CHAPTER 5-3

ECOPHYSIOLOGY OF DEVELOPMENT: PROTONEMATA

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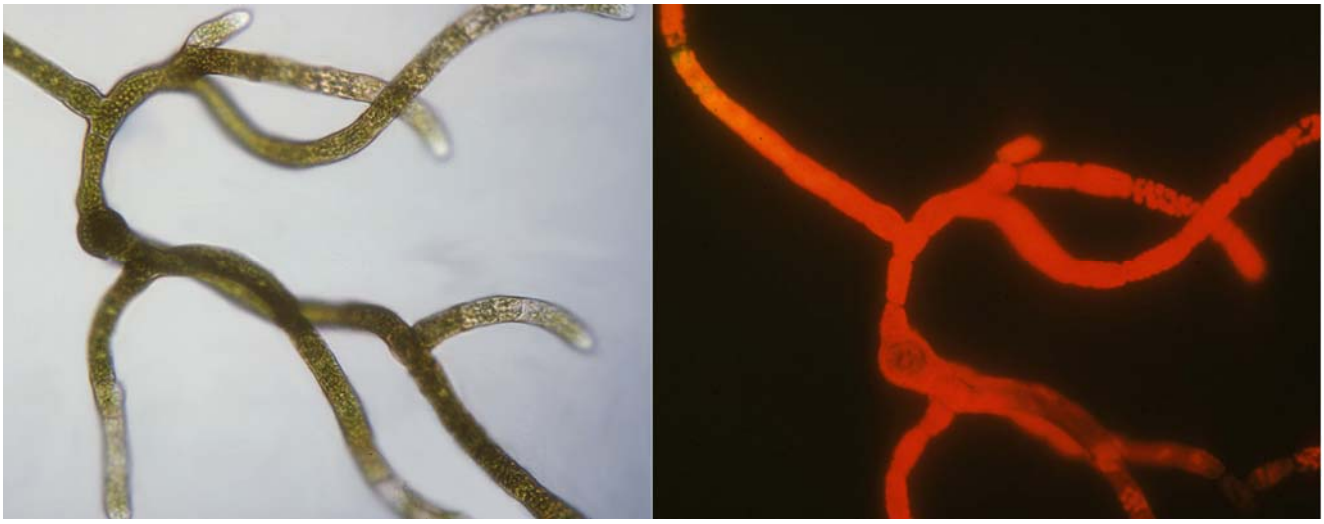

Figure 1. Protonema of ***Fontinalis squamosa***. **Left:** white light. **Right:** UV light showing chlorophyll fluorescence. Photo by Janice Glime.

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.

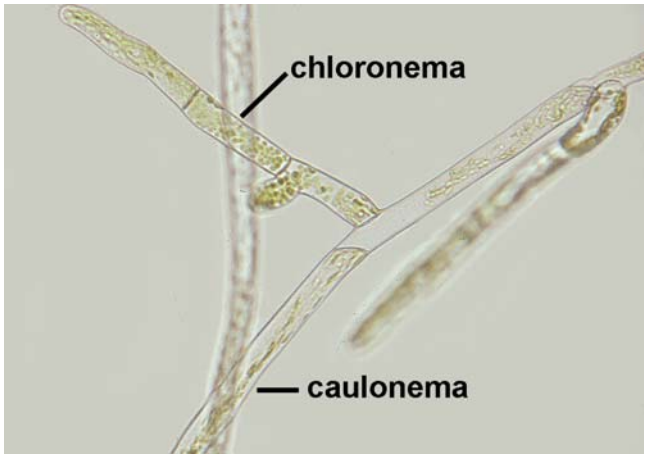

Figure 2. Distinction of chloronema and caulonema on the protonema of ***Funaria hygrometrica***. Photo by Janice Glime.

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.

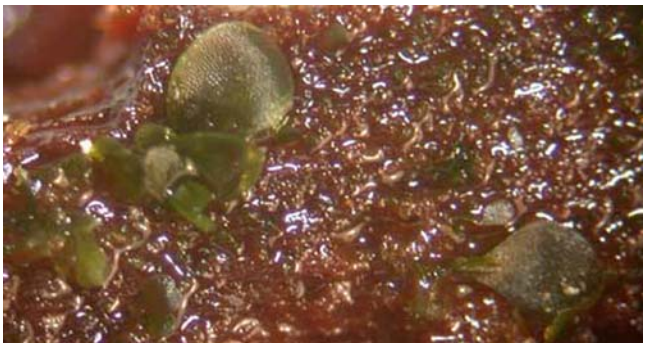

Figure 4. Protonemal flaps of the moss ***Tetraphis pellucida***. Photo from botany website and University of British Columbia, Canada, with permission.

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.

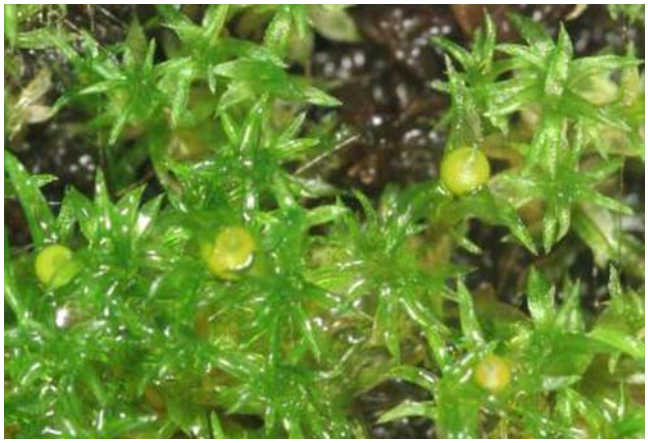

Figure 9. ***Physcomitrella patens*** with capsules, a common research organism because of the ease with which its genes can be manipulated. Photo by David Cove, with online permission.

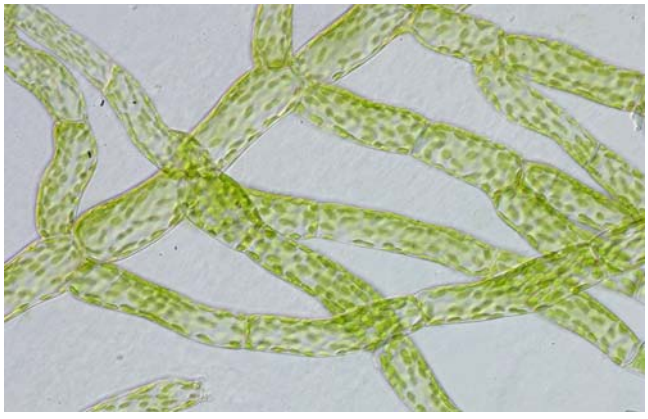

Figure 10. ***Physcomitrella patens*** protonema. Photo from Ralf Reski Lab, through Creative Commons.

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.

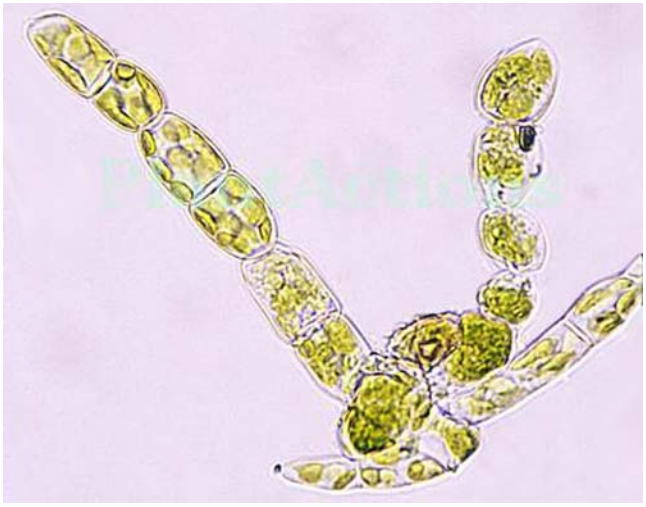

Figure 12. ***Grimmia orbicularis*** protonema. Photo by Eugenia Ron and Tom Sobota, with permission.

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.

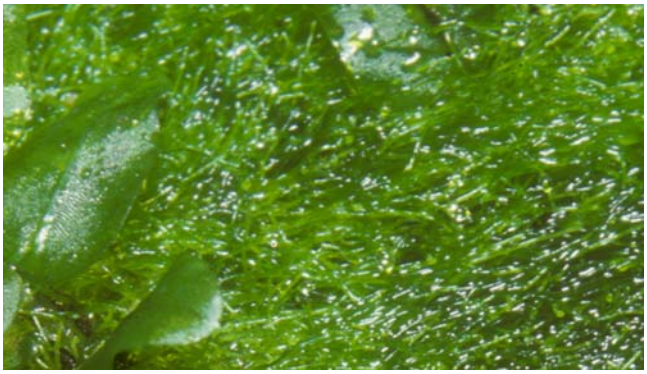

Figure 13. Protonema of ***Plagiomnium*** sp. in the field. Photo by Janice Glime.

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.

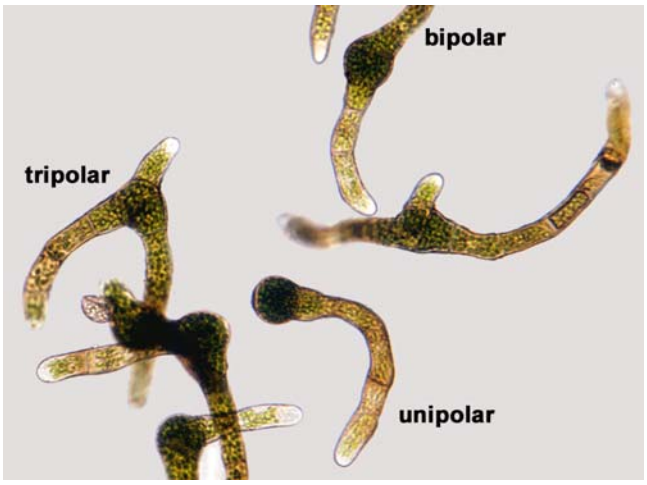

Figure 15. Protonemata of ***Fontinalis squamosa*** showing unipolar, bipolar, and tripolar germination typical at 20ºC and 2100 lux. Photo by Janice Glime.

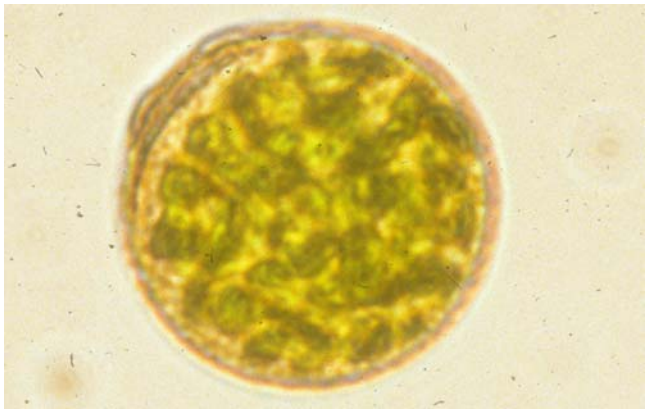

Figure 16. Distended spore of ***Fontinalis squamosa*** as one might find at 3ºC and 120 lux. Photo by Janice Glime.

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.

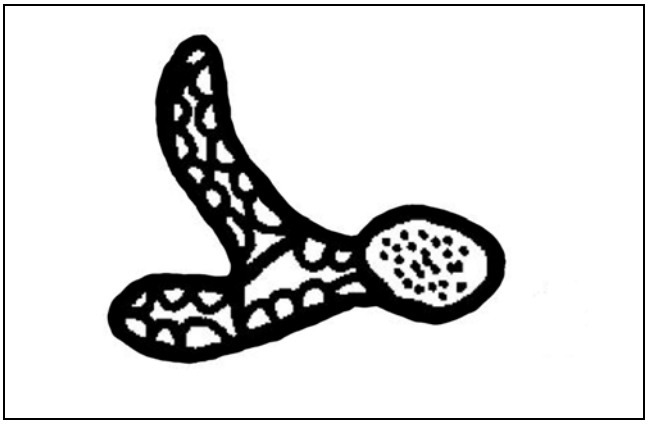

Figure 23. ***Brachythecium velutinum*** protonema branching Redrawn from Herguido & Ron 1990.

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.

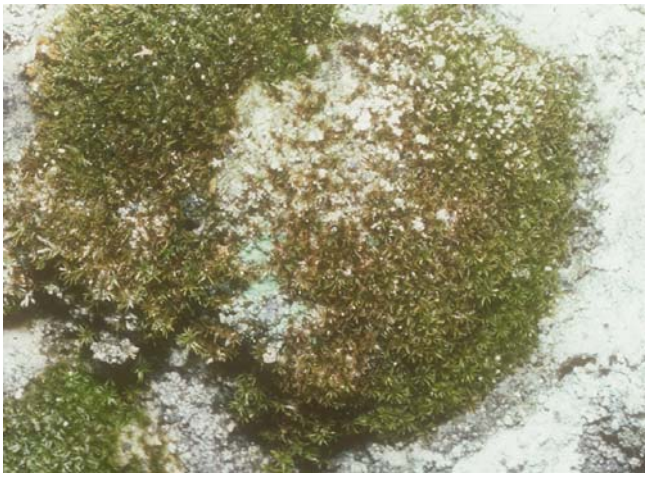

Figure 26. ***Gymnostomum*** forming colony, possibly from multiple buds from one protonema. Photo by Janice Glime.

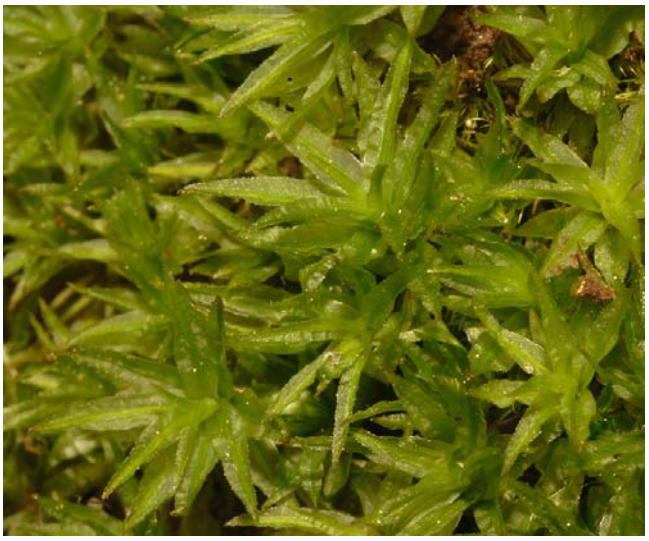

Figure 27. ***Atrichum altecristatum*** drying in an exposed habitat. Photo courtesy of Eric Schneider.

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.

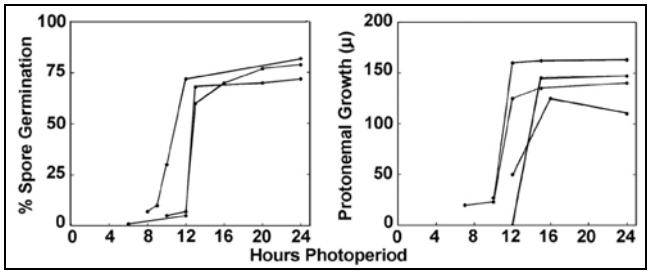

Figure 37. Effect of photoperiod on spore germination after 5 days (**left**) and protonema growth after 3 days (**right**) of ***Bryum pseudotriquetrum***. Redrawn from Kinugawa & Nakao (1965).

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.

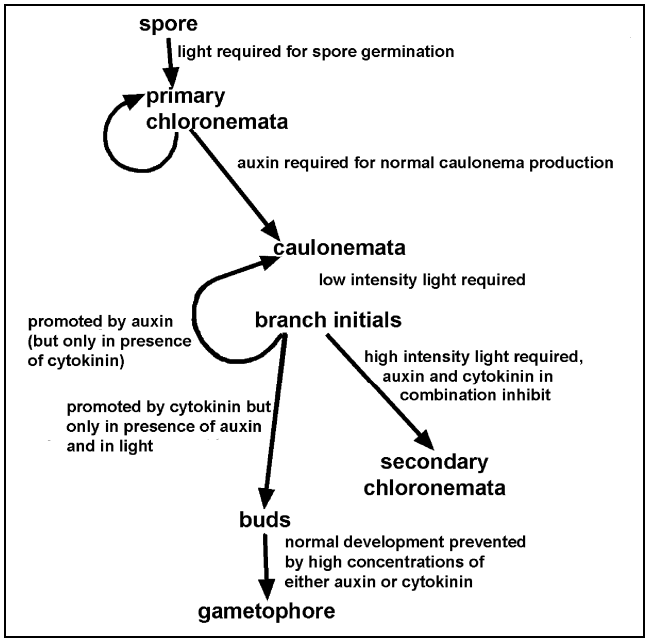

Figure 38. Effects of auxin and cytokinin on ***Physcomitrella patens***. Redrawn from Cove *et al*. (1979).

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).

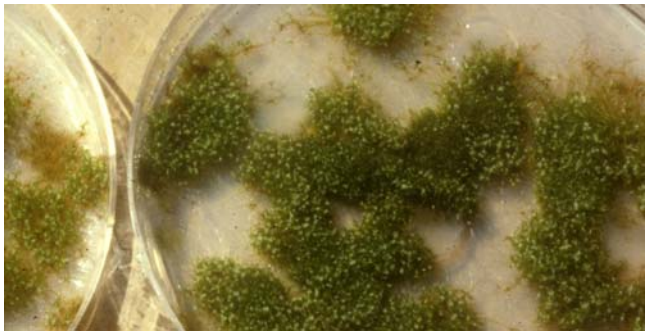

Figure 39. Culture of ***Funaria hygrometrica*** showing distinct colonies resulting most likely from hormonal interaction between clones at the protonemal stage. Each clump is the product of one spore. Photo by Janice Glime.

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.

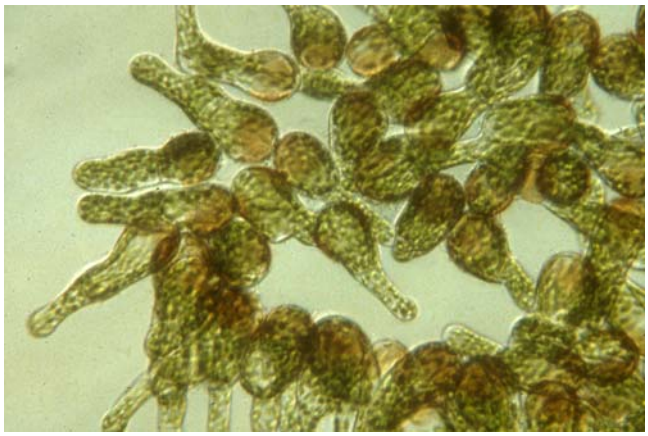

Figure 41. ***Funaria hygrometrica*** spore germination. Photo by Janice Glime.

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.

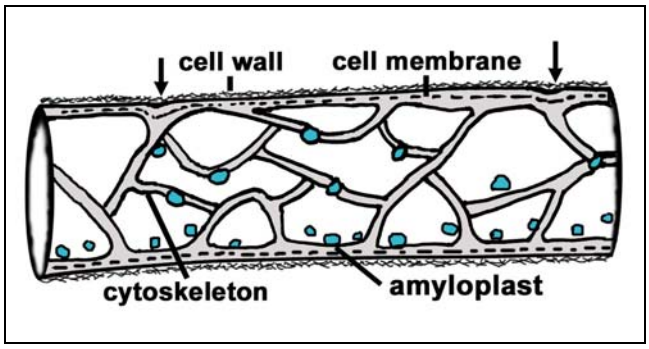

Figure 43. Schematic model of hypothetical relationship of **amyloplasts** (statoliths) of a protonema in response to gravity. Arrows denote pull of **cytoskeleton** on cell membrane. Drawing by Janice Glime.

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.

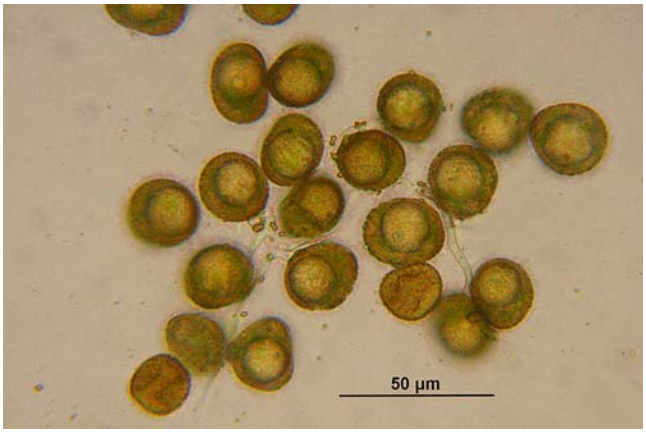

Figure 51. ***Tortula modica*** spores, a species exhibiting amyloplast sedimentation in the sub-apical zone of the protonema. Photo by Hermann Schachner, through Creative Commons.

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.

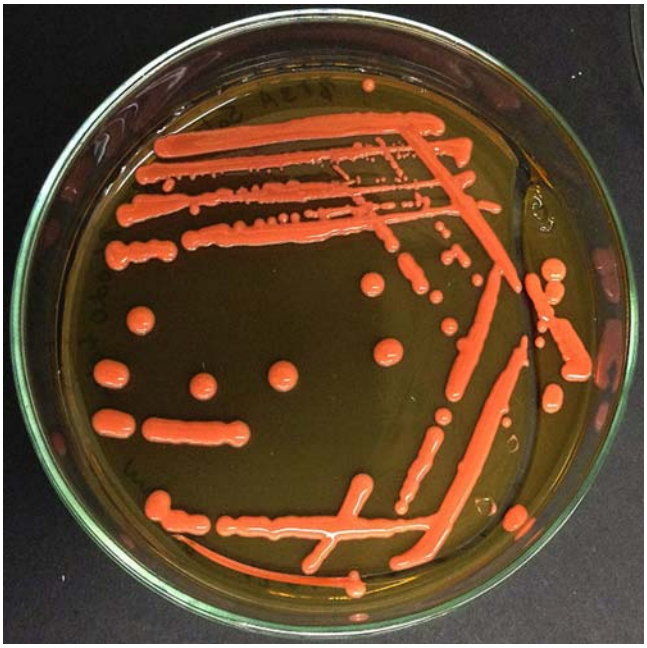

Figure 58. ***Rhodotorula mucilaginosa*** colonies, a yeast species that interacts with protonemata through Creative Commons.

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.

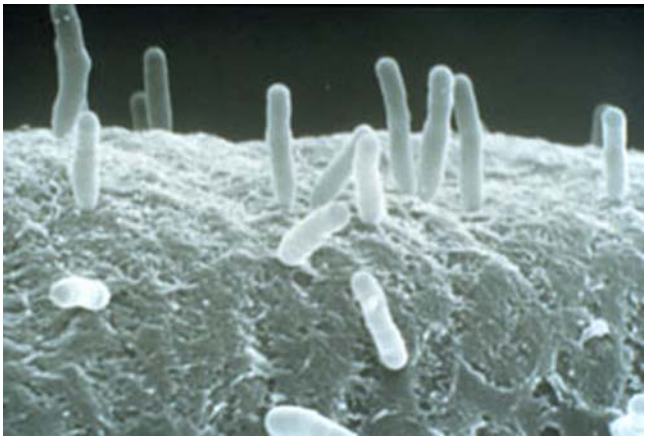

Figure 59. ***Agrobacterium tumefaciens*** on plant cell. Photo by Martha Hawes, University of Arizona.

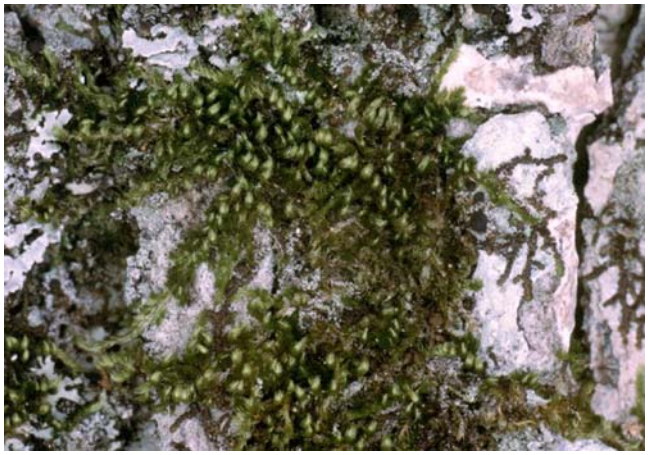

Figure 60. ***Pylaisia selwynii*** on tree bark. Protonema development in this species is enhanced by presence of ***Agrobacterium tumefaciens***. Photo by Jan-Peter Frahm, with permission.

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.

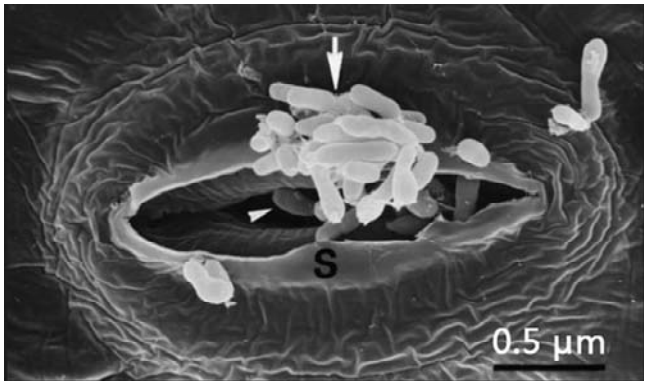

Figure 62. ***Methylobacterium*** in sunflower stoma, a bacterial species that has a positive interaction with protonemata of several bryophyte species. Photo by Kutschera U., through Creative Commons.

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.

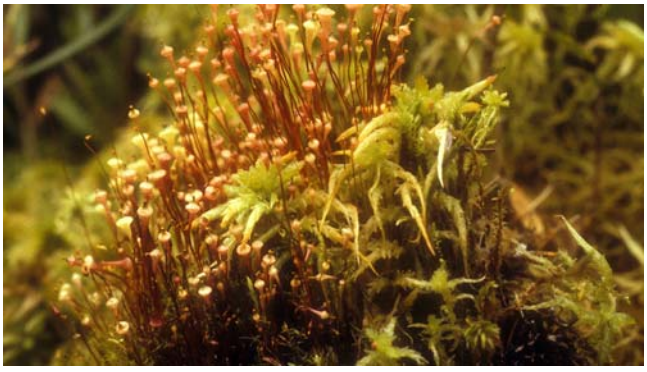

Figure 67. ***Splachnum ampullaceum*** growing among ***Sphagnum*** on dung, where changing dung conditions and fungal exudates influence development. Photo by Janice Glime.

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

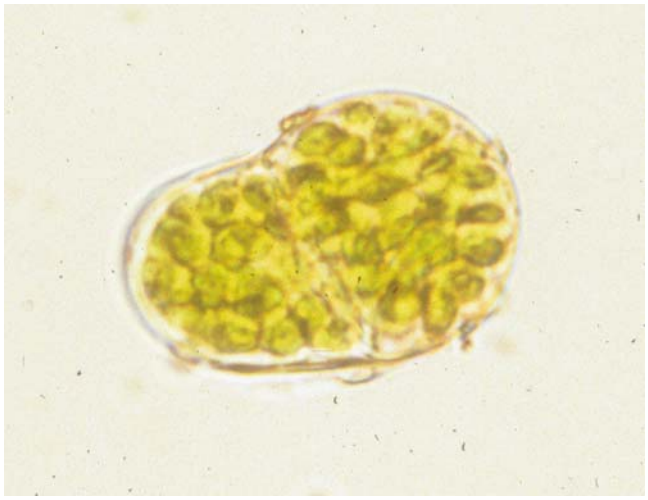

Figure 71. ***Fontinalis squamosa*** spore germination. Photo by Janice Glime.

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.

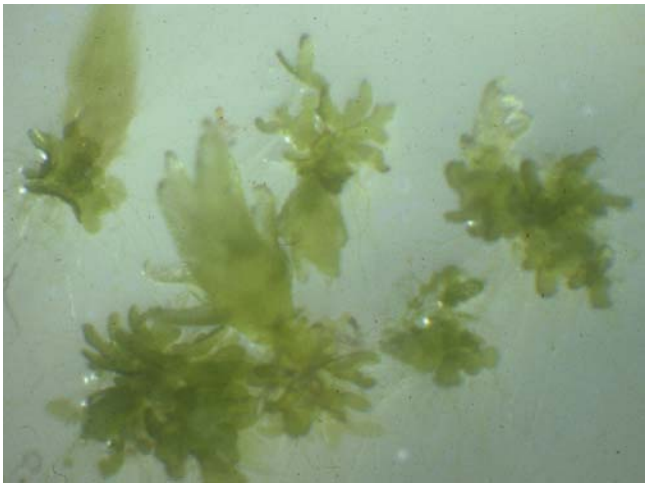

Figure 73. Thalloid protonemata of ***Sphagnum papillosum***. Photo courtesy of Yenhung Li.

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.

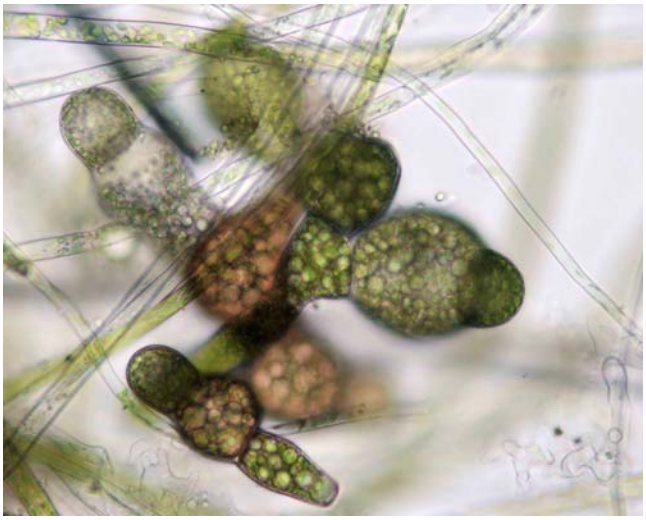

Figure 82. ***Dicranella heteromalla*** protonemata that survived winter in an acid mine lake. Photo courtesy of Jan Fott.

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).

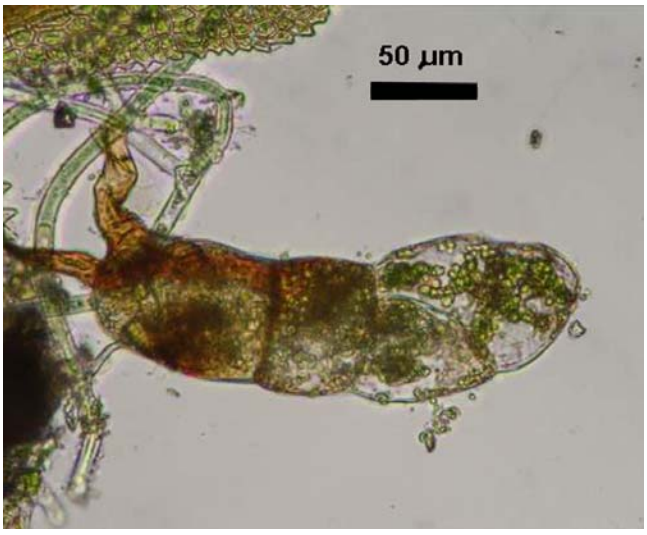

Figure 83. ***Fissidens tenellus*** bud with rhizoids at its base. Photo by Tom Thekathyil, with permission.

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.

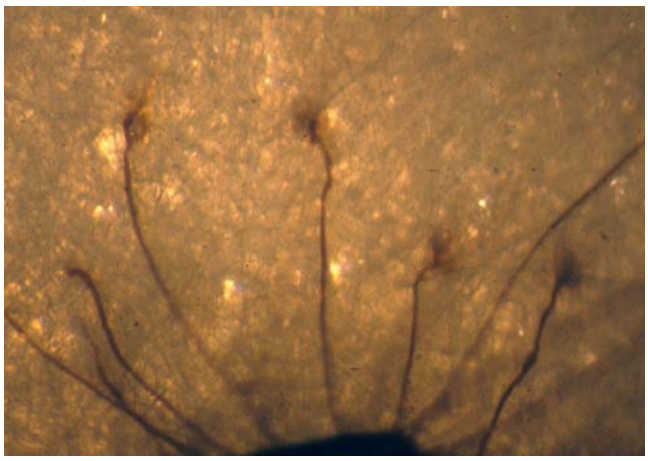

Figure 85. ***Fontinalis squamosa*** rhizoids forming fingerlike tips where they contact the filter paper. Photo by Janice Glime.

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).

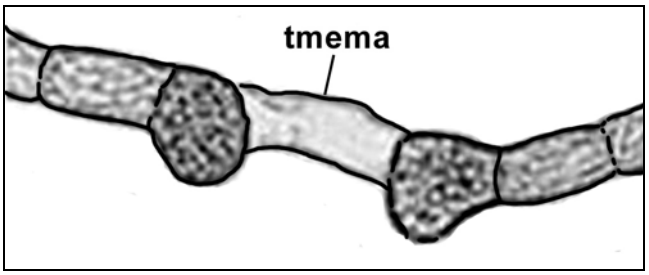

Figure 88. **Tmema** cell in protonema. Redrawn from Decker *et al*. 2006.

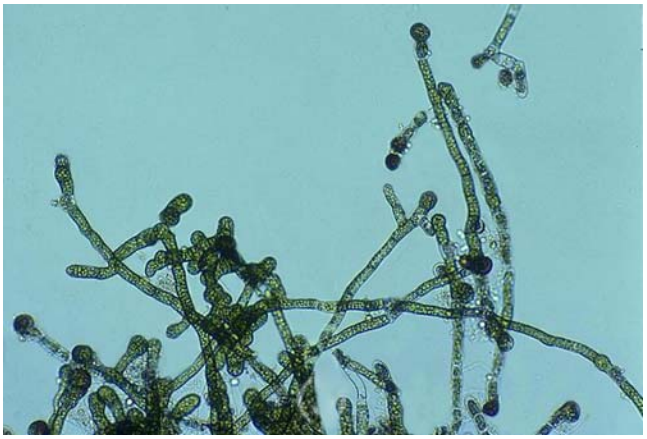

Figure 89. ***Bartramia ithyphylla*** with protonemal gemmae. Photo by Eugenia Ron Alvarez & Tomas Sobota – Plant Actions, with permission

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.

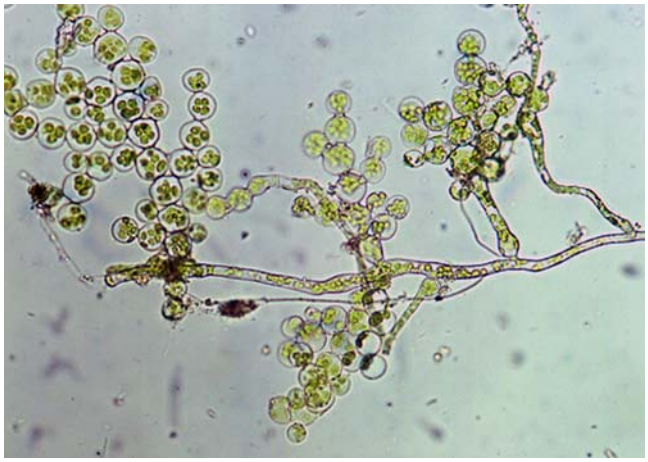

Figure 92. Protonema of ***Schistostega pennata*** showing filamentous protonema and round refractive cells. Photo by Irene Bisang, with permission.

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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Literature Cited

Abeles, F. B. 1973. Ethylene in Plant Biology. Academic Press, New York.

Ahmed, Md. G. U. and Lee, C. H. 2010. Induction of protonemal gemmae and gametophyte of *Cratoneuron decipien* (sic) (Brid.) G. Roth using IAA and kinetin. Plant Omics J. 3: 52-56.

Anderson, L. E. and Crosby, M. A. 1965. The protonema of *Sphagnum meridense* (Hampe) C. Muell. Bryologist 68: 47-54.

Barlow, P. W. 1995. Gravity perception in plants: A multiplicity of systems derived by evolution? Plant Cell Environ. 18: 951-962.

Bhatla, S. C. 1994. Moss Protonema Differentiation. Research Studies Press Ltd., Somerset, England, 296 pp.

Bhatla, S. C. and Dhingra-Babbar, S. 1990. Growth regulating substances in mosses. In: Chopra, R. N. and Bhatla, S. C. (eds.). Bryophyte Development: Physiology and Biochemistry, CRC Press, Ann Arbor, pp. 79-101.

Bierfreund, N. M., Reski, R., and Decker, E. L. 2003. Use of an inducible reporter gene system for the analysis of auxin distribution in the moss *Physcomitrella patens*. Plant Cell Rept. 21: 1143-1152.

Bittisnich, D. J. and Williamson, R. E. 1989. Tip-localised H^+ fluxes and the applicability of the acid-growth hypothesis to tip-growing cells: Control of chloronemal extension in *Funaria hygrometrica* by auxin and light. Planta 178: 96-102.

Bopp, M. 1959. Neue Gesichtspunkte zum Problem der Protonemadifferenzierung. Rev. Bryol. Lichenol. 28: 319-325.

Bopp, M. 1961. Morphogenese der Laubmoose. Biol. Rev. 36: 237-280.

Bopp, M. 1976. External and internal regulation of the differentiation of the moss protonema. J. Hattori Bot. Lab. 41: 167-177.

Bopp, M. 1980. The hormonal regulation of morphogenesis in mosses. In: Skoog, F. (ed.). Plant Growth Substances. Springer-Verlag, Berlin, pp. 351-361.

Bopp, M. 1984. The hormonal regulation of protonema development in mosses: II. The first steps of cytokinin action. Zeit. Pflanzenphysiol. 113: 435-444.

Bopp, M. 2000. 50 years of the moss story. In: Progress in Botany, Vol. 61. Springer, Berlin, Heidelberg, New York, pp. 1-34.

Bopp, M. and Atzorn, R. 1992. The morphogenetic system of the moss protonema. Cryptog. Bot. 3: 3-10.

Bopp, M., Quader, H., Thoni, C., Sawidis, T., and Schnepf, E. 1991. Filament disruption in *Funaria* protonemata: Formation and disintegration of tmema cells. J. Plant Physiol. 137: 273-284.

Chaban, C. I., Kern, V. D., Ripetskyj, R. T., Demkiv, O. T., and Sack, F. D. 1998. Gravitropism in caulonemata of the moss *Pottia intermedia*. J. Bryol. 20: 287-299.

Chopra, R. N. and Dhingra-Babbar, S. 1984. Studies on bud induction in the moss *Trematodon brevicalyx* Dixon. New Phytol. 97: 613-620.

Chopra, R. N. and Kumra, P. K. 1998. Biology of Bryophytes. J. Wiley & Sons, New Delhi, India, 350 pp.

Christianson, M. L. 2000. Control of morphogenesis in bryophytes. In: Shaw, J. A. and Goffinet, B. Bryophyte Biology. Cambridge University Press, Cambridge, UK, pp. 199-224.

Cove, D. J., Schild, A., Ashton, N. W., and Hartmann, E. 1978. Genetic and physiological studies of the effect of light on the development of the moss, *Physcomitrella patens*. Photochem. Photobiol. 27: 249-254.

Cove, D. J., Ashton, N. W., Featherstone, D. R., and Wang, T. L. 1979. The use of mutant strains in the study of hormone action and metabolism in the moss *Physcomitrella patens*. Proceedings of the Fourth John Innes Symposium, pp. 231-241.

Decker, E. L., Frank, W., Sarnighausen, E., and Reski, R. 2006. Moss systems biology en route: Phytohormones in *Physcomitrella* development. Plant Biol. 8: 397-406.

Demkiv, O. T., Khorkavtsiv, Y. D., Kardash, A. P., and Chaban, K. I. 1997. Interactions between light and gravitation in moss protonema tropisms. Russ. J. Plant Physiol. 44: 177-182.

Demkiv, O. T., Kordyum, E. L., Khorkavtsiv, Y. D., Kardash, O. R. and Chaban, C. I. 1998. Gravi- and photostimuli in moss protonema growth movements. Adv. Space Res. 2: 1191-1195.

Duckett, J. G. and Matcham, H. W. 1995. Studies of protonemal morphogenesis in mosses VII. The perennial rhizoids and gemmiferous protonema of *Dicranella heteromalla* (Hedw.) Schimp. J. Bryol. 18: 407-424.

Duckett, J. G., Burch, J., Fletcher, P. W., Matcham, H., Read, D., Russell, A, and Pressel, S. 2004. *In vitro* cultivation of bryophytes: A review of practicalities, problems, progress and promise. J. Bryol. 26: 3-20.

Edwards, S. R. 1978. Protonemal gemmae in *Schistostega pennata* (Hedw.) Web. et Mohr. J. Bryol. 10: 69-72.

Esch, H., Hartmann, E., Cove, D., Wada, M., and Lamparter, T. 1999. Phytochrome-controlled phototropism of protonemata of the moss *Ceratodon purpureus*: Physiology of the wild type and class 2 ptr-mutants. Planta 209: 290-298.

Fangmeier, A., Hadwiger-Fangmeier, A., Eerden, L. V., and Jäger, H. J. 1994. Effects of atmospheric ammonia on vegetation – a review. Environ. Pollut. 86: 43-82.

Fernández, H. and Revilla, M. A. 2003. *In vitro* culture of ornamental ferns. Plant Cell Tissue Organ Cult. 73: 1-13.

Forman, R. T. T. 1964. Growth under controlled conditions to explain the hierarchical distributions of a moss, *Tetraphis pellucida*. Ecol. Monogr. 34:1-25.

Fulford, M. 1956. Sporelings and regenerants of *Ptilidium pulcherrimum* (Web.) Hampe. Rev. Bryol. Lichénol. 25: 247-253.

George, E. F. 1993. Plant Propagation by Tissue Culture. Exergetics Ltd., Edington.

Giles, K. L. and Maltzahn, K. E. von. 1967. Interaction of red, far-red, and blue light in cellular regeneration of leaves of *Mnium affine*. Bryologist 70: 312-315.

Glime, J. M. 1987. The role of tropisms in rhizoid attachment and branch orientation in *Fontinalis*. Lindbergia 13: 85-90.

Glime, J. M. and Knoop, B. C. 1986. Spore germination and protonemal development of *Fontinalis squamosa*. J. Hattori Bot. Lab. 61: 487-497.

Gonneau, M., Pagant, S., Brun, F., and Laloue, M. 2001. Photoaffinity labelling with the cytokinin agonist azido-CPPU of a 34 kDa peptide of the intracellular pathogenesis-related protein family in the moss *Physcomitrella patens*. Plant Molec. Biol. 46: 539-548.

González, M. L., Mallón, R., Reinoso, J., and Rodriguez-Oubina, J. 2006. *In vitro* micropropagation and long-term conservation of the endangered moss *Splachnum ampullaceum*. Biol. Plant. 50: 339-345.

Goode, J. A., Duckett, J. G., and Stead, A. D. 1992. Protonemal morphogenesis of the moss *Tetraphis pellucida* Hedw. in culture and in the wild. Ann. Bot. 70: 519-530.

Goode, J. A., Alfano, F., Stead, A. D., and Duckett, J. G. 1993. The formation of aplastidic abscission (tmema) cells and protonemal disruption in the moss *Bryum tenuisetum* Limpr. is associated with transverse arrays of microtubules and microfilaments. Protoplasma 174(3-4): 158-172.

Gorton, B. S. and Eakin, R. E. 1957. Development of the gametophyte in the moss *Tortella caespitosa*. Bot. Gaz. 119: 31-38.

Hájek, T. and Vicherová, E. 2014. Desiccation tolerance of *Sphagnum* revisited: A puzzle resolved. Plant Biol. 16: 765-773.

Hartmann, E. and Weber, M. 1988. Storage of the phytochrome-mediated phototrophic stimulus of moss protonemal tip cells. Planta 175: 39-49.

Hartmann, E. and Weber, M. 1990. Photomodulation of protonema development. In: Chopra, R. N. and Bhatla, S. C. (eds.). Bryophyte Development: Physiology and Biochemistry, CRC Press, Ann Arbor, pp. 33-54.

Heitz, E. 1942. Die keimende *Funaria*-Spore als Physiologisches Versuchesobjekt. Ber. Deutsch. Bot. Ges. 60: 17-27.

Herguido, P. and Ron, M. E. 1990. Contribución al estudio de la espora de *Brachythecium velutinum* (Hedw.) B., S. & G. Anales Jará. Bot. Madrid 46: 413-420.

Hildebrand, V. R., Kottke, I., and Winkler, S. 1978. Untersuchung über den Einfluss von Pilzen auf die pH-Abhangigkeit von Laubmoosen. Beitr. Biol. Pflanzen 54: 1-12.

Hohe, A., Decker, E. L., Gorr, G., Schween, G., and Reski, R. 2002. Tight control of growth and cell differentiation in photoautotrophically growing moss (*Physcomitrella patens*) bioreactor cultures. Plant Cell Rept. 20: 1135-1140.

Ignatov, M., Ignatova, E., Belousova, A., and Sigaeva, A. 2012. Additional observations on protonemata of *Schistostega pennata* (Bryophyta). Arctoa 21: 1-20.

Imaizumi, T., Kadota, A., Hasebe, M., and Wada, M. 2002. Cryptochrome light signals control development to suppress auxin sensitivity in the moss *Physcomitrella patens*. Plant Cell 14: 373-386.

Jaffe, L. and Etzold, H. 1965. Tropic responses of *Funaria* spores to red light. Biophys. J. 5: 715-742.

Jayaswal, R. K. and Johri, M. M. 1985. Occurrence and biosynthesis of auxin in protonema of the moss *Funaria hygrometrica*. Phytochemistry 24: 1211-1214.

Jenkins, G. I., Courtice, G. R., and Cove, D. J. 1986. Gravitropic responses of wild-type and mutant strains of the moss *Physcomitrella patens*. Plant Cell Environ. 9: 637-644.

Johri, M. M. and Desai, S. 1973. Auxin regulation of caulonema formation in moss protonema. Nature New Biol. 245: 223-224.

Kapur, A. and Chopra, R. N. 1989. Effects of some metal ions on protonemal growth and bud formation in the moss *Timmiella anomala* grown in aseptic cultures. J. Hattori Bot. Lab. 66: 283-298.

Karunen, P. 1973. Studies on moss spores II. Production of chlorophylls in germinating *Polytrichum commune* Hedw. spores. J. Exper. Bot. 24: 1183-1188.

Kern, V. D. and Sack, F. D. 1999a. Irradiance-dependent regulation of gravitropism by red light in protonemata of the moss *Ceratodon purpureus*. Planta 209: 299-307.

Kern, V. D. and Sack, F. D. 1999b. Red light-induced suppression of gravitropism in moss protonemata. Adv. Space Res. 24: 713-716.

Kern, V. D. and Sack, F. D. 2001. Effects of spaceflight (sts-87) on tropisms and plastid positioning in protonemata of the moss *Ceratodon purpureus*. Adv. Space Res 17: 941-949.

Kern, V. D., Smith, J. D., Schwuchow, J. M., and Sack, F. D. 2001. Amyloplasts that sediment in protonemata of the moss *Ceratodon purpureus* are nonrandomly distributed in microgravity. Plant Physiol. 125: 2085-2094.

Kern, V. D., Schwuchow, J. M., Reed, D. W., Nadeau1, J. A., Lucas, J., Skripnikov, A., and Sack, F. D. 2005. Gravitropic moss cells default to spiral growth on the clinostat and in microgravity during spaceflight. Planta 221: 149 – 157.

Kinugawa, K. and Nakao, S. 1965. Note on spore germination and protonemal growth controlled by day length in *Bryum pseudo-triquetrum*. Bot. Mag. (Tokyo) 78: 194-197.

Klein, B. 1967. Versuche zur Analyse der Protonemaentwicklung der Laubmoose. IV. Der Endogene Faktor H und seine Rolle bei der Morphogenese von *Funaria hygrometrica*. Planta 73: 12-27.

Kofler, L. 1958. Contribution a l'etude biologique des mousses cultivees in vitro: Germination des spores, croissance et developpement du protonema chez *Funaria hygrometrica*. Rev. Bryol. Lichenol. 28(1-2): 1-202.

Kofler, L. 1971. Influence de la temperature sur le geotropisme des spores de funaire. C. R. Acad. Sci. (Paris) 272: 1244-1247.

Kofler, L., Dutel, J., and Nurit, F. 1963. Variations in the geotropic sensitivity of germinating *Funaria* spores in

response to some external influences. J. Linn. Soc. London Bot. 58: 311-319.

Kumra, S. and Chopra, R. N. 1985. In vitro studies on spore germination, protonemal differentiation and bud formation in the moss, *Anisothecium molliculum* (Mitt.) Broth. Phytomorphology 35: 223-231.

Kutschera, U. 2007. Plant-associated methylobacteria as co-evolved phytosymbionts. A hypothesis. Plant Signal. Behav. 2(2): 74-78.

Kuznetsov, O. A., Schwuchow, J., Sack, F. D., and Hasenstein, K. H. 1999. Curvature induced by amyloplast magnetophoresis in protonemata of the moss *Ceratodon purpureus*. Plant Physiol. 119: 645-650.

Lamparter, T., Esch, H., Cove, D., Hughes, J., and Hartmann, E. 1996. Aphototropic mutants of the moss *Ceratodon purpureus* with spectrally normal and with spectrally dysfunctional phytochrome. Plant Cell Environ. 19: 560-568.

Lamparter, T., Hughes, J., and Hartmann, E. 1998. Blue light- and genetically-reversed gravitropic response in protonemata of the moss *Ceratodon purpureus*. Planta 206: 95-102.

Larpent-Gourgaud, M. and Aumaitre, M. P. 1980. Photoperiodism and morphogenesis of the protonema of *Ceratodon purpureus* (Hedw.) Brid. Experientia 36: 1366-1367.

Lehnert, B. and Bopp, M. 1983. The hormonal regulation of protonema development in mosses I. Auxin-cytokinin interaction. Zeit. Pflanzenphysiol. 110: 379-391.

Ligrone, R., Duckett, J. G., and Gambardella, R. 1996. Development and liberation of cauline gemmae in the moss *Aulacomnium androgynum* (Hedw.) Schwaegr. (Bryales): An ultrastructural study. Ann. Bot. 78: 559-568.

Maltzahn, K. E. von and MacQuarrie, I. G. 1958. Effect of gibberellic acid on the growth of protonemata in *Splachnum ampullaceum* (L.) Hedw. Nature (London) 181: 1139-1140.

Mehta, P. 1988. In vitro studies on spore germination, protonemal differentiation and bud formation in three mosses grown in vitro. J. Hattori Bot. Lab. 64: 401-410.

Mehta, P. 1990. Studies on the in vitro production of protonemal diffusate by *Hyophila involuta* and its morphogenetic effects in combination with some known growth regulators. Phytomorphology 40:119-123.

Miller, K. and Phillips, T. 2003. Mossy space spirals. National Aeronautics and Space Administration. Exploration Systems Articles. Accessed 22 June 2006 at <http://exploration.nasa.gov/articles/16jul_firemoss.html>

Mishler, B. and DeLuna, E. 1991. The use of ontogenetic data in phylogenetic analyses in mosses. Adv Bryol. 4: 121-167.

Mitra, G. C., Allsopp, A., and Wareing, P. F. 1959. The effects of light of various qualities on the development of the protonema and bud formation in *Pohlia nutans* (Hedw.) Lindb. Phytomorphology 9: 47-55.

Morgan, P. W. and Powell, R. D. 1970. Involvement of ethylene in responses of etiolated bean hypocotyl hook to coumarin. Plant Physiol. 45: 553-557.

Nehira, K. 1966. Sporelings in the Jungermanniales. J. Sci. Hiroshima Univ. B. Div. 2, 11(1): 1-49.

Nehira, K. 1983. Spore germination, protonema development and sporeling development. In: Schuster, R. M. (ed.). New Manual of Bryology, Vol. 1. Hattori Botanical Laboratory, Nichinan.

Nishida, Y. 1978. Studies on the sporeling types in mosses. J. Hattori Bot. Lab. 44: 371-454.

Nomura, T. and Hasezawa, S. 2011. Regulation of gemma formation in the copper moss *Scopelophila cataractae* by environmental copper concentrations. J. Plant Res. 124: 631-638.

Odu, E. A. 1988. Extracellular adhesive substances on bryophyte rhizoids. Acta Bot. Hung. 35(1-4): 273-277.

Odu, E. and Richards, P. W. 1976. The stimulus to branching of the rhizoid tip in *Lophocolea cuspidata* (Nees) Limpr. J. Bryol. 9: 93-95.

Pocock, K. and Duckett, J. G. 1985. On the occurrence of branched and swollen rhizoids in British hepatics: Their relationships with the substratum and associations with fungi. New Phytol. 99: 281-304.

Pressel, S., Ligrone, R., and Duckett, J. G. 2008. Cellular differentiation in moss protonemata: A morphological and experimental study. Ann. Bot. 102: 227-245.

Reiss, H. D. and Herth, W. 1979. Calcium gradients in tip growing plant cells visualized by chlorotetracycline fluorescence. Planta 146: 615-621.

Repp, A., Mikami, K., Mittmann, F., and Hartmann, E. 2004. Phosphoinositide-specific phospholipase C is involved in cytokinin and gravity responses in the moss *Physcomitrella patens*. Plant J. 40: 250-259.

Reutter, K., Atzorn, R., Hadeler, B., Schmülling, T., and Reski, R. 1998. Expression of the bacterial *ipt* gene in *Physcomitrella* rescues mutations in *budding* and in *plastid division*. Planta 206: 196-203.

Rohwer, F. and Bopp, M. 1985. Ethylene synthesis in moss protonema. J. Plant Physiol. 117:331-338.

Rose, S., Rubery, P. H. and Bopp, M. 1983. The mechanism of auxin uptake and accumulation in moss protonemata. Physiol. Plant. 58: 52-56.

Sabovljević, A., Sabovljević, M., Grubisic, D., and Konjevic, R. 2005. The effect of sugars on development of two moss species (*Bryum argenteum* and *Atrichum undulatum*) during *in vitro* culture. Belgian J. Bot. 138: 79-84.

Sabovljević,, A., Cvetić, T., and Sabovljević,, M. 2006. Establishment and development of the Catherine's moss *Atrichum undulatum* (Hedw.) P. Beauv. (Polytrichaceae) in *in vitro* conditions. Arch. Biol. Sci. 58: 87-93.

Sack, F. D., Wagner, T. A., and Kern, V. D. 1998. Gravitropism in moss protonemata. In: Bates, J. W., Ashton, N. W., and Duckett, J. D. (eds.). Bryology for the Twenty-first Century. Maney, Leeds, pp. 247-260.

Sarla. 1992. *In vitro* studies on male clone of *Riccia discolor*: Effect of sugars. J. Hattori Bot. Lab. 72: 141-150.

Sarla and Chopra, R. N. 1989. In vitro regulation of gemma/bud formation by cytokinins in the moss *Bryum capillare* Hedw. Plant Sci. 64: 237–242.

Schnepf, E. and Reinhard, C. 1997. Brachycytes in *Funaria* protonemate: Induction by abscisic acid and fine structure. J. Plant Physiol. 151: 166-175.

Schofield, W. B. 1985. Introduction to Bryology. Macmillan Publishing Co., New York, 431 pp.

Schuster, R. M. 1966. The Hepaticae and Anthocerotae of North America. Vol. I. Columbia Univ. Press, N. Y. 1344 pp.

Schween, G., Hohe, A., Koprivova, A., and Reski, R. 2003. Effects of nutrients, cell density and culture techniques on protoplast regeneration and early protonema development in a moss, *Physcomitrella patens*. J. Plant Physiol. 160: 209-212.

Schwuchow, J. and Sack, F. D. 1990. Microtubule distribution in gravitropic protonemata of the moss *Ceratodon*. Protoplasma 159:60-69.

Schwuchow, J. and Sack, F. D. 1993. Effects of inversion on plastid and gravitropism in *Ceratodon* protonemata. Can. J. Bot. 71: 1243-1248.

Schwuchow, J. and Sack, F. D. 1994. Microtubules restrict plastid sedimentation in protonemata of the moss *Ceratodon*. Cell Motility Cytoskeleton 29: 366-374.

Schwuchow, J., Sack, F. D., and Hartmann, E. 1994. Microtubules restrict plastid sedimentation in protonemata of the moss Ceratodon. Cell Motel Cytoskel. 29: 366-374.

Schwuchow, J. M., Kim, D., and Sack, F. D. 1995. Caulonemal gravitropism and amyloplast sedimentation in the moss *Funaria*. Can. J. Bot. 73: 1029-1035.

Schwuchow, J., Michalke, W., and Hertel, R. 2001. An auxin transport inhibitor interferes with unicellular gravitropism in protonemata of the moss *Ceratodon purpureus*. Plant Biol. 3: 357-363.

Schwuchow, J. M., Kern, V. D., White, N. J., and Sack, F. D. 2002. Conservation of the plastid sedimentation zone in all moss genera with known gravitropic protonemata. J. Plant Growth Reg. 21: 146-155.

Shrimal, S. K. 1975. Antimitotic activity of certain bark extracts. Beitr. Biol. Pflanzen 51: 77-80.

Simon, P. E. and Naef, J. B. 1981. Light dependency of the cytokinin- induced bud initiation in protonemata of the moss *Funaria hygrometrica*. Physiol. Plant. 53: 13-18.

Sironval, C. 1947. Expériences sur les stades de développement de la forme filamenteuse en culture de *Funaria hygrometrica* L. Bull. Soc. Royale Bot. Belgique 79: 48-78.

Sood, S. 1975. Morphogenetic studies on *Pogonatum aloides*. Beitr. Biol. Pflanzen 51: 99-110.

Spiess, L. D., Lippincott, B. B., and Lippincott, J. A. 1971. Development and gametophore initiation in the moss *Pylaisiella selwynii* as influenced by *Agrobacterium tumefaciens*. Amer. J. Bot. 5l8: 726-731.

Spiess, L. D., Lippincott, B. B., and Lippincott, J. A. 1986. Specificity of moss response to moss-associated bacteria: Some influences of moss species, habitat, and locale. Bot. Gaz. 147: 418-424.

Sundberg, S. and Rydin, H. 2000. Experimental evidence for a persistent spore bank in *Sphagnum*. New Phytol. 148: 105-116.

Thelander, M., Olsson, T., and Ronne, H. 2005. Effect of the energy supply on filamentous growth and development in *Physcomitrella patens*. J. Exper. Bot. 56: 653-662.

Thomas, P. A., Proctor, M. C. F., and Maltby, E. 1994. The ecology of severe moorland fire on the North York Moors: Chemical and physical constraints on moss establishment from spores. J. Ecol. 82: 457-474.

Vaarama, A. and Tarén, N. 1959. The effect of gibberellic acid and fungi on spore germination and protonema growth in mosses. Bot. Not. 112: 481-488.

Valanne, N. 1971. The effects of prolonged darkness and light on the fine structure of *Ceratodon purpureus*. Can. J. Bot. 49: 547-554.

Wada, M. and Kadota, A. 1989. Photomorphogenesis in lower green plants. Ann. Rev. Plant Physiol. Plant Molec. Biol. 40: 169-191.

Wagner, T. A. and Sack, F. D. 1998. Gravitropism and gravimorphism during regeneration from protoplasts of the moss *Ceratodon purpureus* (Hedw.) Brid. Planta 205: 352-358.

Wagner, T. A., Cove, D. J., and Sack, F. D. 1997. A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon purpureus*. Planta 202: 149-154.

Walker, L. M. and Sack, F. D. 1990. Amyloplasts as possible statoliths in gravitropic protonemata of the moss *Ceratodon purpureus*. Planta 181: 71-77.

Walker, L. M. and Sack, F. D. 1991. Recovery of gravitropism after basipetal centrifugation in protonemata of the moss *Ceratodon purpureus*. Can. J. Bot. 69: 1737-1744.

Walker, L. M. and Sack, F. D. 1992. Stereological analysis of gravitropism in protonemata of the moss *Ceratodon*. Internat. J. Plant Sci. 158: 24-31.

Walker, L. M. and Sack, F. D. 1997. Stereological analysis of gravitropism in protonemata of the moss *Ceratodon*. Internat. J. Plant Sci. 158: 24-31.

Watson, E. V. 1974. The Structure and Life of Bryophytes. Hutchinson University Library, London, 211 pp.

Watson, M. A. 1981. Chemically mediated interactions among juvenile mosses as possible determinants of their community structure. J. Chem. Ecol. 7: 367-376.

Young, J. C. and Sack. 1992. Time-lapse analysis of gravitropism in *Ceratodon* protonemata. Amer. J. Bot. 79: 1348-1358.

Yu, Y., Guo, S.-L, and Chen, J.-H. 2008. Effects of varying sucrose and ammonium nitrate concentrations on protonemal growth of *Polytrichum commune* (Bryopsida: Musci) *in vitro*. Lindbergia 33: 41-46.

Zhao, J.-C., Huang, S.-L., Li, M., Mamtimin, S., He, J., Zhang, Y.-M., and Li, X. 2004. A study on the characteristics of spore germination and protonemal development in *Lindbergia brachyptera*. Arctoa 13: 223-228.