nigeria, Egunyomi (1982) found that vegetative propagules may succeed where capsules fail. In that species, 41% of the setae had no capsules and 42% of the capsules did not dehisce. The spore germination was 65-88%, but the protonemal growth was abnormal, suggesting that spreading by spores in nature might be rather limited. On the other hand, this species is likely to succeed in dispersal through its numerous axillary propagules.

But production of gemmae usually comes at a price. Sharing of energy can mean no one does well, so it is not surprising that sporophyte development does not coincide with gemma development. In ***Tetraphis pellucida*** (Figure 32), one cannot find gemma cups and sporophytes on the same plant. Both need to occupy the same location at the shoot apex, making it physically impossible. But typically, even the population tends to have these at different times.

Risse (1987) found that among colonist species, propagation is almost entirely vegetative, giving little chance for new combinations of genes. Tubers are common among mosses of disturbed habitats. In ***Leptobryum pyriforme*** (Figure 49), if the protonema is grown in water, gametophore production ceases while tubers and rhizoidal gemmae develop abundantly.

Figure 49. ***Leptobryum pyriforme***, a prolific moss in disturbed areas. Photo by Michael Lüth, with permission.

Hedderson (1995) demonstrated that in the **Pottiales**, production of sporophytes decreases with increasing life expectancy and is negatively associated with production of asexual brood bodies. Among the **Funariales**, **Polytrichales**, and **Pottiales**, dioicous taxa are more likely to produce asexual brood bodies, as are monoicous taxa for which gametangia are unknown. However, production of these brood bodies is positively associated with a longer life expectancy, suggesting that at least the brood bodies do not deplete the plant of its energy supply.

Competition for resources and energy are likely to account for the suppression of gemma production during the production of sexual structures (Terui 1981). In ***Marchantia polymorpha*** (Figure 44), this response can be counter-acted by the application of high sucrose concentrations, thus inducing development of gemma cups.

Because of competing energy requirements, the two genders are likely to differ in their production of gemmae. Female plants require considerably more energy to produce archegonia and sporophytes than do male plants to simply produce antheridia. For example, Laaka-Lindberg (2001) found that in the leafy liverwort ***Lophozia ventricosa*** var. ***silvicola*** (Figure 47), shoots lacking gametangia produced three times as many gemmae as female shoots, and that males produced twice as many. In ***Marchantia polymorpha*** (Figure 44), the number of gemma cups produced by females was less than 1/6 that produced by their male counterparts (Voth 1941). Interestingly, when phosphate supplies decrease to stress levels, the number of cups on male plants decreases while the number on females increases, making them nearly equal!

Ecological Function

Many types of asexual propagules comprise the propagule bank, available to colonize when disturbance brings them to the surface. In this way, taxa such as ***Leptobryum pyriforme*** (Figure 49) and ***Bryum rubens*** (Figure 50) readily colonize disturbed habitats and tip-up mounds (Risse 1987).

As Ross-Davis and Frego (2004) pointed out, our understanding of the role of bryophyte propagules in structuring communities is meager. To address this question, they examined the propagule rain and buried propagule banks of the mature mixed forests in southeastern New Brunswick, Canada. They found 51 taxa in the diaspore rain and buried propagule banks, but only 36 of these were present in the forest floor community. Differences in phenology were evident in the high seasonal variability within the aerial diaspore sources. Considering the hundreds of species available in the geographic region, these propagule sources are relatively limited, undoubtedly to nearby sources. The extant community was most similar to that of the aerial diaspores, suggesting that the buried diaspore bank was reminiscent of a different ecosystem and was ready if that set of conditions returned. Further discussion of brood bodies is in the adaptations subchapter on dispersal.

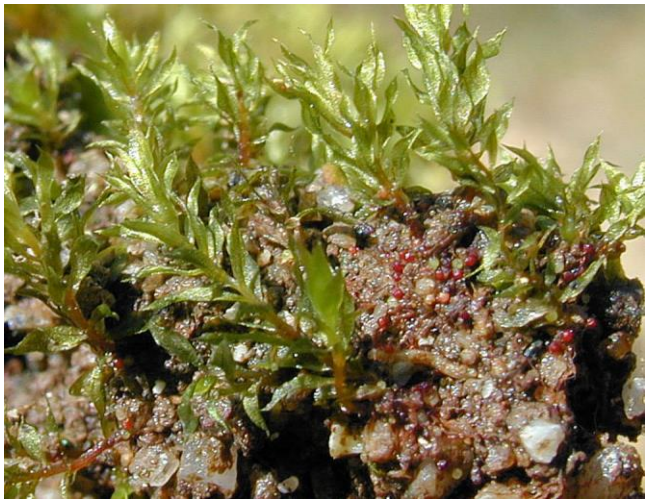


Figure 50. ***Bryum rubens*** showing red rhizoidal tubers in disturbed soil. Photo by Michael Lüth, with permission.

Summary

Brood bodies include both gemmae and propagules (vegetative diaspores). Propagules can be defined as reduced buds, branches, or leaves that serve in reproduction. Gemmae are relatively undifferentiated vegetative reproductive structures and come in a variety of shapes and sizes. Brood bodies provide a safe mode to survive environmental disturbances such as desiccation, physical disturbance, and freezing. Colonist species rely almost entirely on brood bodies to invade newly disturbed habitats. Asexual means are important in colony spread of non-perennial taxa. Brood bodies are most common on dioicous (unisexual) species and compete for energy, thus typically not being present during sporophyte production. As a result, they are often more common on males than on females.

Tubers of mosses occur on the rhizoids, but in liverworts they are extensions of the growing apex and grow toward the ground to serve as a perennating structure. In both cases they provide a diaspore bank that makes the species available when favorable conditions return.

Gemmae seem to require auxin (IAA) to develop and are inhibited from germination by the parent plant, presumably by lunularic acid in liverworts and probably by ABA in mosses. Production is affected by light intensity, wavelength, and moisture availability. These factors plus photoperiod and temperature are known to affect their germination and dormancy as well. Addition of sucrose enhances germination, suggesting the importance of photosynthesis to provide energy.

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CHAPTER 5-8
ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOGENESIS

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ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOGENESIS

Figure 1. Antheridial splash cups of ***Polytrichum juniperinum***. Photo by Janice Glime.

Definition

Gametogenesis – the development of gametes (*genesis* means origin) is the essential process leading to sexual reproduction. In bryophytes, gametes are produced by mitotic division of tissue within multicellular structures, the **antheridia** (male) and **archegonia** (female), collectively known as **gametangia**. The location of these structures on the mosses is the basis for dividing the mosses into two large groups, the **acrocarpous** mosses that produce archegonia at the tips of upright stems, and the **pleurocarpous** mosses that produce archegonia on side branches of a generally horizontal stem. The differences in location of these archegonia can present differences in the ease with which the sperm can reach the archegonium, and hence reach the egg.

Developmental Stages

Lal and Bhandari (1968) described the developmental stages of the sex organs of the moss ***Physcomitrium carpathicum***. The archegonium begins its development in a manner similar to that of the antheridium. In these early stages, it produces a stalk, then the two-sided apical cell gains a third cutting face and the archegonium develops from this cell. The antheridial development is similar to that of other mosses. This chapter will examine the interaction of hormones and the environment as they influence this development.

Environmental Factors

The timing of the induction of gametangia is a critical function in the life cycle of bryophytes. For sexual reproduction to be successful, gametangia must form at a time when they can survive and they must mature at a time when it is safe and sufficient water is present for the sperm to reach the egg. This timing is controlled by external signals in the environment, and this is interpreted internally through such controls as hormones and nutrient levels.

Water Availability

Gametogenesis (development of gametes) must be timed in such a way as to take advantage of the most

critical need in fertilization – water. Because sperm in bryophytes must swim to the archegonium, adequate water is critical, but too much water or rapidly flowing water may dilute or carry off the sperm and make directional movement toward the archegonium all but impossible. In fact, timing of moss reproduction, whether a response to day length or temperature or other environmental stimulus, is often related to the season of proper moisture. Since gametangial initiation can occur several (or many) months prior to the actual time of fertilization, environmental cues other than moisture must trigger the process. It is therefore an expected consequence that different species within a genus respond to different environmental cues for gametogenesis, permitting them to live in different habitats. And even within species, populations can differ widely (Clarke & Greene 1970). But for many bryophytes, water is an important signal for gemetangia to develop, perhaps because it permits the gametophyte to be active and produce sugars needed for energy.

Gametangium Developmental Need for Water

Waterfalls can provide continuous moisture sufficient for sperm dispersal and even contribute to dispersal itself. At Churchill Falls, Labrador, Canada, the bryophytes are very fertile within the spray zone, whereas other vegetation expresses retarded phenology (Brassard *et al*. 1971). It could be that the spray itself induces gametangial production. Kumra and Chopra (1983) found that culture in liquid media favors antheridial induction in ***Barbula indica*** var. ***gregaria*** (Figure 2) and ***Bryum coronatum*** (Figure 3) over that in solid gel culture, greatly hastening it in ***Barbula indica*** var. ***gregaria***.

Figure 2. ***Barbula indica*** var. ***gregaria***, a moss where liquid medium favors antheridial production. Photo by Li Zhang, with permission.

Figure 3. ***Bryum coronatum***, a moss where liquid culture favors antheridial induction. Photo by Michael Lüth, with permission.

Sphagnum (Figure 4) provides a good example of effect of water on gametangial maturation. Sundberg (2002) studied nine sites in Sweden for six years, during which the nine most abundant species produced capsules. Capsule production related most to moisture regime of the previous summer, with more precipitation resulting in more capsules. This presumably relates to success of gametangial formation. Capsule success in wetter pits related positively to spring precipitation in the same year as capsule production, suggesting it was also important for fertilization success. Further discussion of timing of reproduction with moisture availability is in the phenology chapter.

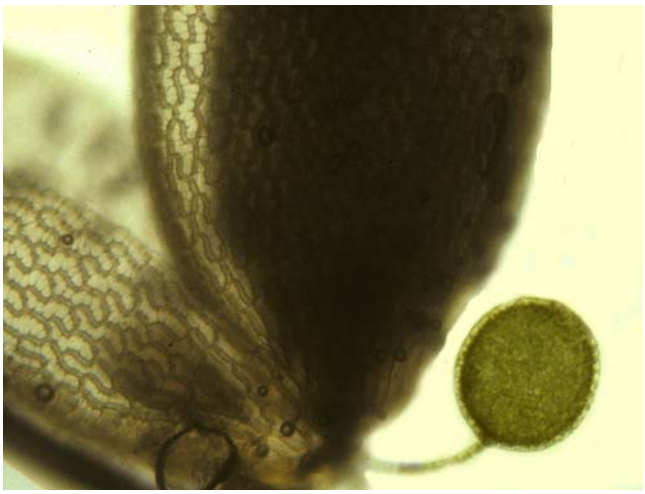

Figure 4. ***Sphagnum papillosum*** antheridium, a species for which moisture is important for gametangial success. Photo courtesy of Yenhung Li.

Swimming Sperm

For sperm to reach the archegonium, they must swim. But a tiny sperm cell (Figure 5) cannot carry that much energy with it, so the distance is limited. Some mosses maximize the effect of rainwater by producing **splash cups** (Figure 1) or **splash platforms** (Figure 6) that house the antheridia.

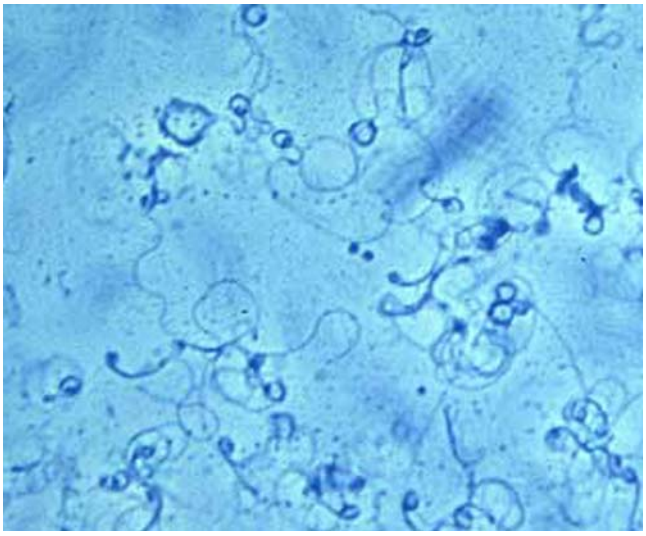

Figure 5. ***Marchantia polymorpha*** stained sperm. Photo from Botany Website, UBC, with permission.

The study by Andersson (2002) on ***Plagiomnium affine*** (Figure 6) provides insight into just how this splash works. He is the only one who has published photographs of the arrival and splash of an actual raindrop, eliminating the problem of laboratory tests where the drops do not reach terminal velocity. The splash is somewhat reminiscent of the expulsion of ***Sphagnum*** Figure 4) spores from a capsule, both demonstrating fluid dynamics. When the raindrop first hits a hard surface (splash cup or platform), it forms a crater many times the diameter of the drop. A jet of water then rises from the center of the crater (Rayleigh's jet). One or more large drops may be pinched off. In a splash cup, this force is typically sufficient to push all the water out of the cup.

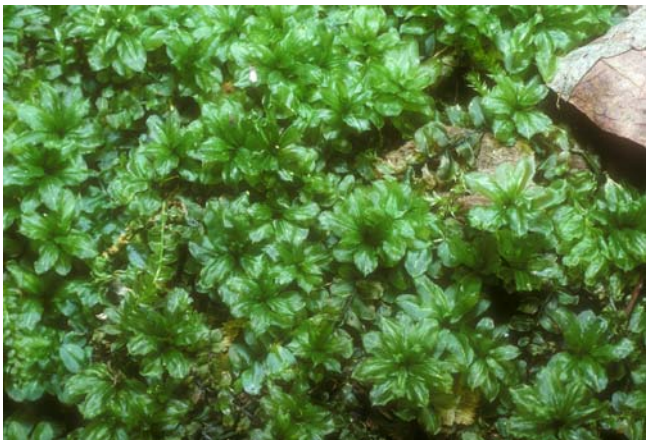

Figure 6. ***Plagiomnium affine*** showing antheridial platforms and runners. Photo by Janice Glime.

Splash cups and platforms are not flat, so the water angles are oblique (Andersson 2002). As the water flows outward from the point of impact, the edge of the water mass bends upward to form a crown. As the drop collapses, the circle of water widens and the crown bends up more. Wave motions travel both vertically and horizontally; a thick cylinder of water forms around the upper rim of the crown and small jets of water extend outward. As these jets become unstable, they break into many tiny droplets that shoot out from the crown with high velocity. The crown collapse occurs after about 8 ms on a wet surface. Most of the droplets are less than 0.5 mm, and many are less than 0.05 mm. The spermatozoids are only about 1 µm (0.001 mm) in diameter and can therefore easily be carried by the droplets of water.

Most experiments with splash cups have not been at distances that mimic terminal velocity. Based on data from Laws (1941), a 3 mm drop would need to be dropped from about 7 m to reach terminal velocity, a height not available in most labs. Reynolds (1980) considered that distances of 30 cm splash from point of impact would not be uncommon.

But does this splash really disperse the sperm? To be dispersed, sperm must be able to exit the antheridium, and this requires that the antheridium must burst. That criterion is satisfied by the first raindrop to strike a mature antheridium (Andersson 2002). But... members of the **Mniaceae** shrivel when dry and do not rewet easily. ***Mnium*** (Figure 7-Figure 8) species may require soaking for an hour before they are ready for making a slide (Koponen 1974), indicating that the leaves in a rainstorm are not ready to make a splash platform in less than an hour. Furthermore, the forest canopy traps many of the raindrops and reduces their velocity (Andersson 2002) or even diverts them so that they run down the trunk instead of striking the forest floor beneath them. Hence, it may take some time before the splash platform is exposed directly to raindrops in a storm, and this might not be achieved at all in a light shower.

Figure 7. ***Mnium spinosum*** wet. Photo by Michael Luth, with permission.

Figure 8. ***Mnium spinosum*** dry. In this condition, it is slow to take in water. Photo by Michael Luth, with permission.

To add further to the complications of reaching a female, the sperm are not released directly as individuals from the antheridium. Rather, they are released in a package, a **vesicle** of fluid. This vesicle must be disturbed by water drops before it will break apart. The vesicles become separated from each other by lipid drops and slowly dissolve, freeing the sperm.

Some seed plants have a chemical delay mechanism to prevent seed germination in a short rain shower, with chemical inhibitors being removed in a more significant rainstorm that is sufficient to sustain the young plant. The intervening factors required for a raindrop to splash the bryophyte sperm successfully seems like a mechanical method to delay sperm dispersal until it is certain there will be sufficient water for the sperm to complete their journey after the splash, with the delay in freeing sperm contributing to this mechanism.

The moss ***Plagiomnium affine*** (Figure 6) is less fortunate than the species with real cups. Its antheridial

platforms succeed only in splashing droplets with sperm about 100 mm (Andersson 2002). Fortunately, most of the females within 80 mm are successfully fertilized, but that does not permit much outcrossing.

In ***Polytrichum ohioense*** (Figure 9), the 2-3 mm cup permits sperm to be splashed 60 cm or more (Brodie 1951). A similar distance is accomplished by the splash platform of ***Marchantia polymorpha*** (Figure 10) (Buller 1942). Even greater distances, up to 230 cm, are achieved by antheridial splash cups of ***Dawsonia longifolia*** (Figure 11-Figure 12) (Clayton-Greene *et al.* 1977; see chapter on sexuality), aided by its greater height (up to 50 cm). These dispersal distances match the observed maximum distances between males and sporophyte-bearing females observed in the field. Very small splashes create an aerosol effect that could permit the sperm to float for considerable distances, and wind can increase the distance downwind.

Figure 9. ***Polytrichum ohioense*** with spent antheridial splash cups producing new growth. Photo by Janice Glime.

Figure 10. ***Marchantia polymorpha*** male splash platforms. Photo by David T. Holyoak, with permission.

Figure 11. ***Dawsonia longifolia*** with perigonia. Photo by Allan Fife, with permission.

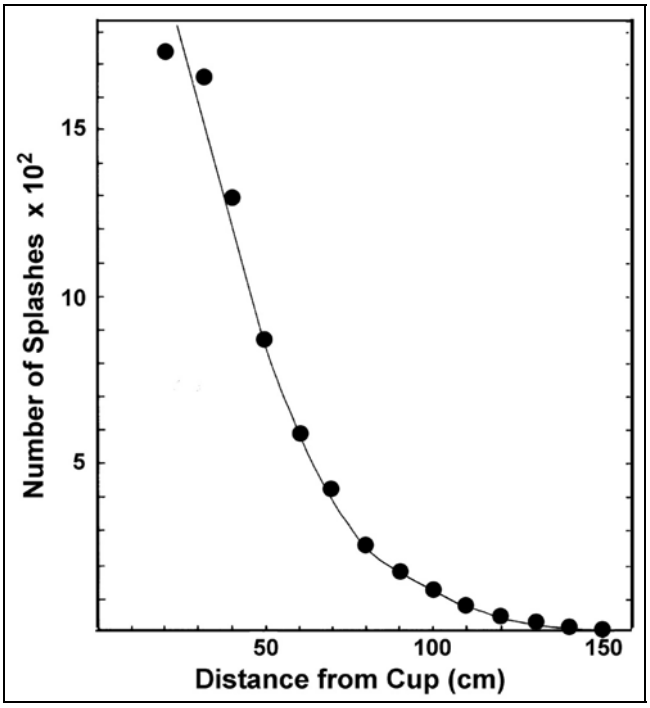

Figure 12. Distance of splashes from 0.055 ml drops dropped from 228 cm and splashed from the splash cup of ***Dawsonia longifolia***. Redrawn from Clayton-Greene *et al.* (1977).

Monoicous species (having male and female organs on the same plant) have a greater chance for fertilization than **dioicous** species because there will always be gametangia of the opposite sex nearby. Rohrer (1982) compared the success of dioicous species with and without splash cups in an aspen forest and a swamp forest of Michigan's northern Lower Peninsula. Those with splash cups had significantly higher sporophyte production (Figure 13). Unfortunately,

splash cups are relatively uncommon, but leaves surrounding antheridia can sometimes act as splash cups or platforms by spreading when hit by a raindrop (reference forgotten ☺).

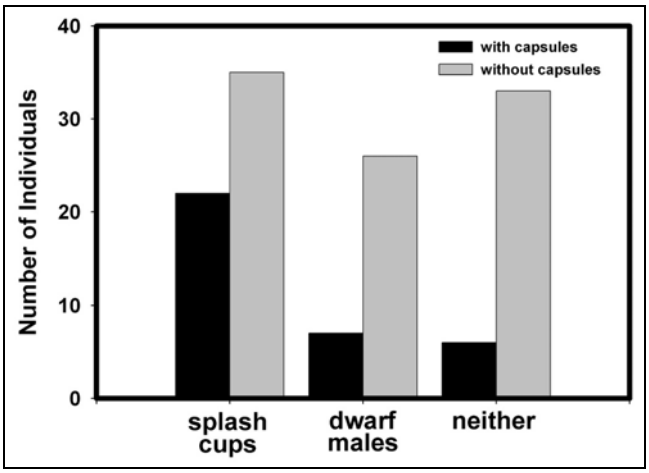

Figure 13. Effect of splash cups and epiphytic dwarf males on number of individuals with sporophytes in dioicous mosses of an aspen forest in the northern lower peninsula of Michigan, USA. Based on data from Rohrer 1982.

Paraphyses

This story is not complete without a discussion of **paraphyses**, those sterile structures, usually filamentous, that accompany most gametangia among the bryophytes. For something to persist this commonly while requiring energy for their development, we usually consider them to have some adaptive function. But little if any testing has been done to show that they make a difference.

Paraphyses usually occur in sufficient density to produce capillary spaces. With this knowledge, we can theorize as to their value. Such spaces would mean that water drops would be drawn between them, providing swimming spaces surrounding the archegonia. For antheridia, these can create water pressure that could aid in the rupture of the antheridium and hence the release of sperm.

But this does not seem to be the only excuse for their continued existence. In the Neckeraceae, structures that can be interpreted as paraphyses develop after fertilization in ***Neckeropsis*** (Figure 14), forming on the perichaetia (Merced-Alejandroa & Sastre-De Jesús 2009). These researchers found that transitions between uniseriate and multiseriate paraphyses occur at different stages in the developing reproductive branch. In early stages they are more typical of paraphyses in most mosses; this stage is the terminal stage in some ***Neckeropsis*** species. In other species, these continue to become multiseriate and ligulate to lanceolate. But what could their function be if they do not develop until after fertilization?

Figure 14. ***Neckeropsis undulata***, a genus in which paraphyses develop after fertilization. Photo by Michael Luth, with permission.

As discussed earlier, Reese (1955) tested a very different function for these paraphyses. He was able to demonstrate their ability to regenerate plants in ***Bryum capillare*** (Figure 15-Figure 16), ***Aulacomnium palustre*** (Figure 17), and ***Funaria hygrometrica*** (Figure 18-Figure 19). Could this be a back-up plan for unsuccessful sexual reproduction? Most likely it is actually a rare occurrence in nature, and thus its most frequent function is most likely that surmised by the early bryologists who considered them to have both a capillary function to draw in water, but also to retain water among the developing gametangia.

Figure 15. ***Bryum capillare*** males with antheridia and paraphyses. Photo by Dick Haaksma, with permission.

Figure 16. ***Bryum capillare*** antheridia, and paraphyses that can regenerate. Photo by Dick Haaksma, with permission.

Figure 17. ***Aulacomnium palustre*** males, a species in which paraphyses can regenerate new plants. Photo by David T. Holyoak, with permission.

Figure 18. ***Funaria hygrometrica*** with antheridia. Photo by Barry Stewart, with permission.

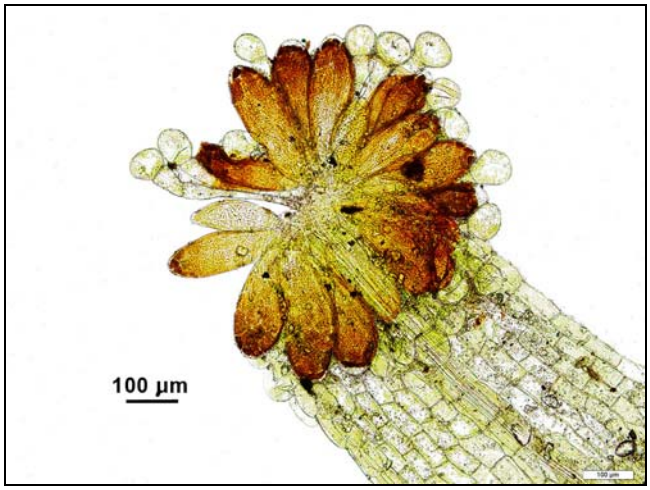

Figure 19. ***Funaria hygrometrica*** antheridia with paraphyses (white) that can regenerate. Photo by Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Photoperiod and Light Intensity

Bryophytes, like flowering plants, can be classified into a variety of short-day and long-day types (Benson-Evans 1964; Maravolo 1980; Glime 1984; Li & Glime 1991). Tracheophyte species that occupy a wide latitudinal range, such as *Typha latifolia*, show population differences in response to day length (McNaughton 1966), and Longton (1972) has demonstrated this for the mosses ***Polytrichum*** (Figure 1, Figure 9) and ***Psilopilum*** (Figure 20). The physiological response mechanism in these two taxa is unknown, and a large number of substances can induce the same response, depending on the species.

In 1983, Chopra and Bhatla contended that mosses, except for ***Sphagnum plumulosum*** (=***S. subnitens***; Figure 21), appeared to be independent of photoperiod for the induction of gametangia. On the other hand, they found that all liverworts tested to date, except ***Ricciella crystallina*** (=***Riccia crystallina***; Figure 22) (Chopra & Sood 1973a), were either long-day or short-day plants. But they clarified this statement – it appears that even in liverworts, the response seems to be quantitative, with greater light intensities increasing the photoperiod response.

Figure 20. ***Psilopilum cavifolium***, member of a genus where populations can show differences in response to day length. Photo by Niklas Lonnell, with permission.

In mosses, other factors such as light intensity and temperature modify the response. For example, ***Bartramidula bartramioides*** [optimum of 3500-4000 continuous light (Chopra & Rahbar 1982)] and ***Leptobryum pyriforme*** (Figure 23) respond linearly to increasing light intensity for gametangial response (Chopra

& Rawat 1977; Chopra & Bhatla 1983), whereas ***Bryum argenteum*** (Figure 44-Figure 45), ***B. coronatum*** (Figure 3), and ***Barbula indica*** var. ***gregaria*** (Figure 2) respond to a specific light intensity for their optimal response (Chopra & Bhatla 1983). In ***Bryum coronatum*** and ***Barbula indica*** var. ***gregaria***, antheridia develop under "ordinary" cultural conditions (Kumra & Chopra 1983), requiring no specific photoperiod for induction, but having a greater response as the photoperiod increases. ***Philonotis turneriana***, on the other hand, remains sterile under "ordinary" conditions. Temperature likewise plays a role, but its role is primarily to constrain the photoperiodic effect within certain temperature limits. However, in ***Philonotis turneriana*** a temperature of 18°C is needed for induction. In ***Barbula indica*** var. ***gregaria*** and ***Bryum coronatum***, the antheridial induction increases as the temperature increases, up to 24°C.

Figure 21. ***Sphagnum plumulosum***, one of the first mosses known to respond to photoperiod for gametangial induction. Photo by J. C. Schou <http://www.biopix.com/>, with permission.

Figure 22. ***Ricciella*** cf ***crystallina*** (=*Riccia crystallina*) Bareilly India. Photo by Michael Lüth, with permission.

Knoop (1984), like Chopra and Bhatla (1983), contends that most mosses seem to be day-neutral. Nevertheless, Benson-Evans (1964) examined a large number of bryophyte taxa with varying environmental influences on initiation of gametangia; photoperiod seemed to be the overriding influence in most cases. In ten liverworts (4 Marchantiales, 6 Jungermanniales), the plants were long-day plants. ***Riccia glauca*** (Figure 24), ***Phaeoceros laevis*** (Figure 25), and ***Sphagnum plumulosum*** (Figure 21) are short-day plants. The moss ***Pogonatum aloides*** (Figure 26) (Benson-Evans 1964) and liverwort ***Ricciella crystallina*** (Figure 22) (Chopra & Sood 1973b) are day-neutral. ***Phaeoceros*** spp. (hornworts) are predominantly long-day induced, a condition that may be true for most hornworts (Schofield 985). Temperature and other external factors can modify these responses, and surely energy will play a role. But are most mosses really day-neutral?

Figure 23. ***Leptobryum pyriforme*** with capsules, a moss that produces more gametangia as light intensity increases. Photo by David T. Holyoak, with permission.

Figure 24. ***Riccia glauca***, a long-day liverwort. Photo by Jan-Peter Frahm, with permission.

Figure 25. ***Phaeoceros laevis***, a long-day hornwort. Photo by Robert Klips, with permission.

Figure 26. ***Pogonatum aloides*** with male splash cups. Photo by David T. Holyoak, with permission.

Despite the tendency for liverworts to be controlled by photoperiod, ***Lophocolea*** (Figure 27) in southern Illinois, USA, is day neutral (Zehr 1979). And the mosses ***Diphyscium foliosum*** (Figure 28), ***Atrichum angustatum*** (Figure 29), and liverwort ***Trichocolea tomentella*** (Figure 31) are long-day plants for gametangial production. ***Nowellia curvifolia*** (Figure 30) is likewise a long-day liverwort, but only for initiation. They will continue to develop unless the process is halted by desiccation.

Figure 27. ***Lophocolea heterophylla*** on a log, a day-neutral liverwort, at least in southern Illinois, USA. Photo courtesy of Betsy St. Pierre.

Figure 28. ***Diphyscium foliosum*** showing female plants with perichaetial leaves and purplish male plants. Photo by Li Zhang, with permission.

Figure 29. ***Atrichum angustatum*** males, a long-day species for gametangial production. Photo by Bob Klips, with permission.

Figure 30. ***Nowellia curvifolia***, a long-day liverwort for gametangial induction. Photo by Michael Lüth, with permission.

Figure 31. ***Trichocolea tomentella***, a long-day plant for gametangial production. Photo by Michael Luth, with permission.

Voth and Hamner (1940) found that photoperiod controlled the development of gemma cups vs gametangiophores in ***Marchantia polymorpha*** (Figure 10). Short days stimulated gemma cup production, whereas long days stimulated more gametangiophores. Miller and Colaiace (1969) found that this species could be grown from gemmae and induced to produce antheridiophores and

archegoniophores in 3-6 weeks under a 24-hour photoperiod at 23°C.

Perhaps ***Fontinalis*** can again give us insight into these seemingly different results. Members of this genus, like ***Fontinalis novae-angliae*** (Figure 32), that are common in fast water of mountain streams face the problem of losing their tiny sperm rapidly downstream as soon as they are released. Goebel (1930) suggests that ***Fontinalis*** can only reproduce when it is in standing water because the water would otherwise wash the sperm away too easily. Hence, it appears that those mosses that live submersed in streams must time their sperm release to coincide with low water levels when the moss is moist, but not in rushing water.

Figure 32. ***Fontinalis novae-angliae*** in a swift mountain stream in New Hampshire, USA. Photo by Janice Glime.

This need for timing of sperm release suggests that a photoperiod response would be beneficial in those regions where low water level periods are somewhat predictable. Indeed, in ***Fontinalis dalecarlica*** (Figure 33), photoperiod seems to control production of gametangia quantitatively, rather than being an on-off signal, with short days causing the maximum number of archegonia to be mature when the moss is above water, but wet, during late summer and early autumn (Figure 34; Glime 1984). Longer days seem to lengthen the time for archegonia production, but aeration (from being above water) is also an important factor, resulting in more archegonia compared to those on submersed stems. Maturation of gametangia when the antheridia and archegonia are located above water, but moist, provides moisture for fertilization but protects the sperm from being washed away by fast water (Figure 35). Perhaps initiation of archegonia is more complex in mosses, causing the appearance of being day-neutral when the combination of stimulating factors is not present.

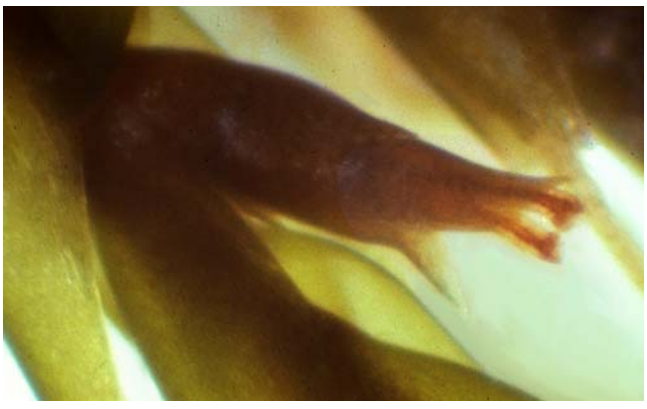

Figure 33. ***Fontinalis dalecarlica*** archegonia, a genus that responds to day length. Photo by Janice Glime

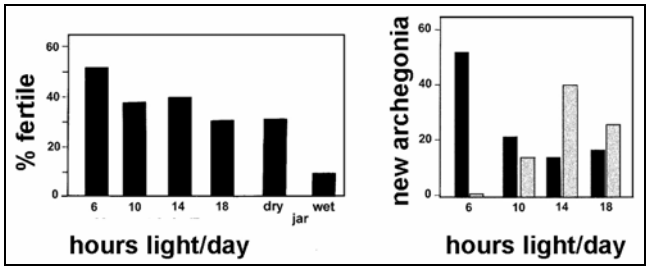

Figure 34. Effects of photoperiod and exposure to air on production of archegonia in ***Fontinalis dalecarlica*** (Figure 33) after 16 weeks of cultivation in artificial streams. **Left:** Day length effect and effect of submersed (wet) vs emergent (dry) at 14-hr photoperiod. Fertility does not differ significantly among the photoperiods, but emergent mosses produced significantly more than wet ones ($p < 0.01$). **Right:** Effect of photoperiod on development time required for archegonia. Black bars ▮ are numbers of archegonia produced during weeks 1-7; gray bars ▒ are numbers produced during weeks 7-16. n = 40 plants in each condition. From Glime 1984.

Leitgeb (1868) found ***Fontinalis antipyretica*** (Figure 35) to produce antheridia from spring until fall, but he did not mention whether the number maturing remained constant. At least for ***Fontinalis dalecarlica*** (Figure 33) from North Carolina, the fact that production is not perfectly responsive to short days, but rather occurs more slowly during longer days, assures the moss of having at least some gametangia ripe whenever water conditions are right (Glime 1984). It is a bet-hedger in the sense of Stearns' (1976) r and K strategies. ***Fontinalis*** can afford to be a bet-hedger because its vegetative parts are both persistent and capable of reproducing by fragmentation. Even a series of years when gametangial maturity does not match the right water level would not cause a serious reproductive problem.

Figure 35. ***Fontinalis antipyretica*** partially above water, providing an opportunity for splashed sperm to locate an archegonium. Photo by Jan-Peter Frahm, with permission.

The suitable photoperiod may be altered by temperature, permitting the plant to be plastic and able to

complete its life cycle in different geographic regions where the photoperiod relationship to temperature is different. For example, ***Fossombronia brasiliensis*** is a short-day plant at 18ºC, requiring 6-12 hours of night, whereas at 10ºC its light requirements are more quantitative (Chin *et al*. 1987). Furthermore, photoperiod affected the sex ratio, with more female gametangia being produced at 10ºC and more male gametangia at 18ºC.

Continuous light can favor some moss gametangial production. For the moss ***Microdus brasiliensis*** (Figure 36), Chopra and Mehta (1987) found that gametangial production increased with increasing photoperiod, with continuous illumination at 18ºC being optimal.

Figure 36. ***Microdus brasiliensis***, a moss in which gametangial production increases with increasing photoperiod. Photo by Jan-Peter Frahm, with permission.

Light intensity can also control fertilization success. ***Phascum cuspidatum*** (Figure 37) has greater fertilization in shade, due to larger antheridia and greater dehiscence, than in sun (Hughes & Wiggin 1969). Since free water is required for fertilization, this mechanism provides a longer period of moisture while the sperm attempts to reach the egg.

Figure 37. ***Phascum cuspidatum*** with capsules, a moss with greater fertilization in shade. Photo by Michael Lüth, with permission.

Little seems to have been done to understand the relationships of photoperiod in gametangial development in the **Anthocerotophyta**. Benson-Evans (1964) reported that this group is comprised of short-day plants, but I haven't found enough references to justify that assertion. She reported that ***Phaeoceros laevis*** (Figure 25) is sterile in 18-hour days, but produces gametangia in 8-12 hour days. Ridgeway (1967) found photoperiod to be the critical factor to induce antheridia and ***Anthoceros*** (Figure 38), ***Phaeoceros***, and ***Notothylas*** (Figure 39), whereas a range of temperatures from 10 to 20°C had almost no effect. However, at 5 and 25°C, the six species studied failed to produce antheridia. At 10°C, none of the species produced antheridia in 18-hour days, whereas all produced them in that photoperiod at 8°C. Most also produced them at 4 and 12°C.

Figure 38. ***Anthoceros agrestis***, a hornwort that produces gametangia in response to photoperiod, shown here with sporophytes. Photo by Jan-Peter Frahm, with permission.

Figure 39. ***Notothylas orbicularis*** with involucres, a species that responds to photoperiod but not temperatures. Photo by Michael Lüth, with permission.

Using single-spore cultures, Lazarenko and Lesniak (1972) found that the long-day (16 hours daylight) ***Desmatodon cernuus*** is sterile in 24 hours of light. Such requirements from the natural environment could eliminate the sexual reproduction in populations that develop in more northern latitudes and may explain the reliance of some species on asexual reproduction. The sibling species ***Desmatodon ucrainicus*** is fully self compatible.

In a more recent study, Lee *et al*. (2010) found that it can actually be the change in photoperiod that induces gametangia. In ***Pohlia nutans*** (Figure 40), changes from long days to short days effected gametangial initiation. It appears we need many more studies before we can assess the importance of photoperiod (and light intensity) on gametangial induction in bryophytes, especially mosses.

Figure 40. ***Pohlia nutans*** with perigonia, a plant that responds to a change in photoperiod to initiate gametangia. Photo by Michael Lüth, with permission.

But it appears that we know little about the effects of light intensity or light quality on the development of gametangia or the success of fertilization. Could it be that in certain wavelengths the sperm are more likely to die, particularly in the UV range?

Photoperiod response is likely to be one of the most frequent differences seen between populations at different latitudes. Wavelength is also likely to be a selection factor, especially at high altitudes. Selection forces would be strong against those individuals that produced gametangia at times when completion of reproduction was unlikely due to low temperatures and possibly strong UV light. Weitz and Heyn (1981) demonstrated that reaction to day length was one of the traits that differed among populations of the ubiquitous moss ***Funaria hygrometrica*** (Figure 41) from various geographic-climatic regions.

Figure 41. ***Funaria hygrometrica*** (Common Cord-moss) male plants with antheridial splash platforms. Photo by Barry Stewart, with permission.

The moss ***Bartramidula bartramioides*** is unusual in having a high nutrient requirement. Chopra and Rhabar (1982) found that it grew best at full strength Knop's medium plus Nitsch's nimor nutrient solution. Gametangial **induction** (initiation of development) occurred at 25±2°C, 3500-4000 lux continuous light.

Nutrients

Nutrient supply as a control of gametogenesis occurs throughout the plant kingdom, although it is probably best developed in the algae. The green algae ***Oedogonium*** (Singh & Chaudhary 1990) and ***Chlamydomonas*** (Figure 42) (Trainor 1959; Matsuda *et al*. 1992) recognize the approach of winter by the diminishing supply of nitrogen in a usable form, developing gametes and creating zygotes (then zygospores) that are able to survive the winter. It is appropriate to ask what role nutrients play in the life cycles for organisms that have quite low nutrient requirements – the bryophytes.

Figure 42. ***Chlamydomonas***, a genus that responds to diminishing N supply by producing gametes. Photo by Janice Glime.

Ramina *et al*. (1979) demonstrated the role of nutrients in *Bougainvillea*, where flower production increased in direct relationship to leaf production but decreased in relation to branch production (which used nutrients without making more). In the aquatic moss ***Fontinalis dalecarlica*** (Figure 33), production of gametangia likewise is inversely related to branch production from 10 August to 14 October (Figure 43), again suggesting an energy limitation (Glime 1984).

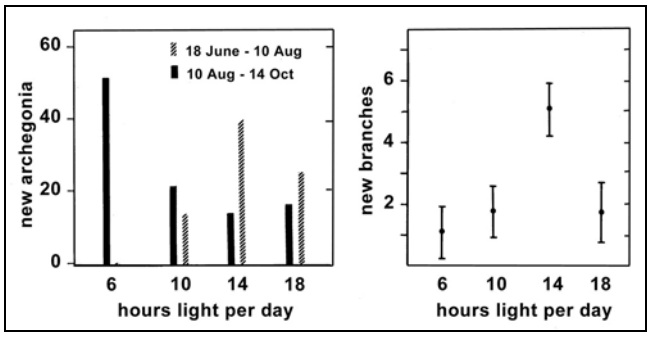

Figure 43. Effect of photoperiod on number of archegonia vs branches in ***Fontinalis dalecarlica***. Redrawn from Glime 1984.

Selkirk (1979) has shown that limited nitrates cause gamete production in several species of the liverwort ***Riccia*** (Figure 24), and Joenje and During (1977) showed that lower nutrients stimulate the production of sex organs

in ***Bryum argenteum*** (Figure 44-Figure 45). A low N:high C ratio in ***Marchantia*** (Figure 10) likewise stimulated production of sexual branches (Lockwood 1975). On the other hand, in ***Fossombronia brasiliensis*** (see Figure 46), N as nitrate caused more gametangial production than when it was supplied as ammonium (Chin *et al*. 1987). Such differences can help to explain differences in habitat preferences among species.

Figure 44. ***Bryum argenteum*** with several plants showing antheridial apices. Photo by Dick Haaksma, with permission.

Figure 45. ***Bryum argenteum*** perigonium showing antheridia. Photo by George J. Shepherd, through Creative Commons.

Figure 46. ***Fossombronia*** sp. ***Fossombronia brasiliensis*** produces gametangia in response to nitrate nitrogen. Photo by Ken-ichi Uedo, through Creative Commons.

Carbohydrates are important for gametangial formation in at least some bryophytes. Whereas ***Bryum argenteum*** (Figure 44-Figure 45), ***B. coronatum*** (Figure 3), and ***Barbula indica*** var. ***gregaria*** (Figure 2) produce gametangia in the absence of carbohydrates in culture, ***Ricciella crystallina*** (Figure 22) and ***Bartramidula bartramioides*** respond to enhanced carbohydrates (Chopra & Bhatla 1983), and addition of sugar in culture seems to be essential for ***Bartramidula bartramioides*** (Chopra & Rahbar 1982). But, as discussed above, Chopra and Bhatla (1983) found that a high carbohydrate:nitrogen ratio was more important than carbohydrates alone in the initiation of gametangia. In particular, bryophytes are likely to respond to depletion of nitrate or ammonium (depending on species), whereas organic nitrogen (amino acids, peptone, urea) affects gametangial formation differently among various species of liverworts.

Amino acids and kinetin, both found in the environment, can alter the photoperiodic response of gametangial induction in the leafy liverwort ***Cephalozia lunulifolia*** (=***C. media***; Figure 56) (Lockwood 1975). Arginine, cysteine, and tryptophan plus kinetin negated photoperiodic control. Those compounds that stimulated asexual reproduction (gemmae) under short photoperiods would also inhibit gametangial activity under long-day conditions. Addition of inorganic nitrogen had no effect on these responses.

Thus, as concluded by Chopra and Bhatla (1983), the importance of the nutrient status varies by species. Generally, however, low nutrient levels seem to be the most important in gametangial induction.

The need for sugar may be an artifact of culture. In their study of the liverwort ***Cryptomitrium himalayense***, Awasthi *et al*. (2013) found that sugar was necessary in the lab for gametangial induction, but when cultured on soil, this species produced gametangia under the same temperature of 21°C and long day (16 hours light) regime with colder nights (8 hours darkness at 15°C), but with no added sugar necessary.

Belkengren (1962) had some rather unusual results in ***Leptodictyum riparium*** (Figure 47). In this species, he was able to induce gametangia by culturing in continuous light, using a CO_2-free period followed by addition of sugar or CO_2. I don't know how this relationship would apply in nature.

Figure 47. ***Leptodictyum riparium***, a species that can produce gametangia in continuous light. Photo by David T. Holyoak, with permission.

I find it interesting that the same nutrient status that favors gametangial production also favors vegetative growth in ***Bartramidula bartramioides*** (Chopra & Rahbar 1982). This was demonstrated using Knop's major nutrients plus Nitsch's minor nutrients at full strength with 1% sucrose. Perhaps the added sucrose gave it the energy it needed to support both.

A low nutrient status in the environment can trigger transport of nutrients from leaves to younger, growing parts in tracheophytes (Salisbury & Ross 1978), and Ogawa and King (1979) have shown that in *Pharbitis nil*, translocation of assimilate is essential for flowering. Perhaps translocation of assimilate accounts for the stimulus to produce gametangia under low nutrient conditions in bryophytes as well, but at present we have no clue that this occurs. By contrast, working with ***Bartramidula bartramioides***, Chopra and Rahbar (1982) showed that optimum conditions for induction of gametangia included full strength nutrient solution.

In ***Ricciella crystallina*** (Figure 22), there was no response in growth of thalli when calcium nitrate concentration was doubled or even quadrupled in Knop's solution (Sood 1974). However, increasing potassium nitrate cause a "considerable" increase in growth. Changing to ammonium nitrate or ammonium sulphate caused the formation of callus tissue. Fe-EDDHA and Fe-EDTA had no effect on thalli, but slightly increased production of archegonia (optimum at 10^{-5} M). Urea as a nitrogen source supported both robust growth and increased archegonial production. Amino acids likewise affected sexuality, with hydroxyproline, serine, threonine, asparagine, glutamic acid, alanine, and leucine causing production of more archegonia. Glycine, tryptophan, aspartic acid, and valine caused production of more antheridia.

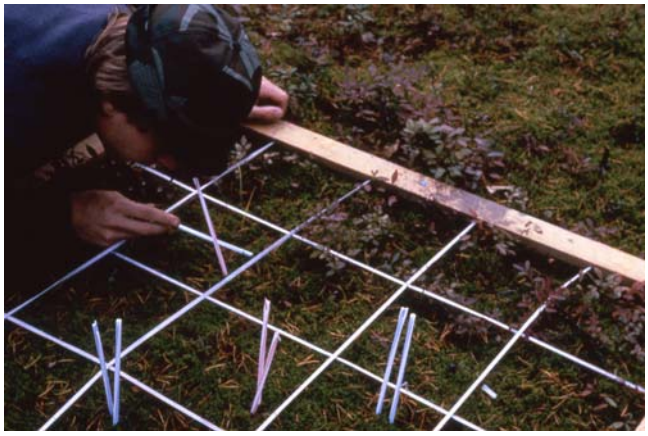

Figure 48. Geert Raeymaekers measuring distances between sporophytes on ***Pleurozium schreberi*** following simulated acid rain treatment. Photo courtesy of Geert Raeymaekers.

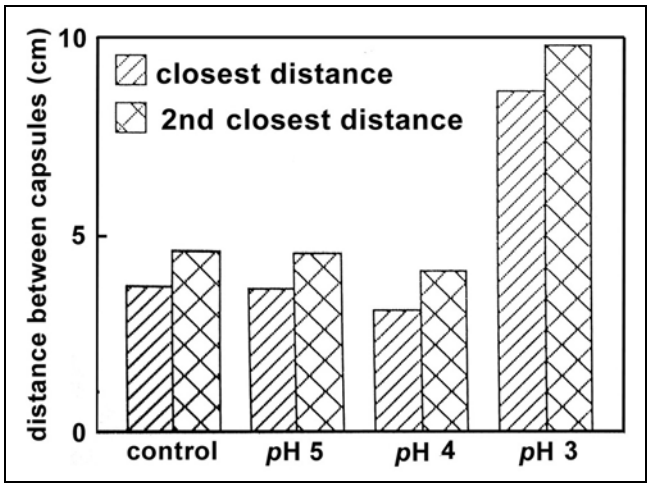

Figure 49. Comparison of distances between sporophytes in ***Pleurozium schreberi*** under simulated acid rain treatments. Redrawn from Raeymaekers 1986.

*p*H

Chopra and Bhatla (1983) concluded that bryophytes exhibit maximal gametangial initiation within a specific *p*H range, and that the *p*H of the medium changes during gametangial production. Bhatla (1981) found that a *p*H of 4.5 inhibited sexual induction in the moss ***Bryum argenteum*** (Figure 44-Figure 45). Raeymaekers (1986) found that a *p*H of 3.5 inhibited formation of capsules (Figure 48-Figure 49) in the acid-loving ***Pleurozium schreberi*** (Figure 50), thus indicating a possible connection with gametangia (Figure 51). Whether pH plays a role in induction of gametangia is unknown, but certainly low pH of acid precipitation can be detrimental to some mosses by interfering with sexual reproduction.

Rhabar and Chopra (1982) found that ***Bartramidula bartramioides*** produced more gametangia in liquid media than on semi-solid media. The two media exhibit different changes in *p*H, but these changes do not affect the time of gametangial induction. However, increasing *p*H, up to *p*H 7.0, increases the percentage of fertile gametophytes.

Figure 50. ***Pleurozium schreberi***, a moss whose sexual reproduction is sensitive to low *p*H. Photo by Bob Klips, with permission.

One interesting correlation in several species of ***Splachnum*** (Figure 66) is that low *p*H, along with low light and nutrient concentration, can favor males over females

(Cameron & Wyatt 1990). This results in clumps of one gender, but the changing *p*H with aging of the dung could favor a change in gender in later populations, ultimately resulting in the presence of both sexes on the same dung. In fact, the ratios on Isle Royale, Michigan, were typically 2:1 females to males.

In the eleven species of bryophytes from a Brazilian Atlantic Rainforest, Maciel-Silva *et al*. (2012) found that monoicous and dioicous species had different responses to *p*H. At sea level, the monoicous taxa were favored by a lower *p*H.

Figure 51. Archegonia of ***Pleurozium schreberi*** showing the loose perichaetial protection they have. Photo by Janice Glime.

Temperature

Temperature induces a variety of responses in flowering plants (Salisbury & Ross 1978), and we might expect even more variety in bryophytes, where some species remain active throughout winter even at high latitudes and altitudes. For example, ***Fontinalis hypnoides*** (Figure 52) produces more gametangia at 15°C than at 1, 5, 10, or 20°C (Glime 1982). Clarke and Greene (1970) showed that the reproductive response of ***Pohlia nutans*** (Figure 40) to day length is dependent upon temperature. In ***Leptobryum*** (Figure 23), low temperature is necessary for induction of antheridia, but once started they are independent of temperature (Chopra & Rawat 1977). On the other hand, for the thallose liverwort ***Ricciella crystallina*** (Figure 22), it appears that temperature is the overriding factor, provided there was a certain minimum photoperiod provided (Chopra & Sood 1973a).

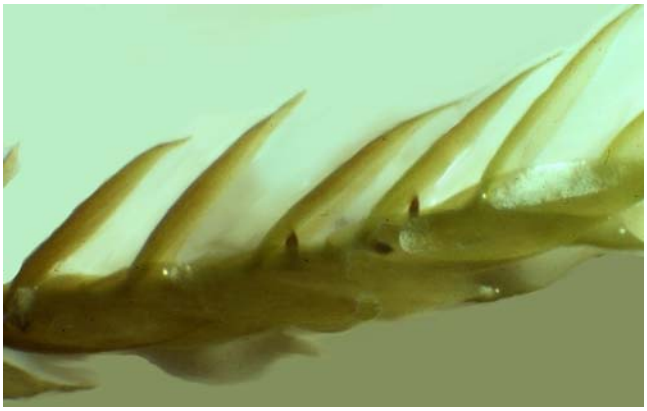

Figure 52. ***Fontinalis hypnoides***, a moss that produces maximum gametangia (seen in early development here) at 15°C. Photo by Janice Glime.

Chopra and Bhatla (1983) suggest that bryophytes operate in a range of temperatures, and that responses to light intensity and photoperiod might only operate within a range of temperatures that are broad in some species and narrow in others. Nevertheless, bryophytes do not seem to require any low temperature pretreatement for the induction of gametangia.

Hohe and coworkers (2002) found that both temperature and day length affect the expression of a MADS-box gene in ***Physcomitrella patens*** (Figure 53). In particular, one gene that was concentrated in the shoot apex and developing sporophytes produced higher RNA under conditions of 15ºC, 8 hours light per day, whereas vegetative growth was predominant at 25ºC, 16 hours light per day, suggesting that lower temperatures and photoperiod were important in sexual reproduction. This interdependence of temperature and photoperiod is an important way to coordinate gametangial production with the appropriate time for sporophyte development.

Figure 53. ***Physcomitrella patens***, a moss that responds to both photoperiod and lower temperatures for gametangial development. Photo by Jan-Peter Frahm, with permission.

Environmental Signalling Interactions

In many cases, perhaps most, the response to photoperiod or temperature or nutrients does not respond to just that one factor. The response is likely to differ in different geographic regions, and this can be the result of selection for a different factor as the trigger, perhaps because one factor cannot be expressed in this

environment. In ***Fossombronia brasiliensis*** (Figure 46), Chin *et al*. (1987) found that at 18°C the plants were short-day plants, requiring a night length of 6-12 hours. (Short-day plants typically are long night plants, measuring number of hours of darkness). When the temperature was only 10°C, this species became a quantitative short-day plant. But temperature also affected the gender expression, with more male gametangia being produced at 18°C and more female gametangia being produced at 10°C. Furthermore, the type of nitrogen available made a difference, with nitrate nitrogen causing production of more gametangia than did nitrogen in the form of ammonia.

In the dioicous moss ***Bryum argenteum*** (Figure 44-Figure 45), temperature, light intensity, and photoperiod all play a role in gametangial formation (Chopra & Bhatla 1981b). Both males and females produce the maximum gametangia at 25±2°C and in the light intensity range of 1800-2000 lux. At higher light intensities, vegetative growth occurs instead. If the temperature is lowered to 10±2°C, the response decreases. Chopra and Bhatla consider this species of ***Bryum*** to be a quantitative day-neutral plant because it is able to produce gametangia in as little as 8 hours of light, increasing production as the day lengthens.

The thallose liverwort ***Asterella tenella*** (Figure 54) requires the right conditions of both temperature and day length (Bostic 1981). For this species, **archegoniophores** (female reproductive branches) were induced under short days (10 hours) with 15°C daytime and 10°C nighttime temperatures.

Figure 54. ***Asterella tenella*** with archegoniophores. Gametangia are induced by short days in this species. Photo by Li Zhang, with permission.

Hormones

These physical cues must somehow be translated into biochemical responses. In the fern *Blechnum spicant*, gibberellic acid is known to illicit production of antheridia (Fernandez *et al*. 1997). In flowering plants, it can cause flowering. Since one known function of GA in flowering plants is increased water uptake (Salisbury & Ross 1978), this role might be important in maintaining an adequate internal water supply during gametogenesis of bryophytes.

Induction of **gametogenesis** by gibberellic acid is consistent with the role of GA_3 in increasing alpha-amylase activity, thus facilitating the metabolism of starch to sugar through hydrolysis. We know from the studies on ***Marchantia*** (Figure 10) (Maravolo 1980) that this starch conversion permits energy-supplying sugars to move to the actively growing regions such as gametangia. This sequel is so consistent with the need for sugar to maintain the sporophyte condition in callus culture (Bauer 1963b) and its requirement for gametophore production (Maravolo 1980), that one is tempted to accept this explanation alone. But how does this relate to photoperiod and temperature? And why do some plants respond to short days and others to long ones? I must conclude, as most flowering plant physiologists have done, that more than one substance is involved. In ***Fontinalis dalecarlica*** (Figure 33), the quantitative response to short days suggests a two-substance response – one present continuously and one that must accumulate as a function of photoperiod/light (Glime 1984).

Salisbury and Ross (1978) state that high auxin concentrations inhibit flowering and Benson-Evans (1961) found that auxins inhibit development of sexual organs in the thallose liverwort ***Conocephalum conicum*** (Figure 55). Growth substances such as 2,4-D and NAA induced receptacle formation but not gametangial production. Application of auxin at 16°C caused cell elongation of the archegoniophore, but not production of new cells. Therefore, it seems that gametogenesis might require the suppression of IAA.

Figure 55. ***Conocephalum conicum*** with antheridia whose development is inhibited by auxins. Photo by Malcolm Storey, through Creative Commons.

IAA seems to have other interesting reproductive functions. For example, in the dioecious hemp, IAA caused predominantly female sex expression (Chailakhyan & Khryanin 1978), but Salisbury and Ross (1978) point out that auxin levels and flowering seldom correlate in any meaningful way. In experiments on the leafy liverwort ***Cephalozia lunulifolia*** (Figure 56), kinetin + IAA inhibited sexual reproduction (Lockwood 1975). Tremaine and Glime (unpub. data) supplied IAA to ***Fontinalis duriaei*** (Figure 57) at concentrations of 10^{-6} and 10^{-8} M on a 12 hr light/12 hr dark cycle and there was no sign of gametangial initiation after 5 weeks. Yet this species usually produces gametangia during short days (personal observations).

Figure 56. ***Cephalozia lunulifolia*** with perianths (light color) enclosing archegonia. Photo by Michael Lüth, with permission.

Figure 57. ***Fontinalis duriaei*** archegonia, a species in which they fail to initiate with added IAA. Photo by Janice Glime.

Cytokinins can also play a role in sexual development. In the liverwort ***Riccia discolor***, 10^{-4} M kinetin proved to be the best concentration for promoting archegonial development as well as enhancing growth (Chopra & Gupta 1992).

Hormones may not affect the antheridial and archegonial inductions equally, possibly explaining how bryophytes manage to begin antheridial development long before archegonial development in most species. Chopra and Bhatla (1983) demonstrated that gibberellins contribute to the stimulation of antheridial formation in the bryophytes they investigated, whereas cytokinins stimulate archegonial induction while inhibiting antheridial induction in ***Ricciella crystallina*** (Figure 22) and ***Bryum argenteum*** (Figure 44-Figure 45). They found that auxins, gibberellins, and cytokinins can interact in controlling the gametangial response – no surprise there.

The hormone **IAA** may likewise have the opposite effects on the two sexes (Chopra & Bhatla 1983). In the thallose liverwort ***Ricciella crystallina*** (Figure 22), IAA increased archegonial induction, but in the mosses tested [***Bryum coronatum*** (Figure 3), ***B. argenteum*** (Figure 44-Figure 45), ***Barbula indica*** var. ***gregaria*** (Figure 2)], it favored antheridial induction.

Bhatla and Chopra (1981; Chopra & Bhatla 1981a) examined hormonal regulation of gametangial induction in ***Bryum argenteum*** (Figure 44-Figure 45) and found that both IAA and gibberellins (GA3) increase the induction of male gametangial branches while inhibiting the female clones in this dioicous moss. Cytokinins (kinetin, DMAAP) increased gametangial induction in the female clone while slightly inhibiting it in the male clone. When IAA and kinetin were both present, they were able to nullify the inhibitory capacity of each other. Cyclic AMP prevented kinetin from inhibiting male gametangial induction but stimulated the kinetin effect on females. ABA served as an inhibitor of both growth and gametangial induction in both sexes. Females proved to be more sensitive to ABA than males.

Cyclic AMP is one factor that may help in the control of hormone action and hence in controlling gametangial formation (Chopra & Bhatla 1983). This compound is a common mediator of hormone action in animals and is now known to increase gametangial induction in the moss ***Bryum argenteum*** (Figure 44-Figure 45). Cyclic AMP also increases antheridial induction in ***Bryum coronatum*** (Figure 3) and ***Barbula indica*** var. ***gregaria*** (Figure 2). To further confuse the investigator, it can overcome the inhibitory effects of ammonium ions and concentrations of sucrose that are too high, hence increasing gametangial formation, as Chopra and Bhatla have shown in ***Bryum argenteum***.

Environmental Hormone Interactions

Interactions with the environment can supply bryophytes with hormones, such as yeast extract and sex hormones from animals (Chopra & Bhatla 1983). These can increase the induction of both antheridia and archegonia.

Basile *et al*. (1969) found that the leafy liverwort ***Scapania nemorea*** (Figure 58) regularly associates with the bacterium ***Pseudomonas estorquens***. This association provides it with stimulation for both larger growth and earlier reproductive maturity than sterile cultures.

Figure 58. ***Scapania nemorea***, a liverwort that associates with ***Pseudomonas estorquens*** that stimulates earlier reproductive maturity. Photo by Li Zhang, with permission.

Sugars

Chopra and Rhabar (1982) found that sugar (1%) was necessary for gametangial induction in ***Bartramidula bartramoides***. On the other hand, ***Bryum argenteum*** (Figure 44-Figure 45) has markedly lower gametangial induction in 4% sucrose (Bhatla & Chopra 1979). Adding cyclic 3',5'-AMP neutralized the effects of the sucrose, but the concentrations are different for male (10^{-7}) and female (10^{-5}).

Overall Physiology

In summary, metabolic changes are needed for the initiation of gametangia (Chopra & Bhatla 1983). Liverworts may have an increase in cellular levels of carbohydrates, auxins, RNA, and proteins as the gametangial development begins. Enzymes and their concentrations change. Phenolic compounds change. And new colors develop. Reynolds and Maravolo (1973) found that two of the phenolic compounds inhibited IAA oxidase activity and two enhanced it in ***Marchantia polymorpha*** (Figure 10). The significance of this interaction in gametangial development seems still to be a mystery.

Both vegetative growth and gametangial development are regulated by and favored by iron and copper chelating agents such as EDTA and EDDHA (Chopra & Bhatla 1983). But it is interesting that in ***Riccia*** (Figure 24) these chelates favor archegonial development more than antheridial formation, whereas the opposite is true in ***Bryum argenteum*** (Figure 44-Figure 45) (Chopra & Bhatla 1983). Salicylic acid (the effective compound in aspirin) inhibits gametangial formation in most bryophytes, probably by chelating iron and copper or other metals involved in needed enzymes. We know that in ***Bryum argenteum*** there are marked changes in iron and copper levels. Iron seems to induce the reproductive phase, but copper inhibits it. In ***Bartramidula bartramioides***, on the other hand, salicylic acid enhances both vegetative growth and gametangial formation.

Cyclic AMP enhances antheridial production in the moss ***Bryum coronatum*** (Figure 3) and ***Barbula indica*** var. ***gregaria*** (Figure 2) and overcomes the inhibitory effects of ammonium ions and high levels of sucrose on gametangial development in ***Bryum argenteum*** (Figure 44-Figure 45) (Chopra & Bhatla 1983).

In an attempt to understand the physiological changes leading to development of gametangia in liverworts, Rao and Das (1968) studied ***Exormotheca tuberifera***, ***Plagiochasma articulatum***, ***Reboulia hemisphaerica*** (Figure 59), ***Fimbriaria angustata***, and ***Pallavicinia canara***. In ***Fimbriaria angustata***, a sharp rise in respiration and a doubling of the C:N ratio accompanied the transition from vegetative to reproductive state in females. Formation of archegoniophores occurred with an increase in the plant's own IAA, RNA, and protein. Carbohydrates accumulated in the archegoniophore at the expense of the gametophyte as the sporangia developed. By contrast, the antheridial production was correlated with a decrease in levels of IAA, RNA, and protein, and unlike the females, there was no notable increase in the C:N ratio.

Color Changes

Both antheridia and archegonia are often recognizable first by the addition of red coloration as they develop. In archegonia, this is often present in the neck canal cells (Figure 33, Figure 57). In antheridia, the color can be so intense that it is visible through the surrounding leaves, making branch tips red in some species of ***Sphagnum*** (Figure 60). In ***Marchantia berteroana*** (Figure 61), production of the flavone acacetin stops and instead 8-hydroxyapigenin and 8-hydroxyluteolin glycosiduronic acids (previously absent) become the predominant flavonoids (Markham *et al*. 1978). Acacetin seems instead to be important during the asexual phase.

Figure 59. ***Reboulia hemisphaerica*** male & female gametangiophores. Photo by Bob Klips, with permission.

Figure 60. ***Sphagnum*** with red antheridial branches. Photo by Janice Glime.

Figure 61. ***Marchantia berteroana*** antheridial heads showing red color. Photo by Clive Shirley, Hidden Forest, with permission.

Delay of Gametogenesis

But suppose that gametogenesis is **not** a process to be **initiated**, but rather it is a natural process that **must be stopped**. Sexual reproduction is ancient. It no doubt began with like cells bumping into each other and managing to stay together long enough to fuse. No special

male and female existed; no special inducers were needed. Perhaps something was needed to cause the two membranes to lose their integrity at the region of contact. Then the process became more sophisticated. Attracting substances drew cells together; different strains arose, some repelling and others attracting. Ultimately, special structures housed these one-celled gametes, and then some control was possible. As this scenario continued, the process became more complex and more controlled. The joining and dividing cycle of primitive cells was then subject to controlled delays. Whole sequences of differentiation were interjected to delay the sexual process. These sequences are the ramifications by which we identify species, genera, even phyla of plants. Therefore, it is reasonable that gametogenesis is controlled by inhibitors, factors of the surrounding tissues that retard gamete production and allow productivity of the organism to increase.

It follows that the multitudinous environments for the many species have caused this problem to be solved in multitudinous ways (see Stebbins & Hill 1980). Thus in one species a high concentration of IAA prevents gametogenesis, whereas in another the lack of alpha-amylase or GA deprives the prospective gametangia of the necessary energy source. As long as the raw ingredients (*e.g*. energy, nitrates, amino acids) are being diverted to other sources, gametogenesis is retarded. Such a multitude of ways can accomplish this that surely no consistent pattern could be recognized or even expected. The possibilities of combinations of concentrations and mobilities necessary to override the limits caused by the parent plant are almost limitless.

Male vs. Female

It is often considered a paradox that bryophytes tend to have female-biased sex ratios, whereas flowering plants usually have male biased sex ratios (Rydgren *et al*. 2010). Early control over gender was most likely simple. Internal environment may have been important. For example, Bhandari and Lal (1968) observed abnormal archegonia in ***Physcomitrium immersum*** that behaved as antheridia. Each had an egg, ventral canal cell, and neck canal cells as would be found in a normal archegonium, but in some these divided repeatedly, forming instead a mass of antheridial cells. They suggested that this is evidence of common origin of the two sexual organs.

Such behavior is somewhat suggestive of sex determination in maple (*Acer*) flowers. In these plants, the concentration of plants affects the ethylene concentration as the flower develops and determines the sex ratio by abortion of one of the parts. Factors related to sex ratio in bryophytes have been discussed in the chapter on sexuality. Therefore, they will be covered only briefly here.

We have noted that bryophytes, or at least many of them, do have sex chromosomes, a phenomenon known for plants first in the liverwort genus ***Sphaerocarpos*** (Figure 62) (Allen 1930; Anderson 2000). The gender is expressed only in the gametophyte generation by having either a small Y chromosome (male) or an X chromosome (female). This determination is made at meiosis, providing two male and two female spores. The monoicous (bisexual) taxa seem to have been derived mostly from polyploidy in which the chromosome number is duplicated and both X and Y chromosomes are present.

Figure 62. ***Sphaerocarpos michelii***, member of the genus where X and Y sex chromosomes were first discovered. Photo by Jan-Peter Frahm, with permission.

When the sexes are separate, *i.e*. dioicous/unisexual taxa, it is not unusual to find all male or all female populations, derived from a single spore carrying genes for only one gender. In other cases, one gender may outcompete and overgrow the other. Such is the case with ***Marchantia papillata*** subsp. ***inflexa*** (Figure 63), a dioicous thallose liverwort that lives on rock and bark surfaces (McLetchie *et al*. 2001). In this case, the females seemed to benefit from light to moderate disturbance and gradually eliminated the males. However, at high disturbance levels, the males dominated. This change in dominance seemed to result from dispersal of gemmae within the patch. We have seen in the brood body chapter that females typically produce fewer gemmae, instead spending energy to support the female reproductive organs and developing sporophyte.

Figure 63. ***Marchantia papillata*** subsp. ***inflexa***, a species in which females can outcompete males in disturbed areas. Photo by Scott Zona, with permission.

McLetchie *et al*. (2001) found that in ***Marchantia papillata*** subsp. ***inflexa*** (Figure 63) spores were needed to colonize large areas following disturbance, and that sexual reproduction predominated. However, as the population grew and the space became fully occupied, reproductive effort shifted to less sexual and more asexual means. Does this strategy predominate? It would seem more advantageous to reproduce asexually to fill the area, then reproduce by more widely dispersed spores when it gets crowded.

Maintaining the sexual specificity can get complicated in regenerants. Bauer (1963a) explained that sex determination in regenerated tissue can take two forms:

1. Sex determination is restored following de-differentiation, as in ***Funaria hygrometrica*** (Figure 18-Figure 19).
2. Sex determination is disturbed, causing the sexual balance to remain permanent or to slowly return to normal, as seen in members of **Splachnaceae** (Figure 64).

In the **Splachnaceae**, as the tendency toward femaleness is weakened, the male expression becomes more common until eventually only male plants can arise (Bauer 1963a). Surprisingly, this can occur even in species such as ***Splachnum rubrum*** (Figure 64) wherein sex determination is genetic. This species produces dwarf males, but these are usually sterile. The change in gender from vegetative offspring could be from cytoplasmic or genetic changes. However, Bauer reasoned that the constant changes among intermediate kinds of sex determination provides evidence against gene mutation.

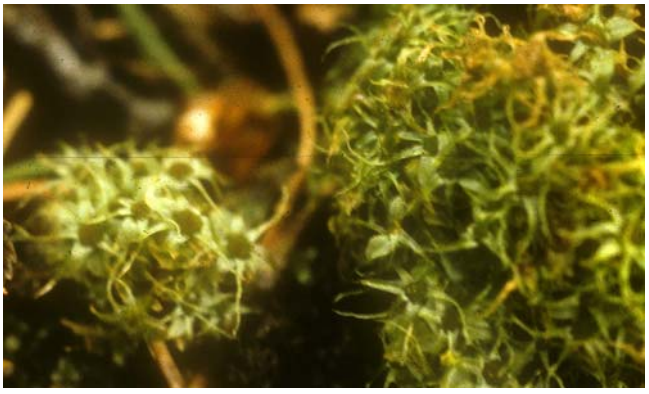

Figure 64. ***Splachnum rubrum*** with antheridial splash platforms, a species where gender is genetically determined. Photo by Janice Glime.

Rydgren *et al*. (2010) explored the ability of maintaining a female-biased sex ratio by testing it in ***Hylocomium splendens*** (Figure 68), a dioicous pleurocarpous moss that is common on the boreal forest floor. They found that males had a slightly lower production and survival of vegetative offspring than did the non-sporophytic females. This bias is important in a species such as this where sporophytes are uncommon. The slightly better success of males permitted them to expand into female clones, thus facilitating reproduction.

Differential Survival

Not all sex ratio differences are the result of adult competition. Shaw and Gaughan (1993) studied eleven populations of the moss ***Ceratodon purpureus*** (Figure 65) and found that at the time of germination female gametophytes outnumbered males 3:2, suggesting differential survival rates of spores or germlings. Furthermore, female clones formed much more biomass than did male clones, further increasing the bias. Nevertheless, male clones produced more stems, permitting them to provide additional gametangia and sperm.

Figure 65. Prolific production of capsules exhibited by ***Ceratodon purpureus***, suggesting a predominance of females. Photo by Michael Lüth, with permission.

Sex ratio can often change dependent upon growing conditions, even in species where gender of an individual is genetically predetermined. Shaw and Beer (1999) observed that despite chromosomal sex determination in ***Ceratodon purpureus*** (Figure 65) that would produce equal numbers of male and female cells at meiosis, the sex ratio varied considerably among families of offspring. Some genetically identical individuals (*i.e.*, grown from a single spore) that maintained a nearly 1:1 gender ratio had progeny that produced either predominately male or predominately female offspring.

This discrepancy between offspring sex ratios of two families of siblings suggests that there is a differential germination of spores, most likely related to environmental factors. Additional factors that may be relevant are the differences in size, maturation rates, and reproductive output of the male and female gametophytes in this species.

One factor that can account for highly biased sex ratios is simply the gender of the spore that lands there. Generally, one spore will produce multiple gametophores of one gender. However, Cameron and Wyatt (1990) rejected this as an explanation of the highly biased sex ratio in ***Splachnum***. They concluded that the unbiased and abundant dispersal by flies precluded such a bias by ensuring that both genders would arrive on the substrate. But even more interesting is the fact that in ***Splachnum ampullaceum*** (Figure 66), a single spore can give rise to both male and female gametophores. Instead, it is low light, *p*H, and nutrients that favor production of males over females.

Figure 66. Massive number of capsules of the dung moss ***Splachnum ampullaceum*** resulting from the guaranteed close proximity of males. Photo by Michael Lüth, with permission.

There is some evidence that at least in some bryophytes gender may be determined like that of crocodile eggs – by temperature. For the liverwort ***Sphaerocarpos texanus*** (Figure 67), sex ratios showed female bias among spores that broke dormancy after treatment at 25/15°C for 1-8 weeks (McLetchie 2001), despite a 1:1 ratio of male:female among spores produced (McLetchie 1992). In both field and laboratory-grown cultures, pure female clones were most common, followed by mixed sex, and least frequently, pure male (McLetchie 1992). It appears that the male spore has a lower survival and germination rate that continues into the gametophyte stage.

There seems also to be a physiological gender bias that depends in part on ecological conditions. In ***Mnium hornum*** (Figure 77) and ***Plagiomnium undulatum*** (Figure 76), only female regenerants from fragments survived desiccation (77%) (Newton 1972b). Such a strategy could soon create a population of predominantly females.

McLetchie and coworkers (2001) demonstrated that competitive interactions between genders could account for some sex differences at gametophyte maturity in the dioicous thallose liverwort ***Marchantia papillata*** subsp. ***inflexa*** (Figure 63) in Trinidad. Using differential equations, they modelled interactions of the two genders under various disturbance regimes. They found no way to stabilize the sex ratio, but rather, under conditions of low to moderate disturbance, females would gradually eliminate males. Under high disturbance conditions, males would eliminate females. Successful germination of gemmae dispersed within the patch played an important role. Since females of this species have only a narrow window in which to produce gemmae without interfering with energy needed for sexual reproduction, they would have less opportunity for successful gemma dispersal and establishment under large disturbance, but under conditions of small disturbance, already established female thalli might be able to outgrow male thalli. Although gemmae appear to be the most important means of maintaining replacement due to disturbances within patches, spores are the primary means for colonizing areas of major disturbances. Production of spores among initial colonizers when the patch becomes fully occupied is maximal, but that production subsequently declines as the sex ratio drifts toward one or the other gender.

Additional information on the costs and tradeoffs of producing archegonia vs antheridia is covered in Chapter 3 of this volume.

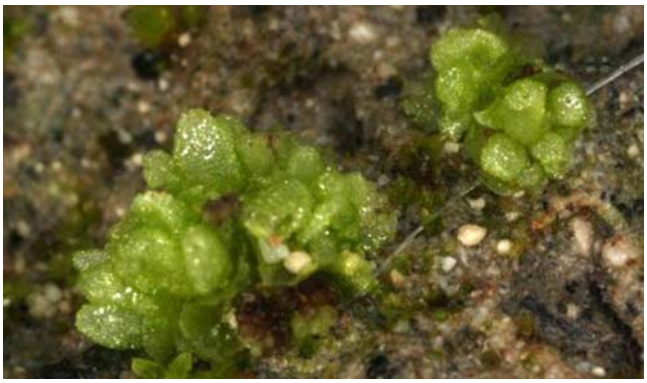

Figure 67. ***Sphaerocarpos texanus***, a species in which females seem to outcompete males. Photo by Jan-Peter Frahm, with permission.

Bisexual Gametangial Differentiation

Differentiation of a single cell such as a spore ultimately into an organism with cells of many functional types is always intriguing. Differentiation of parts of an organism into male and other parts into female is no less intriguing. What determines which branch, which gametangial cluster, will become male and which female? If we can understand these processes in plants, perhaps we can begin to understand the complexities that contribute to cross-gender behavior in humans.

Using the dioicous ***Hylocomium splendens*** (Figure 68) as a model, Rydgren and Økland (2002) examined a Norwegian population for five years. During that time, the tissue devoted to gametangia differed. That population had a 4:1 ratio of male to female branches. Of those females, ~30% produced sporophytes. Production of sporophytes varied three-fold during the five years, relating to weather favorability for growth and development. Large segments with high relative growth rates were more likely to produce sporophytes, with a distinct lower size threshold. Although the size limit increased in years with low sporophyte production, the lowest limit was ~2 mg segment dry weight. Furthermore, production of sporophytes was much greater in upper parts of plants, regardless of size differences, suggesting a role for light in initiation of sexual branches.

Figure 68. ***Hylocomium splendens*** bearing sporophytes. Photo by Janice Glime.

Hormonal Regulation of Gender

In some trees, such as *Acer*, ethylene concentration affects the male:female ratio. But in the small space of a bryophyte mat, could such a high concentration accumulate? There seems to be no evidence that packing of gametophytes, hence higher ethylene production, is a sex determinant. Nevertheless, lab evidence demonstrates that ethylene control is a possibility. Location of sexual structures on the bryophyte could result from a balance among IAA, ethylene, and GA_3. Chopra and Sood (1973b) showed that GA_3 plus ethrel (which produces ethylene in water) enhanced antheridia production, whereas IAA + cyclocel (CCC) enhanced archegonia production in ***Ricciella crystallina*** (Figure 22). This is consistent with the role of IAA in favoring femaleness in flowers (Salisbury & Ross 1978). If this relationship holds true, a strong apical dominance, concomitant with apical production of IAA, should produce archegonia at the apex. This is exactly the correlation seen in acrocarpous mosses. Conversely, lack of apical dominance should result in archegonia on side branches, as we see in pleurocarpous mosses. However, Schofield (1985) reminds us that IAA is not involved in sex determination in the same way in all taxa, inducing female sex organs in the liverwort ***Riccia*** (Figure 24) and male organs in the mosses ***Barbula*** (Figure 2) and ***Bryum*** (Figure 45). Because it is common in the environment, IAA could serve as an environmental control, interfering with sexual coordination and hence sporophyte production for some taxa in some habitats. It is likely that hormones interact and that concentrations or relative concentrations are important in gender determination.

Dwarf Males

Dwarf males present an interesting modification to sexual differentiation. In theory, the presence of dwarf males should increase the success of fertilization for a species, particularly among dioicous taxa. However, in two habitats in Michigan, USA, the presence of dwarf males had no significant impact on sporophyte production of dioicous mosses (Rohrer 1982). Dwarf males have been discussed in detail in Chapter 3; this chapter will concentrate on physiological relationships.

In the moss ***Trachybryum megaptilum*** (=***Homalothecium megaptilum***; Figure 69), males are typically dwarf, but this is a function of being on a female plant (Wallace 1970). Occasional full-sized males are found growing alone, but dwarf males never occur on these full-size males. Despite differences in gametophore appearance, there is no morphological difference between male and female spores. Wallace suggested that some substance released from the female plant might inhibit growth of the male plant.

In ***Dicranum*** (Figure 70), it appears that female plants present a growth-inhibiting substance that keeps their epiphytic males small (Loveland 1956). On the other hand, in ***Macromitrium*** (Figure 71) it is genetically determined in those taxa that are truly **anisosporous** (having a bimodal distribution of spore sizes with smaller spores generally producing males), whereas isosporous taxa again seem to be affected by hormones from females (Une 1985). Auxin, applied as 2,4-d, results in dwarf males, suggesting again a role for IAA.

Figure 69. ***Trachybryum megaptilum***, where dwarf males form on female plants. Photo through Creative Commons.

Another puzzle that has physiological implications suggesting hormonal concentration gradients is development of morphs among gametangia of a single reproductive head. In ***Plagiomnium medium*** (Figure 72), antheridia typically surround archegonia. In the border zone between the two sexes, Bryan (1927) always found at least one abnormal gametangium in each of the 100's of heads examined, from nearly perfect to possessing a combination of antheridial and archegonial cells. This likewise suggests some sort of hormonal control that involves concentrations or interaction – or both.

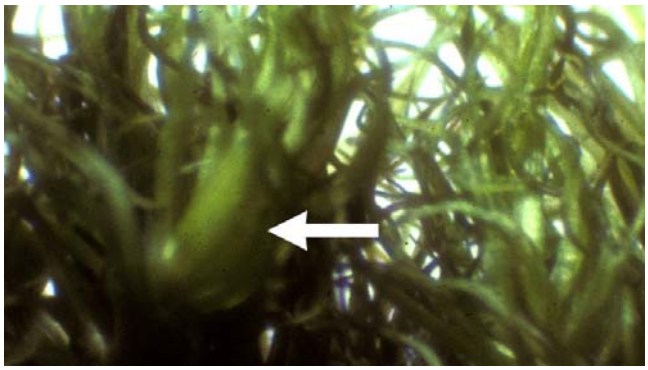

Figure 70. Dwarf male (**arrow**) of ***Dicranum polysetum*** growing on a female plant. Photo by Janice Glime.

Figure 71. ***Macromitrium piliferum*** with capsule, an autoicous moss in a genus where isosporous spores may form dwarf males in the presence of auxin. Photo by Jan-Peter Frahm, with permission.

Figure 72. ***Plagiomnium medium***, a moss in which antheridia usually surround the archegonia. Photo by Jan-Peter Frahm, with permission.

Different Controls

One consequence of sexual differences is that antheridia and archegonia can be under different controls. This can result in maturation of males and females at different times, perhaps accounting for sterility in many populations. Allsopp (1964) suggested that nutritional factors cause male and female production at different times on monoicous species. Lockwood (1975) found that amino acid additives promoted maleness and inhibited femaleness in ***Cephalozia lunulifolia*** (Figure 56); ammonium nitrate plus citrate also inhibited female gametangia. Machlis (1962) found that males of ***Sphaerocarpos donnellii*** (Figure 73) dropped the *p*H of their media from 5.3 and 7.1 to 4.1 in 15 days, whereas females raised the *p*H, suggesting physiological and possibly nutritional differences. Riemann (1972) suggested that mild, humid winters may result in maturation of the male and female of ***Rhytidiadelphus triquetrus*** (Figure 74) at different times, whereas harsh winters regulate their timing. Berthier (1966) has shown that antheridial production in ***Fontinalis*** (Figure 75) is greater under conditions of minimal growth and greater dominance by the main axis; fewer antheridia occurred in high light at 15ºC, whereas 8ºC and 90% light produced the most antheridia. It is likely that a wide variety of these mechanisms play a role in **protandry** (male gametangia mature first) and **protogynandry** (female gametangia mature first) among bryophyte species.

Figure 73. ***Sphaerocarpos*** sp. ***Sphaerocarpos donnellii*** can lower the *p*H of its medium to 4.1. Photo by Belinda, through Creative Commons.

Figure 74. ***Rhytidiadelphus triquetrus***, a moss that may have males and females mature at different times when winters are mild but mature together when they are harsh. Photo by Janice Glime.

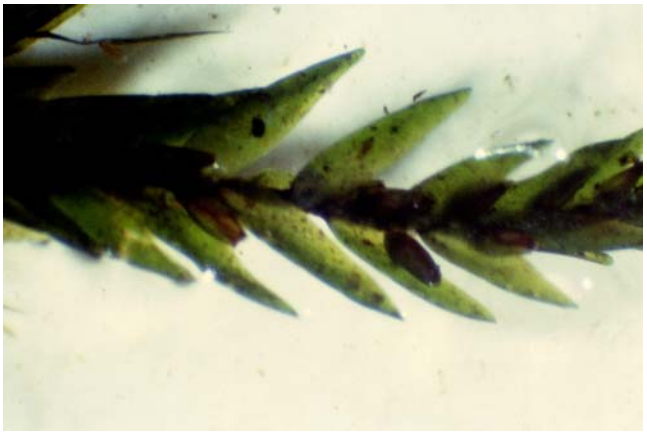

Figure 75. ***Fontinalis antipyretica*** var ***gigantea*** with perigonia (male). Antheridia are produced during times of minimal growth. Photo by Janice Glime.

One selective factor at work is that antheridia generally require a longer time to mature than do archegonia, as discussed earlier regarding phenology. Therefore, they must begin development sooner to mature when the population of female structures is receptive. ***Plagiomnium undulatum*** (Figure 76) has met this challenge with very different signals to initiate gametangia. Males require long days and diurnal temperature fluctuations to produce gametangia, whereas females require a short 7.25- to 12-hour day at 10ºC or warmer (Newton 1972a). We have already seen that more male gametangia are produced by the liverwort ***Fossombronia brasiliensis*** at 18ºC and more female gametangia at 10ºC. These differences in temperature and/or photoperiod can permit male gametangia to start development sooner and be ready when female gametangia are ready. But such timing differences meet new challenges when spores are dispersed to new locations where timing of day length and temperature can be different from that of the parent location, so that males and females are no longer synchronized. If only vegetative reproduction follows after introduction by a single spore, no opportunity arises for selection of synchronized variants; instead the species may persist sterile for centuries.

Figure 76. Vegetative branches of ***Plagiomnium undulatum***. Photo by Michael Lüth, with permission.

Newton (1972a, b) found that male and female plants themselves differed in photoperiod response in ***Mnium hornum*** (Figure 77). In short days, the number of males and females was about equal, but in intermediate and long days significantly more female plants arose from buds and produced mature plants. Even regeneration favored females. Thus, in northern areas where sufficiently warm temperatures may occur only during long days, a disproportionate number of females can result. This shifts the population toward dependence on regeneration, due to insufficient fertilization, further promoting females. Similarly, Longton and Greene (1969a,b) found that ***Pleurozium schreberi*** (Figure 50-Figure 51) produced capsules predominantly in coniferous forests due to lack of males elsewhere.

Figure 77. Male plants of ***Mnium hornum***, exhibiting a splash platform. Photo by Michael Lüth, with permission.

The problem of separate stimuli for the two sexes is further complicated by non-sex-related selection pressures after dispersal. Van Zanten and Pocs (1981) concluded that monoicous species of Jungermannidae have better dispersal than dioicous species because the percentage of monoicous species with a bipolar distribution is greater than that of dioicous species. This is reasonable since production of spores is more likely to occur in monoicous taxa, and these are dispersed more easily than asexual propagules due to the smaller size of spores. Since selection pressures related to sporophyte development are absent in isolated dioicous individuals, selection pressures would revert to gametophyte benefits. These could easily be different in male and female plants because of different amounts of time required for development of male and female gametangia.

Day length and **temperature** influence the onset of gametogenesis, and we have good evidence that responses to these variables vary within a species throughout the world (Monroe 1965, Clarke & Greene 1970). In dioicous species, vegetative reproduction is the only mechanism for reproduction until a second spore arrives. By that time environmental selection pressures and genetic drift in these small populations have had ample time to make the two sexes uncoordinated. If the signal for gametogenesis is different in the two sexes, there might never be an opportunity for the two gametes to meet. I would predict what van Zanten and Pocs (1981) have already illustrated, that long range dispersal of dioicous species would result in a large number of physiological species with low or no sexual reproduction.

All of these controlling factors suggest that Dan Norris may have been right in his comments to Bryonet on 2 May 2003 – the conditions of monoicy and dioicy and all their subsets may not be as distinct as we present them. The expression of gender may be under control of the environment and not any predetermined genetic distinction.

Numbers of Gametangia

Although each female branch typically produces only one sporophyte, archegonia occur in clusters within **perigonia**. One might ask why all this wasted energy to produce multiple archegonia if only one is successful. Even if all get fertilized, only one embryo succeeds in emerging from its archegonium. Could it be that multiple archegonia are needed to produce sufficient attractant for the sperm to find the location? Or might there be dangers lurking as sperm enter the archegonia, making backups necessary? Have we examined them closely enough to know that all eggs are simultaneously receptive, or might it be that this is a way to insure that one of the eggs is ready at the time of successful sperm dispersal?

The male gametangia generally outnumber female gametangia, but not always (Table 1). Since males must disperse the sperm, with nearly all of them being unsuccessful in fertilizing an egg, large numbers are necessary to provide enough chances for a few to succeed. Note in Table 1 that the ratio of male to female gametangia is considerably higher in the dioicous taxa.

Table 1. Mean number of gametangia per inflorescence, based on data for inflorescences that had gametangia in immature to dehisced stages. From Une & Tateishi (1996).

Physcomitrella patens subsp. *californica*	♀	2.0	Paroicous	Une & Tateishi 1996
	♂	7.2		
Astomum crispum	♀	3.3	Autoicous	Deguchi & Hidaka 1987
	♂	14.1		
Aulacopilum japonicum	♀	2.2	Autoicous	Deguchi & Hidaka 1987
	♂	3.3		
Venturiella sinensis	♀	3.6	Autoicous	Deguchi & Hidaka 1987
	♂	5.1		
Fabronia matsumurae	♀	2.7	Autoicous	Deguchi & Hidaka 1987
	♂	5.8		
Entodon challengeri	♀	5.5	Autoicous	Deguchi & Hidaka 1987
	♂	8.0		
Pogonatum inflexum	♀	3.4	Dioicous	Imura 1994
	♂	64.4		
Atrichum rhystophyllum	♀	4.6	Dioicous	Imura 1994
	♂	76.4		
Trachycystis microphylla	♀	9.8	Dioicous	Imura & Iwatsuki 1989
	♂	43.1		
Bryum argenteum	♀	5.5	Dioicous	Miles *et al*. 1989
	♂	10.6		
Pleurozium schreberi	♀	8.2	Dioicous	Longton & Greene 1969a
	♂	6.1		

In the survey of literature presented by Une and Tateishi (1996), ***Pleurozium schreberi*** (Figure 50-Figure 51) had more female than male gametangia per inflorescence, and ***Bryum argenteum*** (Figure 44-Figure 45) had little difference between them. Perhaps this is possible because these species are so successful at vegetative reproduction. In Canada, large geographic areas have only one gender of ***Pleurozium schreberi***, yet the species is still quite successful. ***Bryum argenteum*** is easily spread by broken tips.

Gender Recognition

Recognizing the gender of a bryophyte is often difficult if reproductive structures are absent. For mosses like ***Polytrichum***, old splash cups may be present, with new growth proceeding from the center (Figure 78). But even these can eventually change sex and thus determination of the sex of the moment may be less convincing. Size often plays a role, but this is affected by growing conditions as well, so one must assess it for each population. In ***Marchantia polymorpha*** (Figure 10), the male plants are narrow compared to females if one examines the thallus ~1 cm back from the tip, but then one needs both genders at hand to make the assessment (Voth 1941). Voth has observed another difference that I have not confirmed – the female plants have a smoother upper surface and reflect more light than male plants, at least in culture, but again, one really needs the male plants for comparison.

Figure 78. ***Polytrichum juniperinum*** with new growth from the antheridial splash cups. Photo by Janice Glime.

Yet, somehow, through biochemical means, a sperm is able to recognize a female of its own species, be it on a separate plant or the same one, and travel in that direction. As discussed in the chapters on life cycles of bryophytes, this recognition is facilitated by a concentration gradient from the disintegrated neck canal cells of the archegonium. But the nature of that exudate, and particularly what makes it specific for that species, remains a mystery.

Fertilization

Success of fertilization varies widely from very successful monoicous annual taxa to poorly successful dioicous perennials (Rohrer 1982). Rohrer found that success varied by habitat, with only 19.3% of the populations of the dioicous, vs 75.9% of monoicous taxa producing sporophytes in a dry aspen (*Populus*) forest. In a wet coniferous forest, the success of monoicous taxa increased to 84.1%, whereas that of dioicous taxa decreased to 12.3%. Surprisingly, having dwarf males epiphytic on female plants did not significantly increase the production of sporophytes in dioicous taxa.

Although several archegonia are typically present on a branch or stem tip, in most species only one sporophyte develops. Stark and Castetter examined the archegonia of ***Trichostomum planifolium*** (= ***T. perligulatum***) at the end of the fertilization season and found that 8% of the archegonia and 7% of the antheridia were abortive. In 13 of the 47 fertilized perichaetia they examined, there was at least one aborted embryo in addition to the developing embryo. Only two had more than two fertilized archegonia. There were no cases where more than one embryo developed. The abortions were all in early developmental stages. Hughes (1979) found that in ***Phascum cuspidatum*** (Figure 37) archegonial initiation ceases when one of the archegonia has been fertilized. The archegonial abortion raises the question of causes of this abortion. Is there an inhibitory substance produced by the first developing embryo that stops the others? Is there insufficient energy for more than one to continue? Could the hybrid status enter into the success or failure?

A more in depth discussion of fertilization is in Chapter 3.

Self-incompatibility

Fertilization is the termination of the gametogenesis development phase. Successful fertilization must be followed by successful development of the embryo to the mature sporophyte. We know that seed plants have a variety of mechanisms that prevent self-fertilization, either as prezygotic mechanisms that prevent the sperm from reaching and penetrating the egg or from postzygotic mechanisms that interfere with development of the embryo or mature sporophyte. This self-incompatibility has barely been explored in bryophytes.

We have suggestive evidence that self-compatibility exists among bryophytes. Boisselier-Dubayle *et al*. (1996) found the monoicous leafy liverwort ***Plagiochasma rupestre*** (Figure 79) to be self-compatible based isozyme markers of progeny. Lazarenko and Lesniak (1972) cultured two species of ***Desmatodon*** to determine their self-compatibility. ***Desmatodon cernuus*** was sterile in 24 hours of light, being a long-day plant at 16 hours of illuination and requiring low temperatures in the dark for normal sporophyte development. On the other hand, ***Desmatodon ucrainicus*** was completely self compatible in 24 hours of light, successfully producing sporophytes in single-spore cultures. However, this study raises a caution. One must reproduce the conditions of gametangial development, fertilization, and sporophyte development to test self-compatibility or other conditions involving reproduction.

When a spore travels to a new geographic area, it can encounter changes to the environmental signals needed for its normal development. Failing these signals, the reproductive state might never be initiated. Absence of such developmental signals seems to interfere with sexual reproduction in ***Desmatodon cernuus*** (Lazarenko & Lesniak 1972).

Jesson *et al*. (2011) considered that both polyploidy and monoicism could strongly depress inbreeding. They tested this in 21 populations of ***Atrichum undulatum*** (Figure 80). In one population, using allozyme markers, they found that the rates of selfing were greater than zero, despite the population having only one-third monoicous individuals. Lazarenko (1974) found that an inbred clone of ***Tortula cernua*** (=***Desmatodon randii***; Figure 81) was able to persist through 15 generations. This clone also gave rise to a sterile line that thus forth reproduced vegetatively, but also by producing apogamous capsules through 14 generations because the few spores, despite lacking an exosporium, were able to germinate. These studies suggest that self-incompatibility is not strong among bryophytes and that self-fertilization is possible.

Figure 79. ***Plagiochasma rupestre***, a self-compatible monoicous liverwort. Photo by Michael Lüth, with permission.

Figure 80. ***Atrichum undulatum*** males with splash cups and antheridia. This is a long-day plant. Photo by Janice Glime.

Figure 81. ***Tortula cernuua*** with capsules, a species that can survive 15 generations of inbreeding. Photo by Lars Hedenäs, with permission.

Stark (1983) reported that the autoicous ***Entodon cladorrhizans*** (Figure 82) was self-fertile and protandrous on a given stem. He found that approximately 90% of the perichaetia developed sporophytes and that this was independent of the number of perichaetia per stem, attesting to a high success rate for fertilization. Since only one archegonium typically develops a mature sporophyte in any given perichaetium, this is a good percentage. Self-fertilization is evidenced by significantly higher frequency of fertilization on bisexual stems than on those with only perichaetia, by the tendency for unfertilized perichaetia to be near the end of the stem away from perigonia, and by the highest fertilizations occurring on stems with perigonia.

Figure 82. ***Entodon cladorrhizans***, an autoicous moss with abundant sporophytes. Photo by Bob Klips, with permission.

Trichostomum planifolium is a protogynous monoicous desert moss, but it has a period of gametangial overlap, ending with a period of only ripe male gametangia

(Stark & Castetter 1995). Based on their observations of the population in southern New Mexico, USA, Stark and Castetter concluded that this moss is self-compatible, with common occurrences of fertilization from gametangia on the same stem. They supported this conclusion by the fact that stems that lacked a sporophyte had fewer antheridia and had no perigonia (n=3) and that all stems that produced sporophytes had at least one perigonium. The evidence is circumstantial and not definitive, but does suggest self-compatibility.

Zieliński (1986) used two peroxidase alleles to indicate presence of self-fertilization. He found that 38 of the 40 progeny examined in ***Pellia epiphylla*** (Figure 83) subsp. ***borealis*** were monomorphic for one of the two alleles involved and interpreted this to mean that self-fertilization had occurred. But we really need to know more than just the constancy of two alleles. Logic would suggest that in many cases the heterozygosity resulting from cross-fertilization would make those individuals more fit, consequently selecting against those individuals lacking a mechanism to prevent self-fertilization. But does this exist among bryophytes?

Figure 83. ***Pellia epiphylla***, a species wherein identity of alleles suggests selfing. Photo by Li Zhang, with permission.

We know that seed plants often (usually?) are self-sterile. They have several mechanisms during and following pollination/fertilization to prevent success of self-fertilization, and these can provide suggestions for possible mechanisms in bryophytes:

- different maturation times of male and female parts
- dispersal vector behavior – moving from mature females to mature males (several animal vectors are now known)
- sperm unable to swim in neck of archegonium
- failure of self-fertilized embryo to develop
- rejection of self-fertilized embryos by plant
- better competition by hybrid embryos
- failure of next generation to reproduce

But do we know that any of these mechanisms occur in bryophytes? Gemmell (1950) suggested that all monoicous species were obligate inbreeders. This seems unlikely since evolution from dioicous to monoicous is a common direction in bryophytes. Lazarenko and Lesnyak (1972) disproved the suggestion of Gemmell by demonstrating cross breeding in ***Desmatodon*** (Figure 84), including cross breeding between two different species in the genus. Now we are raising the question whether monoicous bryophytes actually have mechanisms to ensure outbreeding in at least a portion of the population.

Figure 84. ***Desmatodon latifolius*** with abundant capsules, a species in which hybrids among species in the genus are known. Photo by Michael Lüth, with permission.

Just in time for this writing, Stark and Brinda (2013) published their study on ***Aloina bifrons*** (Figure 85), a dioicous moss living in the dry Mojave Desert, USA. Despite being dioicous in an environment unfriendly toward fertilization by water, this moss had frequent sporophyte production, leading the researchers to question its dioicous status (Stark & Delgadillo M. 2001). They found that it could, at least occasionally, be **rhizautoicous**. They found **ramets** (individuals in clone of genetically identical individuals that have grown in given location, originating vegetatively from single plant), connected by single rhizoids, that produced both **perichaetia** (archegonial groupings) and **perigonia** (antheridial groupings).

Figure 85. ***Aloina bifrons***, a moss that is apparently facultatively autoicous. Photo from Proyecto Musgo, through Creative Commons.

But all is not well for self-fertilization because it leads to all those dangers of inbreeding that make the offspring less fit. Rather, Stark and Brinda (2013) found that ***Aloina bifrons*** (Figure 85) actually practices self-incompatibility. First, it practices **protandry** – a condition wherein the male reproductive structures mature before the female structures. There was some overlap in maturity times between archegonia and antheridia, and self-fertilization did occur within single clones. However, sporophytes aborted during the embryonic development. Stark and Brinda did allow for the possibility that these cultures might require a resting phase to continue their sporophyte development, so we are still left wondering.

It appears that we know little about incompatibility mechanisms in bryophytes. Let's recall that the monoicous condition in bryophytes is aparently derived from the dioicous condition. Hence, the mechanisms had to arise anew after the monoicous taxa arose. We should perhaps expect that self incompatibility is an imperfect condition that is still evolving. But for now, there are no studies to determine if more embryos abort from self-fertilizations than from outbreeding. There is no evidence to determine the effect of self-fertilization on future generations. There is no study that has examined the success of sperm from the same plant vs different plants in reaching and penetrating the egg. Hence, we have no idea how extensive or important self-incompatibility is in bryophytes.

Geographic and Habitat Relationships

Certainly physiological evolution has occurred as species have broadened their ranges to more and more distant locations. ***Pleurozium schreberi*** (Figure 50-Figure 51) often is without capsules because no male plants are present. Longton and Greene (1969a,b) found that females are more abundant worldwide, causing us to ponder on the cause. Could it be that male expression requires a temperature and photoperiod combination that is not available in their more cosmopolitan distribution?

Working with ***Macromitrium*** (Figure 71), Une (1985) found a possible explanation for the absence of mature males in some species. In isosporous ***Macromitrium***, female protonemata developed buds at 10ºC, but after 160 days the males had failed to produce buds, making it impossible for them to complete a life cycle in a short growing season.

Two ***Pohlia*** (Figure 86) species provide evidence to suggest that changes in the reproductive response are possible mechanisms for survival in widespread locations, and this plasticity may explain the abundant capsules seen on some ***Pohlia*** species. Clarke and Greene (1970) found that gametangial maturation was faster in the Arctic and sub-Arctic than in Britain, permitting these species to complete their maturation in the shorter Arctic summer. Lewis Smith and Convey (2002) indicated that in the Antarctic sexual reproduction likewise was highly successful, suggesting that the severe climate with its low temperatures and short growing season is not a severe detriment to successful gametangial production. They consider that microhabitats make this reproduction possible. Most of the fertile species are monoicous, short acrocarpous species on rather calcareous soils. Could it be that calcium is an important part of the reproduction story?

Figure 86. ***Pohlia filum*** growing in an alpine area and producing abundant sporophytes. Photo by Michael Lüth, with permission.

In the Brazilian Atlantic Rainforest, an altitudinal cline permits us to compare reproductive performance. Maciel-Silva *et al*. (2012) monitored eleven species for fifteen months at sea level and a montane site to compare reproductive performance. The highest level of reproduction was among monoicous taxa, especially for sexual branches and fertilized gametangia. At sea level, there were more females and more sexual branches than at the montane site. But these differences seemed only to compensate for other factors because the sporophyte frequency was similar in both sites. Microhabitats like decaying wood were important in maintaining sufficient water levels for good gametangial production. Water availability and maintenance may have been the major factor influencing the success of sporophyte production.

Another geographic problem is that timing that is ideal in one locality may be all wrong in another. Signals for production of gametangia may come from photoperiod, signalling an upcoming rainy season, but in another, the rainy season may be during a different part of the year. For example, ***Octoblepharum albidum*** (Figure 87) in Brazil times its reproductive maturity to coincide with the rainy season (Pôrto & Oliveira 2002). The capsules begin their development during the rainy season, but complete it during the subsequent dry season when they disperse their spores. In this case, the rainfall seems actually to enhance development of gametangia, hence ensuring the correct timing. The behavior of ***Sematophyllum subpinnatum*** (Figure 88) in these tropical lowland forests is similar (Oliveira & Pôrto 2001). Although both antheridia and archegonia develop and mature throughout the year, they increase in number during the rainy season. Subsequent appearance of sporophytes primarily from June to September indicates that most fertilization events occur during the rainy season.

Odu (1981) found similar timing in tropical Africa. The perennial ***Racopilum africanum*** (Figure 89), ***Fissidens weirii***, and ***Thuidium gratum***, and an annual ***Stereophyllum*** sp. (Figure 90) all develop their gametangia at the onset of the rainy season, complete fertilization during that season, and produce mature capsules ready for spore dispersal at the onset of the dry season.

Figure 87. ***Octoblepharum albidum***, a moss in which rainfall seems to enhance gametangial production. Photo by Niels Klazenga, with permission.

Figure 88. ***Sematophyllum subpinnatum***, a species in which antheridia and archegonia are produced throughout the year, but increase in the rainy season. Photo by Michael Lüth, with permission.

Figure 89. ***Racopilum africanum*** with young sporophytes that are initiated near the beginning of the rainy season and mature at the beginning of the dry season. Photo by Jan-Peter Frahm, with permission.

Figure 90. ***Stereophyllum radiculosum***, a moss that develops its gametangia at the beginning of the rainy season. Photo by Scott Zona, with permission.

For the mosses ***Bryum coronatum*** (Figure 3), ***Hyophila involuta*** (Figure 91), and ***Barbula indica*** (Figure 2) in southwestern Nigeria, gametangia development starts at the onset of the rainy season (March), providing them sufficient water to mature (Fatoba 1998). But their maturation requires 8-10 months (ending November – January), whereas the rainy season ends in mid October. The southwestern Nigerian rainy season has a "little dry season" (mid-July to mid-September, but mostly in August) (Adejuwon & Odekunle 2006), although the length decreases away from the coast. This little dry season might influence the persistence of the long developmental period for these gametangia. Temperatures typically range 26-28°C annually, so they have little influence on the bryophyte timing. This 8-10 months for maturation of gametangia places time of fertilization so that it permits the capsules to mature and spores to be dispersed in October – November, early in the regular dry season.

Figure 91. ***Hyophila involuta***, a moss that begins gametangial development at the beginning of the rainy season in Nigeria. Photo by Niels Klazenga, with permission.

In desert habitats, even timing can fail to provide an opportunity for gametangial production. The desert moss ***Syntrichia caninervis*** (Figure 92) had 85% non-sexual ramets in a 10-hectare study area in the southern Mojave

Desert of Nevada, USA (Bowker *et al.* 2000). Those that had sexual expression were associated mostly with shaded microsites, higher soil moisture content, and taller ramets. The taller ramet may have been a result of the greater moisture available, but it also may have been the size that had reached the required threshold for available energy as discussed earlier in this chapter.

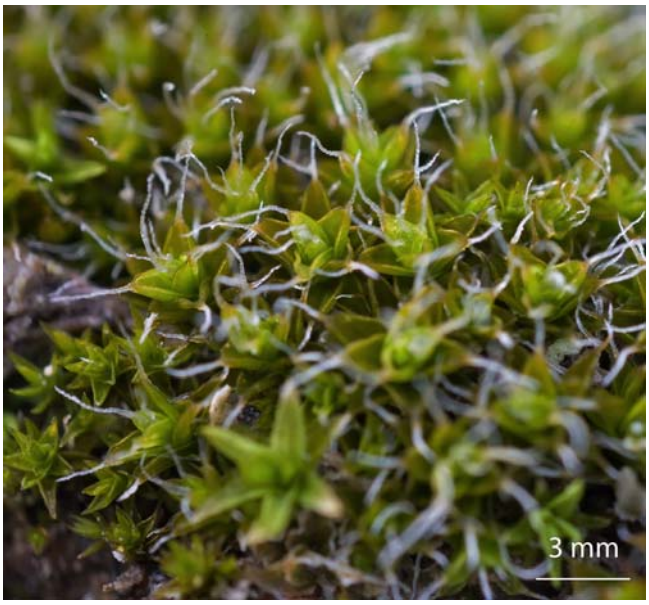

Figure 92. ***Syntrichia caninervis***, a moss with 85% non-sexual ramets in the Mojave Desert. Photo from Proyecto Musgo, through Creative Commons.

In another desert moss, ***Syntrichia inermis*** (Figure 93), also from the Mojave Desert, more than 90% of the plants are monoicous (Stark 1997). In this species archegonia are initiated and receptive in the same winter, whereas antheridia require 1-3 years to reach maturity. Abortion is only 3-4% for both gametangia, but only 50% of the current cycle of perichaetia become fertilized. The slowest growth rates known, an 18-month dormancy period during sporophyte maturation, and the longest known period for antheridial maturation attest to limitations placed on reproduction in this moss by its desert habitat.

Figure 93. ***Syntrichia inermis*** with capsules, showing high sporophyte production of this monoicous moss. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Tradeoffs – Cost of Reproduction

Reproduction of any type comes at a price. Sexual reproduction requires considerable energy, and it benefits a plant to maximize success of its gametes in achieving fertilization. Actual measures of energy costs for any process in bryophytes are rare. The cost of reproduction can be indicated indirectly by its apparent effect on production of other structures and growth. For example, in ***Marchantia polymorpha*** (Figure 10), gemmae cups are generally not produced on the same portions of a colony as are the sexual structures (Figure 94) (Une 1984). But Une suggested that this might actually be due to age of the thallus, or to available nutrients, assuming that the interior of the colony where the gametangial branches occurred was the older and hence may have used up more of the available nutrients.

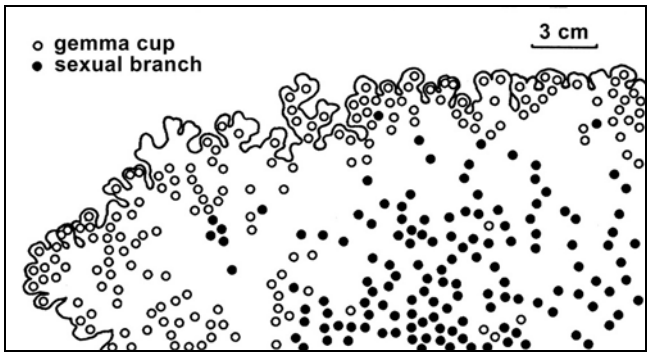

Figure 94. Location of gemma cups and archegoniophores in a colony of female ***Marchantia polymorpha***. Modified from Une 1984.

The example of the leafy liverwort ***Lophozia ventricosa*** (Figure 95) var. ***silvicola*** demonstrates the high cost of being female (Laaka-Lindberg 2001). Female plants allocated 24% of their biomass to sexual reproduction whereas their male counterparts expended only 2.3%. The cost to the female was reduced stem length and both genders exhibited modified branching of gametangial shoots. When compared with asexual shoots, both genders had reduced stem length. Predictably, asexual plants produced the most gemmae (mean 2100), males next (1360), and females least (800).

Figure 95. ***Lophozia ventricosa*** with gemmae, a species with a high cost for gametangia. Photo by Michael Lüth, with permission.

The desert moss ***Syntrichia inermis*** (Figure 93) seems to tell a different story. In that species, it is more costly, by an order of magnitude, to produce male sexual organs than female ones (Stark *et al*. 2000). Stark *et al*. attributed this extra cost to the longer time required for development, greater number of male gametangia per perigonium than for archegonia per perichaetium, and presence of paraphyses among antheridia in that species. It would be interesting to see if this sex ratio could be modified by providing the limiting resources, presumably sugar.

Stark and coworkers (1998, 2001) found other indications of tradeoffs resulting from sexual reproduction in ***Syntrichia caninervis*** (Figure 92). Interestingly, non-sex-expressing individuals exhibited lower biomass, shorter total stem length, fewer branches, and shorter ramets than sex-expressing individuals; all individuals weighing more than 2 mg dry weight were sexually expressing, suggesting a threshold size needed for reproduction in order to provide sufficient energy. Furthermore, when inflorescence number was considered, the biomass of males and females did not differ.

McLetchie (1996) found that distance between male and female plants, as expected, decreased sexual success of the plants, but he also found that smaller males were less successful in accomplishing successful fertilization in the dioicous, thallose ***Sphaerocarpos texanus*** (Figure 67). From this he concluded that successful fertilization is sperm-limited. One might also argue that these could represent maturity differences.

For the epiphyte ***Neckera pennata*** (Figure 96), Wiklund and Rydin (2004) found a similar indication of minimum size. The first reproduction occurred at a colony size of 12-79 cm^2, requiring an estimated 19-29 years until the plants were sexually active! These apparent thresholds suggest that a critical size is important for sex expression. This implies that an energy threshold is required, and thus there must be a tradeoff between stored energy and sexual productivity.

Not only is production of gametangia expensive, but the ensuing production of sporophytes likewise is costly. It is therefore not surprising that Stark and coworkers (2001) found that 63% of the fertilized perichaetia of ***Syntrichia caninervis*** (Figure 92) had abortive sporophytes. This need for energy to produce the sporophyte seems to be subject to high selection pressure, as most bryophytes produce only one sporophyte per apex despite having multiple archegonia.

Relative fitness of sexual and asexual individuals can depend on the environmental conditions. In ***Marchantia papillata*** subsp. ***inflexa*** (Figure 63), Fuselier and McLetchie (2002) found that light intensities can shift sexual fitness and alter the timing of asexual reproduction. There were negative tradeoffs between the asexual and sexual fitness of females at some light intensities. In high light intensities, female plants suffer a sex-specific cost for their plasticity in timing, and asexual fitness shifts the population toward monomorphism of sexes. Fuselier and McLetchie concluded that opposing selective forces on sexual vs asexual expression could explain persistence of sexual dimorphism despite selection against dimorphism in the pre-adult phase.

Bisang and Ehrlén (2002) clearly demonstrated costs of sexual reproduction in female plants of the polysetous ***Dicranum polysetum*** (Figure 97). They used a retrospective method to estimate photosynthetically active gametophyte biomass present at the onset of the sporophyte cycle and determined that reproductive effort, that is the proportional investment into reproductive structures, was 16% when sporophytes were successfully produced and only 1.3% when no fertilization occurred. The reproductive output of capsule number and dry weight were positively correlated with vegetative apical growth, whereas the reproductive effort was inversely related to dry mass of the annual segment preceding sporophyte initiation, indicating that energy was evidently shunted from that apical gametophyte tissue into the sporophyte. But even the next growth cycle paid the price of that reproduction; the probability of initiation of subsequent perichaetia was reduced as a result of sporophyte development, and when new perichaetia did develop, they were reduced in mass. In plants with sporophytes, investments in innovations were negatively correlated with reproductive structures. And, more sporophytes per plant resulted in reduced mass per sporophyte.

Figure 96. ***Neckera pennata***, a moss that requires 19-29 years before plants are sexually active. Photo by Jan-Peter Frahm, with permission.

Figure 97. ***Dicranum polysetum*** showing multiple sporophytes from a single stem. Photo by Janice Glime.

Summary

Gametes in bryophytes are produced in **antheridia** (sperm) and **archegonia** (eggs). The location of these structures divides mosses into **acrocarpous** mosses with terminal gametangia and **pleurocarpous** with side- branch gametangia. Water is needed for dispersal of sperm and in some cases this is aided by the presence of splash cups or splash platforms. Once released the sperm swims to the archegonium, attracted by some factor released when the neck canal cells of the archegonium disintegrate.

Both **monoicous** and **dioicous** taxa of bryophytes exist, and chromosome numbers suggest that monoicous taxa are derived through **polyploidy**. Sex determination is under genetic control in at least some bryophytes, with either an X or a small Y chromosome programming females vs males, respectively. There are implications that expression of these genetic differences is manifest in **IAA** differences, but it appears that **ethylene** could interact with IAA or that concentrations or relative concentrations may be important.

Some ***Macromitrium*** taxa have two spore sizes that translate into dwarf males from small spores, but generally dwarf males seem to be determined by some factor from the female upon which they land. Gender survival ratios, already discussed in the chapter on sexuality, are altered by spore survival, protonemal survival, competition, and survival of the gametophores. It may furthermore be altered by the environment to express one or the other sex.

Initiation of gametangia may be an ancient event that must be controlled by inhibition rather than initiation. The apparent initiation could instead be a set of conditions that override or immobilize inhibitors. Initiation of gametangia can be triggered by light intensity, photoperiod, temperature, and water availability, but it appears that many bryophytes, especially mosses, may respond to some combination of these. Liverworts seem to be more dependent on photoperiod. Other factors that influence gametangial development and gender expression include *p*H and form and availability of N. There may be a minimum size, at least for some taxa, before gametangia will develop, implying need for sufficient energy supply. Antheridia typically initiate before archegonia and take longer to develop. Because these two gametangia are initiated at different times, they are often under different controls that can cause a mismatch in maturity times. This can be particularly problematic when they disperse to a new geographic region and may account for absence of sporophytes on particular species in some geographic regions.

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