

CHAPTER 5-5

ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOPHORES

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CHAPTER 5-5

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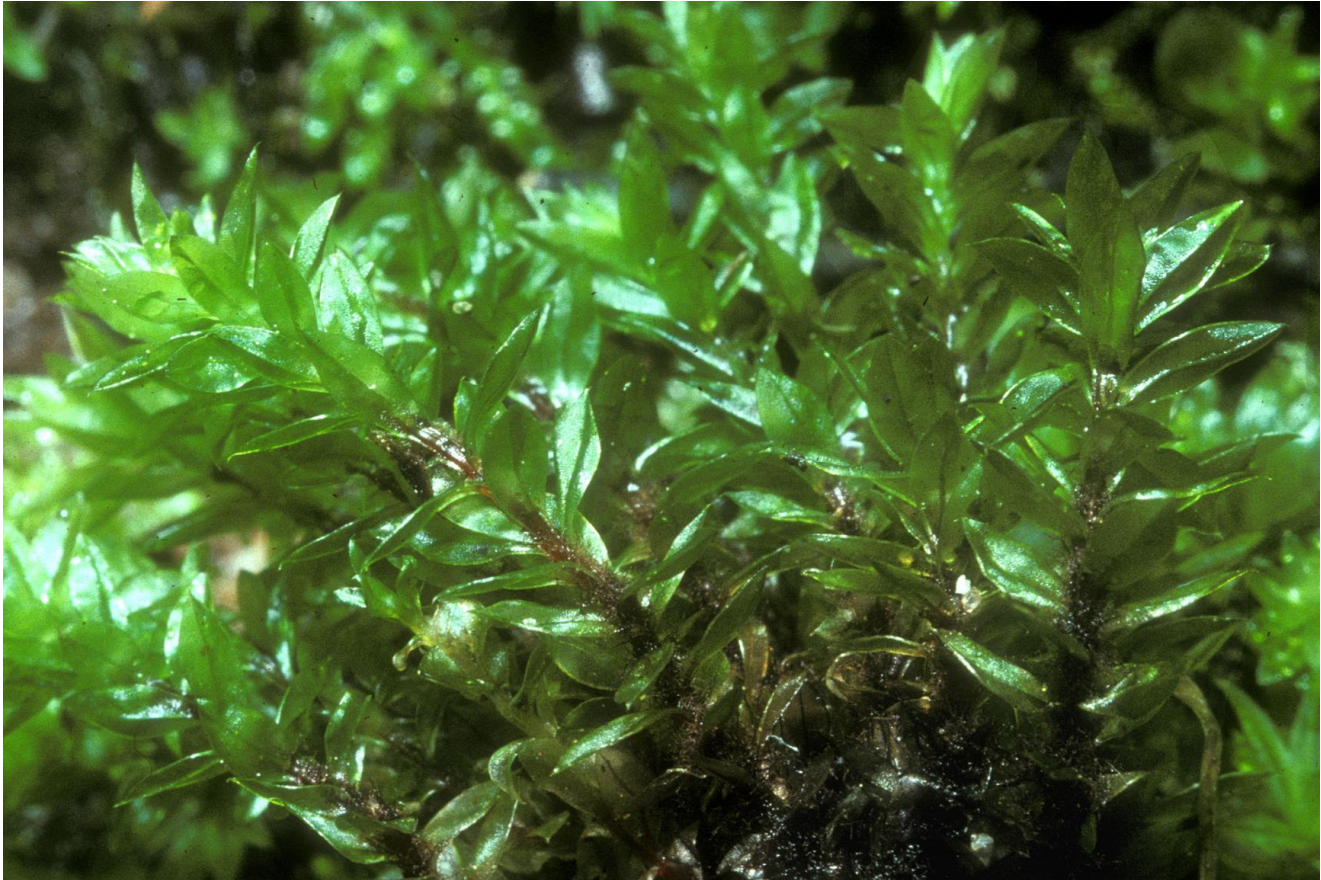


Figure 1. *Bryum pseudotriquetrum* gametophores, showing leaves, stems, and rhizoids. Photo by Janice Glime.

Growth

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

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

Stem Growth

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

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

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

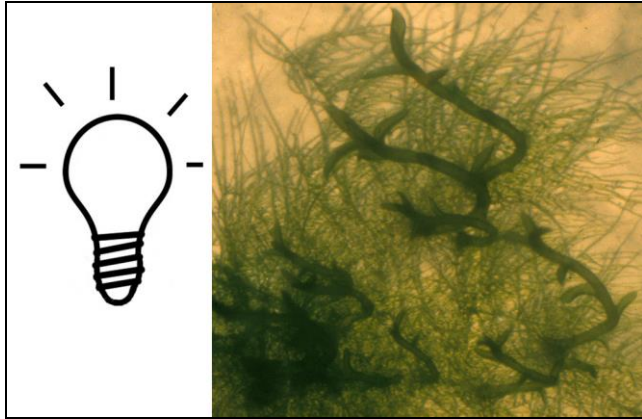


Figure 2. Culture of *Funaria hygrometrica* with Petri plate covered on top and the only light source from the side of the plate. Note the etiolated appearance of the shoots in this dim light compared to those in Figure 3. Photo by Janice Glime.



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

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

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



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

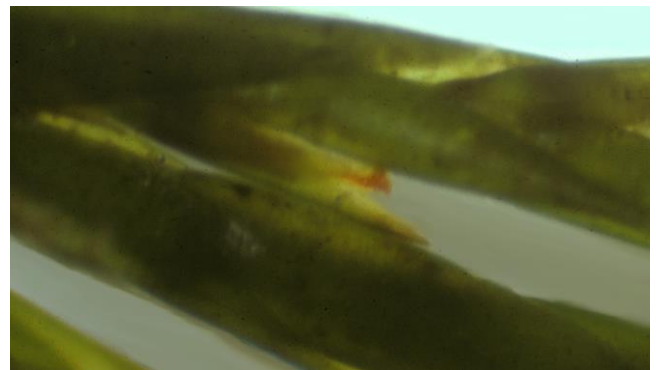


Figure 5. *Fontinalis dalecarlica* with archegonium, a phenomenon that coincides with a slowing of vegetative growth. Photo by Janice Glime.

Water

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



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



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

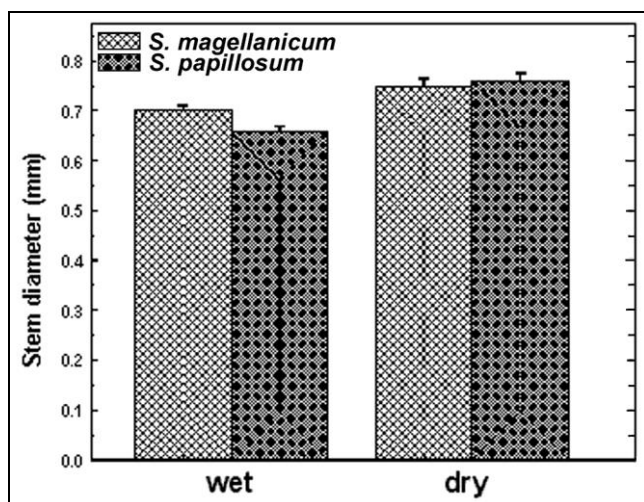


Figure 8. Effect of water level on stem diameter due to number of hyaline cell layers. **Wet** indicates stem tip starting at level 3 (7 cm) above the water; **dry** indicates stem tip starting at level 5 (15 cm) above the water. Based on Li *et al.* 1992.

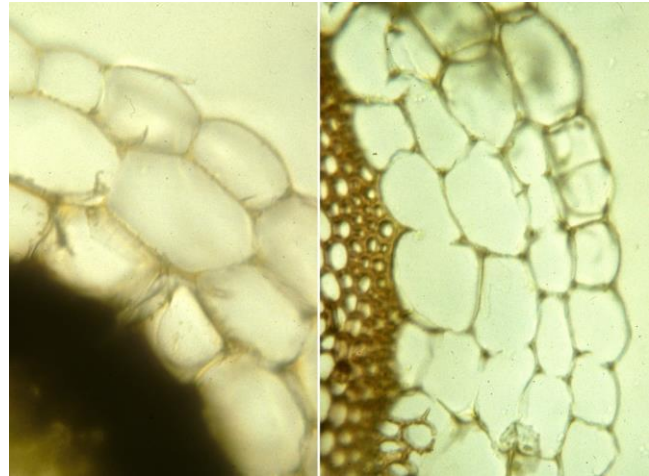


Figure 9. Effect of water level on stem width due to number of hyaline cell layers for *Sphagnum magellanicum*. **Left:** Stem at level 3 above the water (wet), showing only three rows of hyaline cells. **Right:** Stem at level 5 above the water (dry), showing four rows of hyaline cells. Based on Li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

Light

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



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

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

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



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

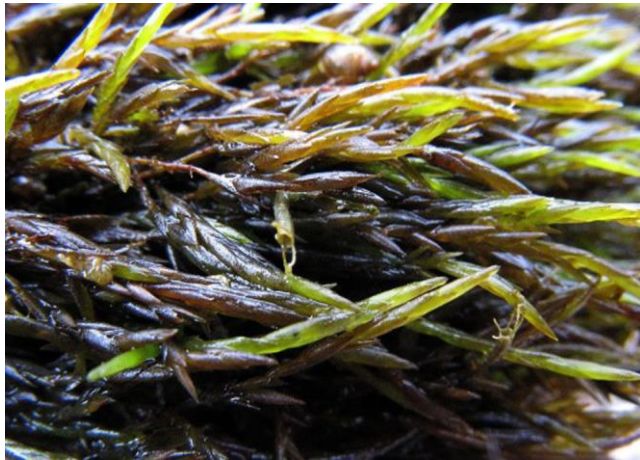


Figure 12. *Fontinalis duriaei*, an aquatic species where photosynthesis attenuates at low light levels (5400 lux). Photo by Jan-Peter Frahm, with permission.

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



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

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



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

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



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

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

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

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

Tropisms

It seems that most of the research on tropisms has been done on the protonema. **Phototropism** and **gravitropism** are most likely common for bryophyte stems, but aside from field observations, we know almost nothing about them in mature plants. However, it is clear that stems grow up and rhizoids grow down, just as do stems and roots of tracheophytes. One would expect tropisms in acrocarpous mosses, and surely something is causing their normal upright growth. Yet there seem to be a number of acrocarpous mosses that grow on vertical substrata and do not respond to gravity, and perhaps not to light. Genera

such as *Orthotrichum* (Figure 16) typically grow outward from their tree trunk habitat and even the sporophyte seems oblivious to gravity. And at least some species of *Pogonatum* (Figure 17-Figure 18) and *Oligotrichum* (Figure 19) seem to lack a strong gravitropism or phototropism in their gametophytes when growing on a vertical substrate, whereas their sporophytes do bend upward. On the other hand, the stem of the pleurocarpous aquatic moss *Fontinalis* exhibits positive phototropism (bends toward light; Figure 20). A strong phototropism is seen for the acrocarpous *Funaria hygrometrica* in Figure 3.



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



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



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



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



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

Photoperiod

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

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

But *Sphagnum* (Figure 6-Figure 7) is not alone in showing short-day dormancy, and control appears to be

unrelated to temperature. In the liverwort *Reboulia hemisphaerica* (Figure 21), long days caused archegoniophore elongation at either 15°C or 25°C, whereas short days induced no response at any temperature (Koevenig 1973b). Even application of IAA, NAA, VA, and GA₃ could not break the effect of short days. This leaves us to wonder what ultimately controls the response, and is the controlling factor the same in all bryophytes?



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

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

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

Temperature

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



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

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



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

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

Growth Regulators

Hormones in plants seem to defy definition (Christianson 1999). In plants, using the terminology of "growth regulators" permits us to define them as substances produced in one place in the organism that acts in small quantities to affect another part. But Christianson contends that this definition does not work well for the "untidy bundle of phenomena in plants." Rather, plant hormones

can act locally or be transported and often have numerous roles, interact with other hormones, or are concentration dependent for their functions.

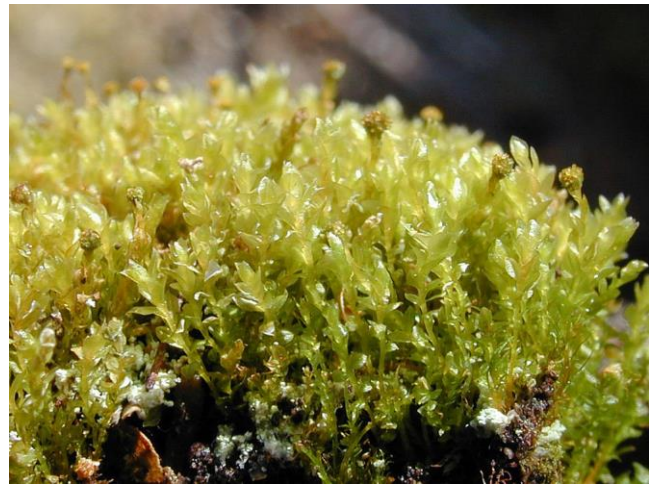


Figure 24. *Tetraphis pellucida* with gemmae, a moss in which growth is controlled by temperature rather than light. Photo by Michael Lüth, with permission.



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

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

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

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

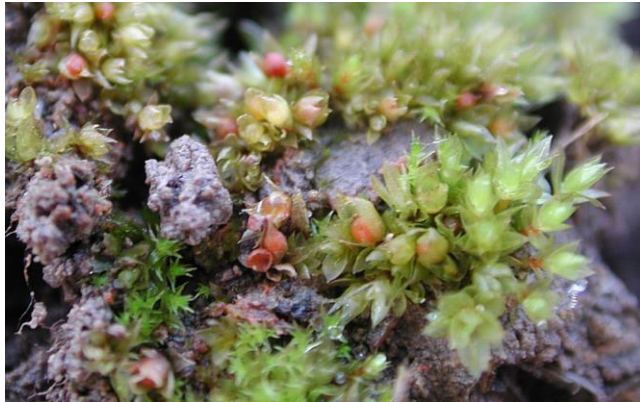


Figure 26. *Physcomitrella patens* with capsules, a moss that has demonstrated the concentration of auxin at the tip and base of the upright gametophore, with distribution throughout the stem. Photo by Michael Lüth, with permission.

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

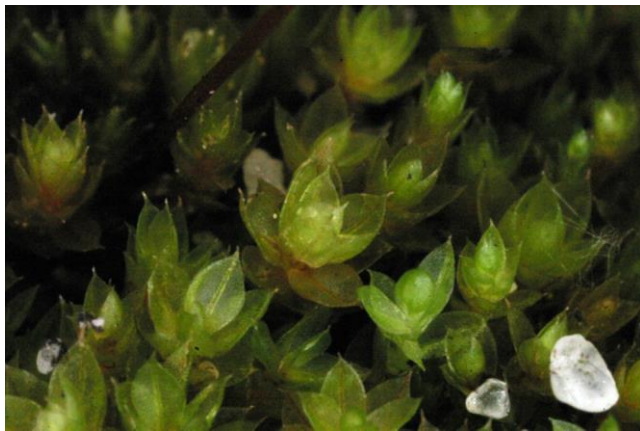


Figure 27. *Bryum atrovirens*, a species that exhibits abnormal development at higher concentrations of auxins. Photo by Jan-Peter Frahm, with permission.

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

While working with *Avena* (wheat) and two liverworts, Kaufman *et al.* (1982) found several basic generalities in

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

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

Bryophytes seem to respond to different concentrations and respond at different rates from those exhibited by tracheophytes. While working with *Avena* (wheat), Kaufman and coworkers (1982) discovered that a tenfold increase in the growth rate of *Avena* internodes appeared about three hours after application of 10^{-5} M **GA₃**, but that 10^{-5} M **IAA** had no effect. On the other hand, when working with the liverworts *Pellia epiphylla* (Figure 28) and *Conocephalum conicum* (Figure 29), they found that the setae and archegoniophore stalks responded to 10^{-5} M IAA with a two-fold increase in growth rate within 10-15 minutes. Many higher plants also show this rapid response to IAA, but this depends again on the concentration (Osborne 1974; Muir 1974). The rapid response in the liverworts suggested to Kaufman and coworkers (1982) that IAA had a direct effect on the cell membrane, allowing expansion by drawing water into the cell, since growth of the cytoplasm would require slow protein synthesis. We now know that IAA probably works on the cell wall (Goodwin & Mercer 1983), most likely by facilitating the breakdown of **calcium pectate** so the fibers can slide and expand, and this most likely involves an acid efflux via the proton pump from the cells, hence the H^+ observed by Kaufman *et al.* (1982). The freed Ca^{++} is then available to enter the cell, most likely accounting for the observed increase in Ca^{++} there.



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



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

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



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

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

Ethylene is likely to have an early role in gametophore development. We know that seedlings produce ethylene in response to physical contact (Abeles 1973). Thus, if an emerging seedling encounters dense soil or rock, ethylene production inhibits mitosis, thus halting meristematic

activity, and the cells respond by less elongation and by growing wider and thicker, giving the stem greater strength. This greater strength, coupled with continuing but reduced cell elongation, can dislodge small obstructions or push through dense soil. If the obstruction is a rock, ethylene production on the side of contact slows elongation on that side, resulting in plant curvature around the rock.

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

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



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



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

Submersed mosses [*Fontinalis* (Figure 31), *Drepanocladus* (Figure 33-Figure 34)] often possess widely spaced leaves and thin stems, whereas the same species in shallow water will have thick stems and overlapping leaves. Fuchsig (1926) observed that this gives the shallow water individuals a greater resistance to desiccation with weight loss during desiccation being greatest in the deep water form. Two factors would implicate ethylene and IAA as the controlling factors here. In deep water, light is dim and no light inhibition of IAA should occur since UV light in particular is filtered out. Therefore an etiolation response is expected. At the surface, two factors known to enhance ethylene production occur: (1) stress due to wave action and alternate wetting and drying; (2) a high ratio of O₂:CO₂ relative to deep water. Endogenous ethylene could easily account for thicker cells and greater stem strength at the water surface.



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



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

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

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

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

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

Branches and Apical Dominance

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

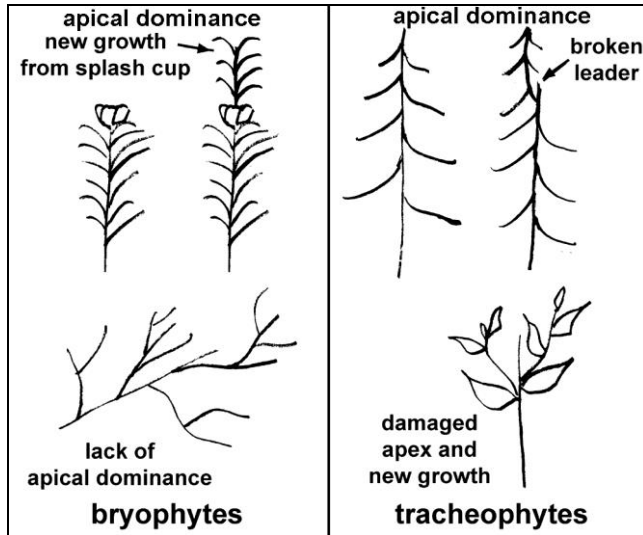


Figure 35. Effects of apical dominance on growth forms of bryophytes and tracheophytes. Drawings by Janice Glime.

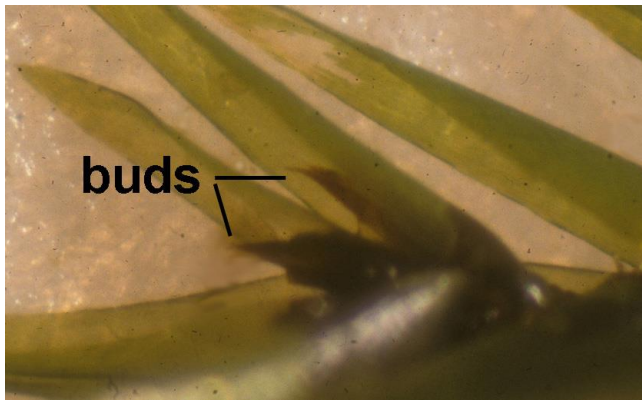


Figure 36. Branch buds developing near the broken tip of *Fontinalis squamosa*. Photo by Janice Glime.

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

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

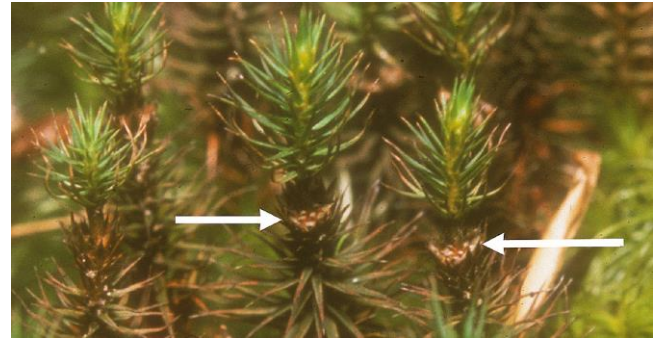


Figure 37. New growth from a senescent antheridial splash cup of *Polytrichum ohioense*. Photo by Janice Glime.

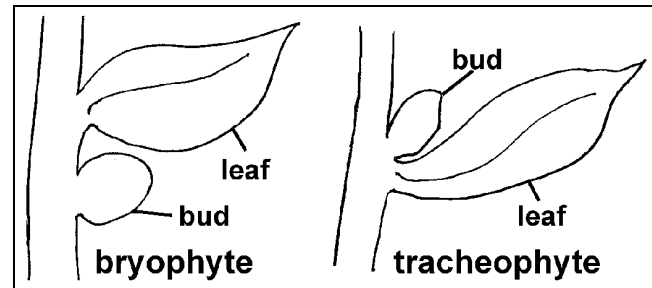


Figure 38. Position of branch buds in bryophytes vs. tracheophytes. Drawing by Janice Glime.

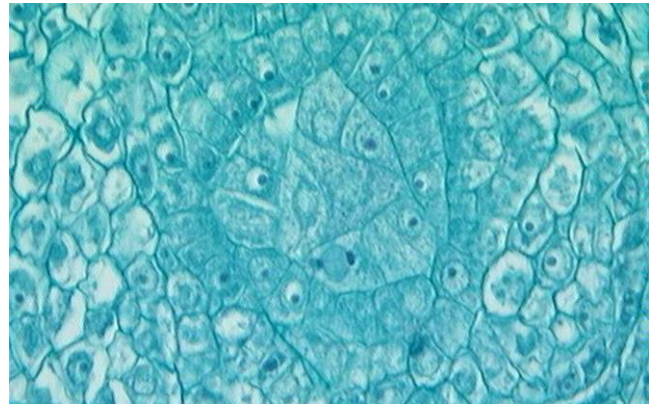


Figure 39. *Polytrichum* stem apex cross section showing three cutting faces. Photo by Magda Turzańska, with permission.

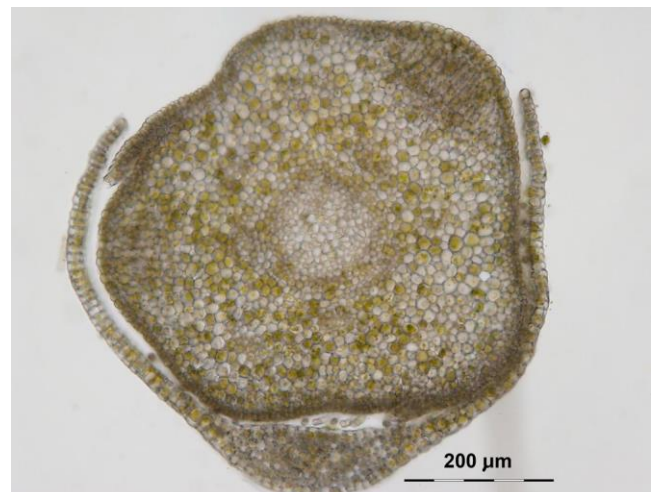


Figure 40. Mature *Polytrichum* stem cross section. Photo by Magda Turzańska, with permission.

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

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



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

Environmental Factors

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

Chopra and Rashid (1969) likewise found that increased light intensity promoted lateral bud formation in

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

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

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



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

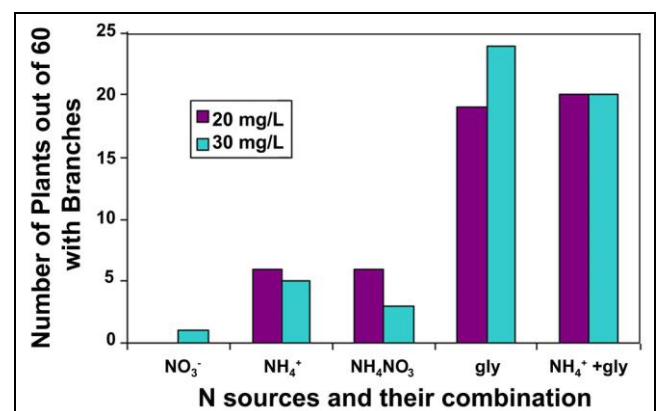


Figure 43. Effects of different types of nitrogen source on branch production in the Java moss, *Taxiphyllum barbieri*. gly = glycine. Graph from Alghamdi 2003.

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

Growth Regulators

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

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



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

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



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



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

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

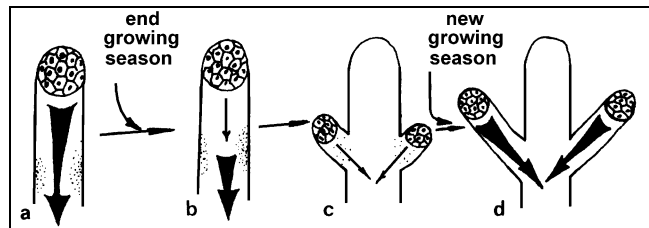


Figure 47. Theoretical relationship of auxin (IAA) and cytokinin in controlling branch production. **a)** Apical region during active growing season shows large production of IAA (arrow), inhibiting localized concentrations of cytokinin. **b)** End of growing season slows apical activity and production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

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

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



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

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

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

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

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

Pleurocarpous Mosses

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



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

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

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

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

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



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



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

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

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

on branching. Since this variety does little branching normally, it may have been an inappropriate taxon to test.

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

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



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

Thallose Liverworts

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

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

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

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

Nutrients

Koevenig (1973a) suggests that the growth hormones IAA, NAA, BA (6-benzyladenine, a cytokinin), and GA₃ may only aid in elongation but not actually induce it, implying that other substances are needed, such as the metals. Many compounds influence plant growth. Sharma *et al.* (1960) reported that *Haplomitrium* (Figure 53) gametophytes grew better on media containing various amino acids, indicating that organic material must be present in the substrate. Copper can stimulate growth of some bryophytes at elevated concentrations (0.01 ppm), presumably through greater photosynthesis (Sommer 1931; Glime & Keen 1984), wherein it is needed in plastocyanin, a chloroplast protein. Nevertheless, it soon becomes inhibitory at higher concentrations.



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

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

nature. However, etiolation due to lower light intensity in the laboratory cannot be ruled out.



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

Leaves

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



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

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

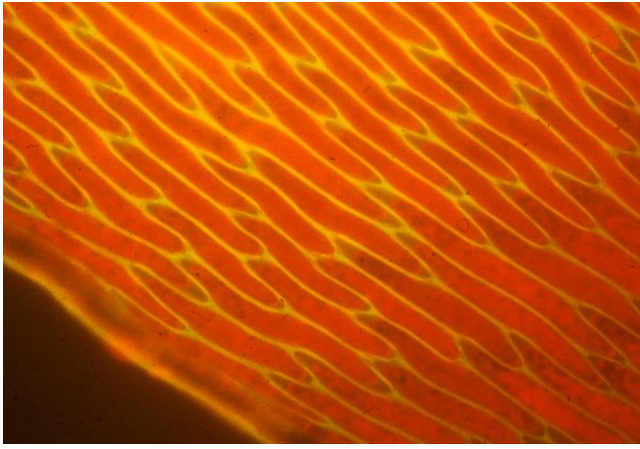


Figure 56. Fluorescence of cell walls under UV light in a leaf of *Fontinalis antipyretica*. Photo by Janice Glime.

Light

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



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

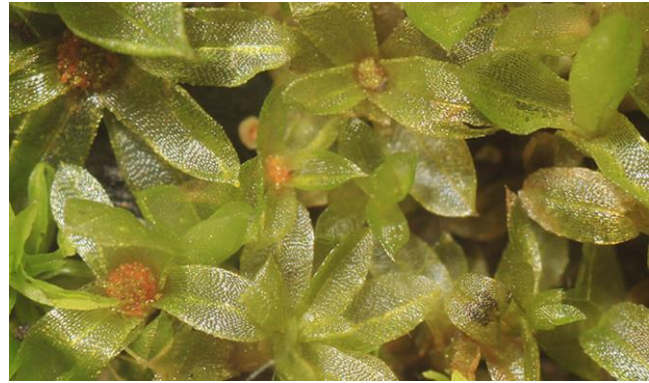


Figure 58. *Funaria hygrometrica*, a species for which light quality changes did not change leaf shape. Photo by Barry Stewart, with permission.

Water

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



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

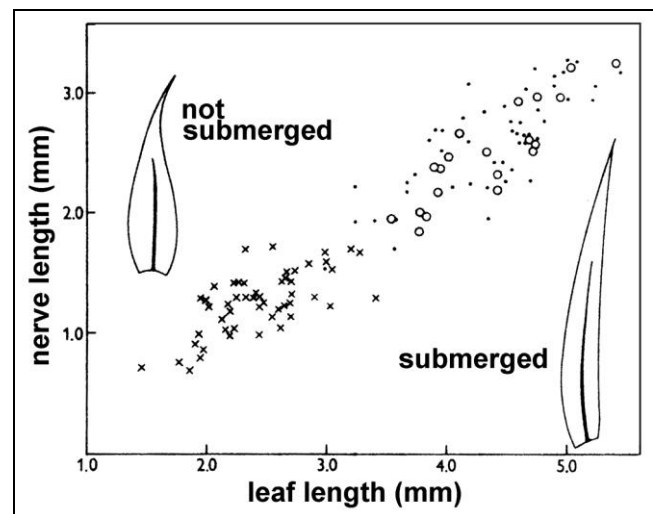


Figure 60. Modifications in leaf morphology of *Drepanocladus fluitans* due to submergence, in this case causing elongation. Redrawn from Lodge 1959.

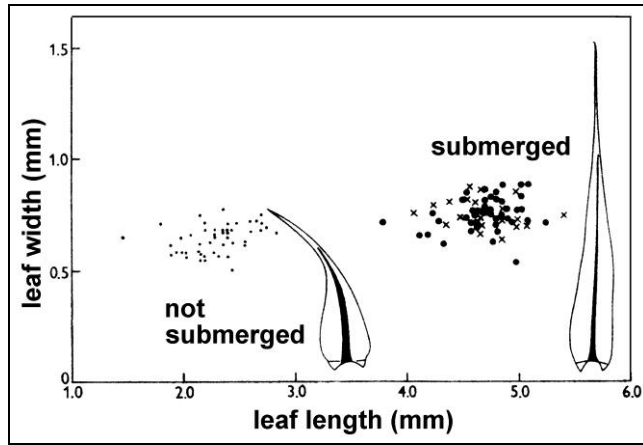


Figure 61. Modifications in leaf morphology of *Drepanocladus fluitans* due to submergence, in this case causing loss of falcation. Redrawn from Lodge 1959.

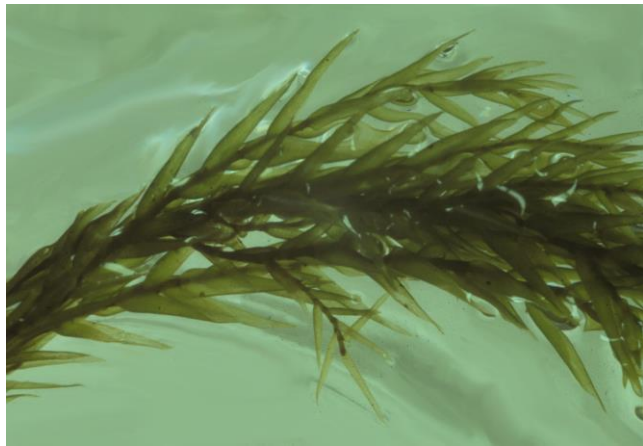


Figure 62. *Fontinalis novae-angliae* with normal submerged leaves. Photo by Janice Glime.



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

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

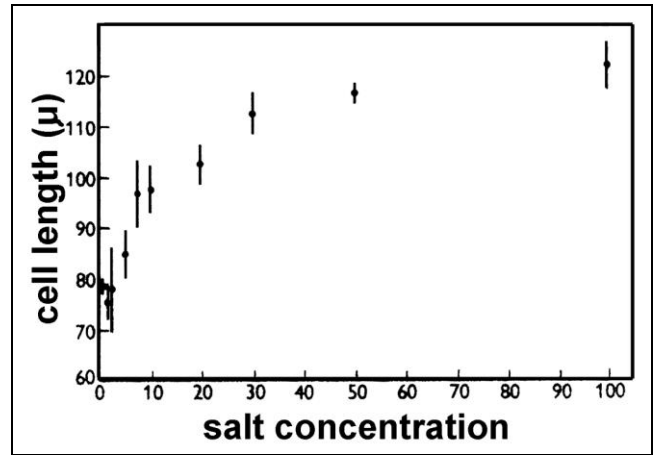


Figure 64. Relationship between leaf cell length and salt concentration in *Drepanocladus fluitans*. Concentrations are relative percents of highest concentration with individual ions kept in same proportions. Redrawn from Lodge 1959.

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

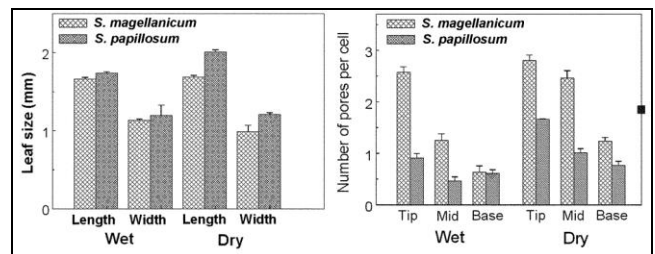


Figure 65. Effect of water level (water availability) on **left:** leaf length and **right:** number of pores per cell in *Sphagnum magellanicum* (Figure 6) and *S. papillosum* (Figure 7). Wet denotes 0 cm initial distance of capitulum from water; dry denotes 10 cm initial distance. Bars represent standard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

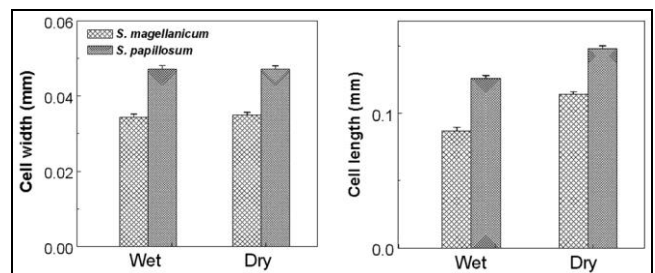


Figure 66. Effect of water level (water availability) on hyaline cell width and length in *Sphagnum magellanicum* and *S. papillosum*. Wet denotes 0 cm initial distance of capitulum from water; dry denotes 10 cm initial distance of capitulum from water. Bars represent standard error. From Li *et al.*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

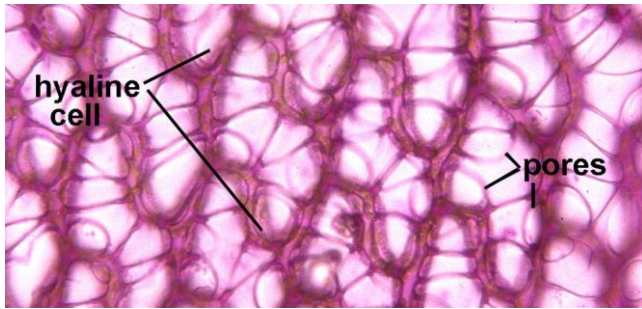


Figure 67. *Sphagnum papillosum* leaf showing hyaline cells and pores. Photo courtesy of Yenhung Li.

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



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

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



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



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



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

Nutrients

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



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

Growth Regulators

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

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

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



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

In nature, such events are likely to occur in response to leaf litter cover, ice, snow, and other physical barriers. By preventing diffusion of ethylene, unequal concentrations of ethylene result around different parts of plants, and as ethylene buildup occurs, contorted growth can result. An ethylene-induced growth differential between stems and leaves could explain the appearance of reduced leaves on

stolons (horizontal stems from which upright stems arise) of certain species of *Fontinalis* (Glime 1980). If these stolons are a response to burial in a sandy substrate, or even burial among other *Fontinalis* branches that impede flow, ethylene production and accumulation could be the biochemical agent.



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

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

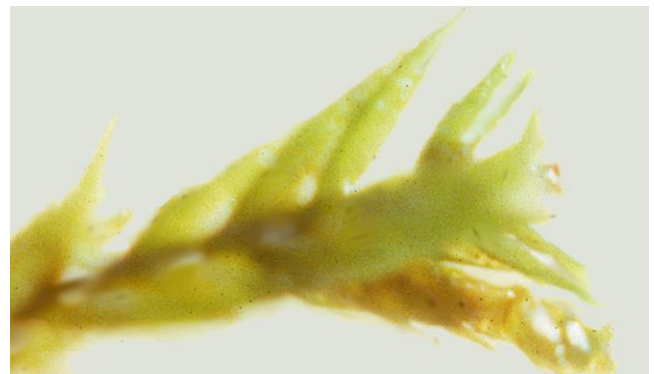


Figure 75. Effects of ACC (and presumably ethylene) on the shoot apex of *Fontinalis squamosa*. Note truncated tip where leaves did not elongate while nearby leaves continued growth. Photo by Janice Glime.

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

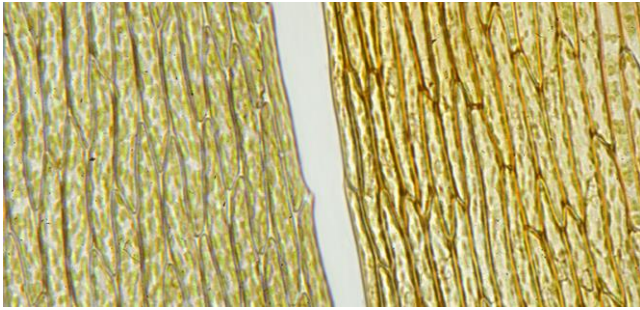


Figure 76. Effect of ACC on leaf cell wall color in *Fontinalis antipyretica*. **Left:** Normal cells. **Right:** Cells subjected to 10^{-4} M ACC. Photo by Janice Glime.

As noted above, *Fontinalis* also can develop a modified leaf shape when grown exposed to air. When it is submersed during growth, leaves are straight, but in our lab cultures where it grew in a thin film of water and continuously received exposure to air while remaining wet, leaves became falcate (curved like a sickle; Figure 63). This may have been another example of ethylene production in the high oxygen, low CO₂ environment of air, as opposed to that in water. It is interesting that the other two genera in the family, *Brachelyma* and *Dichelyma* (Figure 77), have falcate leaves and grow most of the year out of the water.



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

Liverwort Leaf Suppression

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

Ethylene seems to have played a major evolutionary role in these bryophyte leaf arrangements. Basile and Basile (1983a, b, 1984, 1994) have shown that **hydroxyproline** (crystalline amino acid abundant in major glycoprotein of plant primary cell wall) will induce underleaves of liverworts to reach the size of lateral leaves, and in some cases induce development of underleaves when they are unknown in nature. They contend that loss of normal-sized underleaves in bryophytes, such as seen in

Haplomitrium (Figure 78), is an evolutionary result of inhibition by ethylene, because ethylene antagonists such as hydroxyproline can induce these bryophytes to produce normal leaves where small underleaves would normally be. This is consistent with the widespread belief that 3-ranked leafy liverworts (Figure 78) are the primitive form, with 2-ranked ones being derived (and as implied here, derived due to suppression of the third row that results in reduced underleaves typical of many leafy liverworts; Figure 79).



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

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

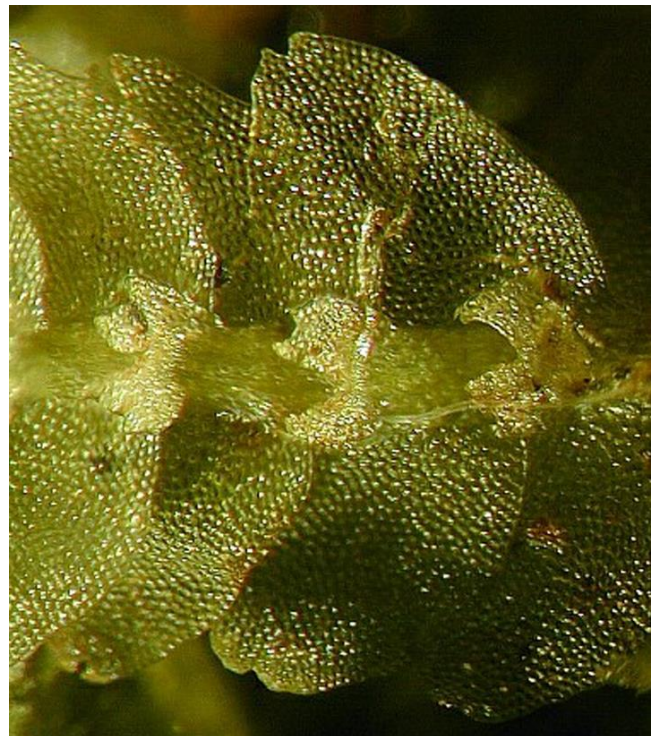


Figure 79. Ventral view of *Calypogeia fissa*, a leafy liverwort with the underneath row of leaves suppressed. Photo by Michael Lüth, with permission.

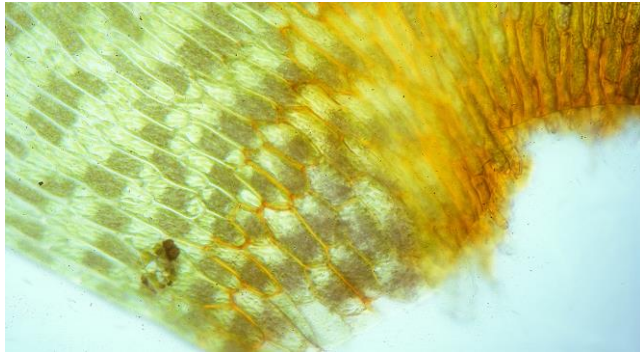


Figure 80. Plasmolyzed basal leaf cells in *Fontinalis antipyretica* subjected to 10^{-3} M ACC. Photo by Janice Glime.

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

Cuticle

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

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

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

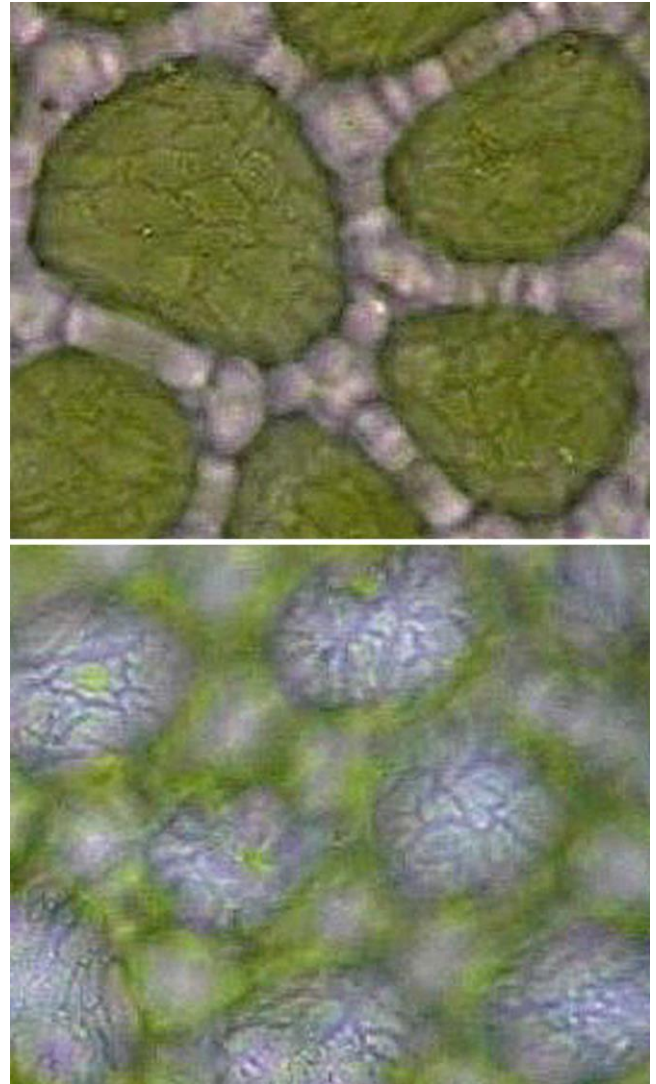


Figure 81. *Mylia anomala* showing cuticle. Photo by Paul G. Davison, with permission.

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

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

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

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

Calyptrae

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

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

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

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

Rhizoids

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

Temperature

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



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

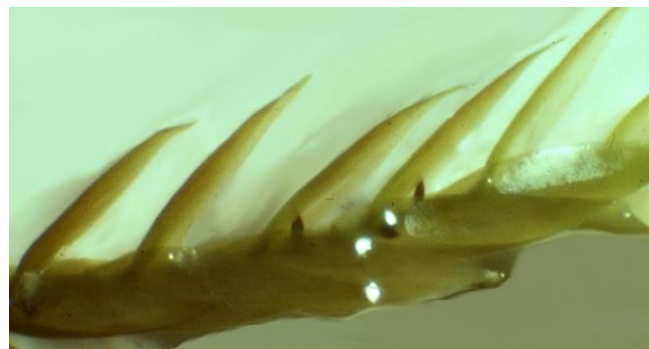


Figure 83. *Fontinalis hypnoides*, a species that lives in both streams and lakes. Photo by Janice Glime.

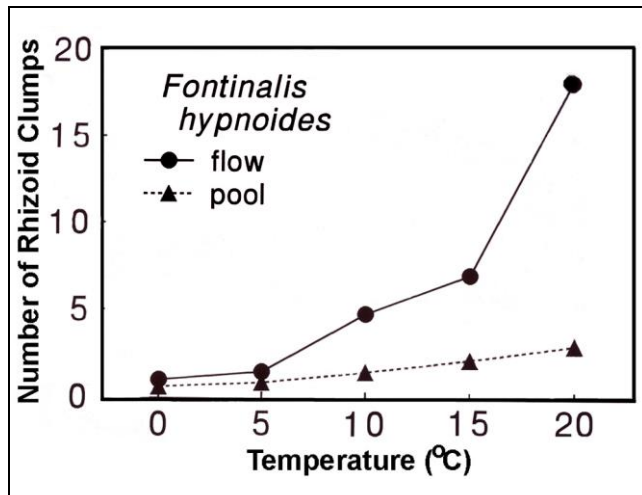


Figure 84. Flow and temperature effects on mean number ($n=40$ stem tips in each condition) of rhizoid clumps in *Fontinalis hypnoides* from the Keweenaw Peninsula of Michigan, USA, after 15 weeks in flowing water and pool conditions in artificial streams. From Glime & Raeymaekers 1987.

Light

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



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

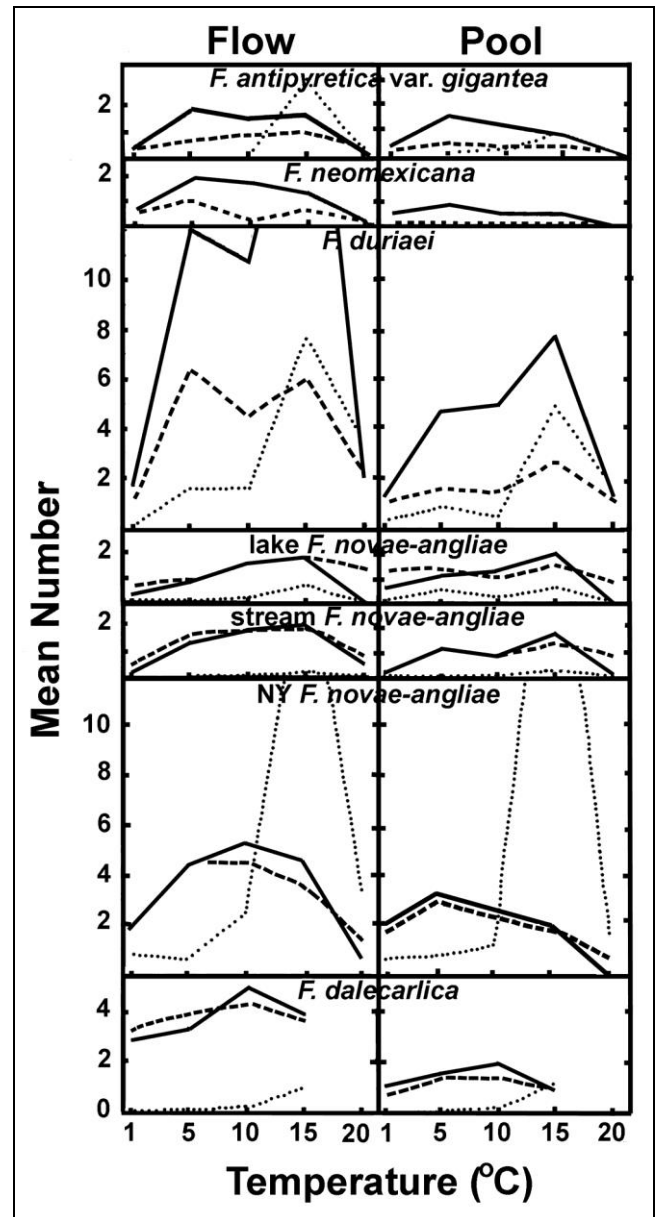


Figure 86. Flow and temperature effects on mean number (40 replicates at each condition) of rhizoid clumps (**dotted line**), branches per cm (**dashed line**), and cm growth of stem + branches (**solid line**) after 15 weeks in flowing water and standing water (pool) conditions in artificial streams.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



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

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



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

Tropisms

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

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

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

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

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

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

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

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



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

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



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

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

One might suspect that **gravitropism** (directional growth in response to gravity) could be a cue for direction of growth in *Fontinalis* rhizoids, but I have not been able to induce a gravitropic response in *Fontinalis antipyretica* or *F. squamosa* (Glime 1987c). Instead, a strong negative phototropism occurs, even when it means rhizoids must grow pointed toward the stem apex, as in Figure 90. *Funaria hygrometrica* (Figure 2-Figure 3, Figure 54), on the other hand, has positively gravitropic rhizoids (Figure 91) that are indifferent to light (Kofler 1958). *Funaria* does not grow on vertical substrata, so gravitropism would be an adaptive feature for *Funaria*, whereas in *Fontinalis* it could be maladaptive for a plant that tends to grow on

vertical faces on downstream sides of rocks. On the other hand, light will always be from above in habitats suitable for *Funaria*, so absence of phototropism may have no selective disadvantage.



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

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



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

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

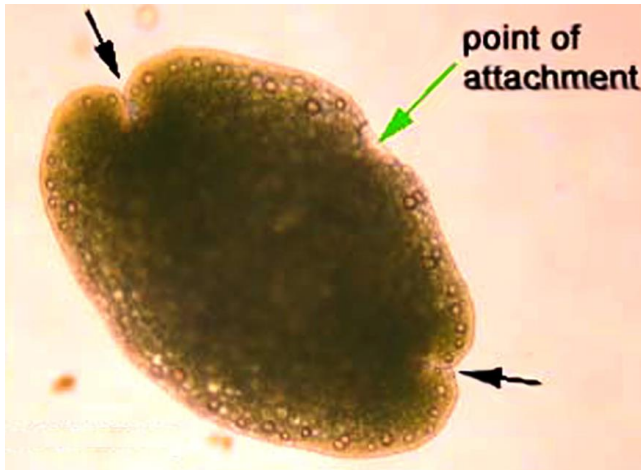


Figure 93. *Marchantia polymorpha* gemma. Black arrows indicate apical notches that serve as growing points. Photo by Kavita Uttam, Botany website, UBC, with permission.

Adhesion

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

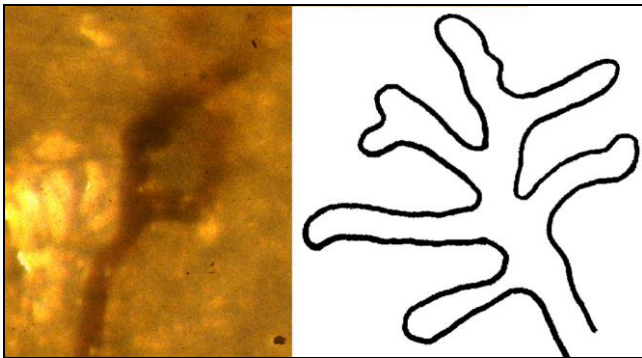


Figure 94. Branched tip of *Fontinalis squamosa* rhizoid in response to contact. Photo by Janice Glime; drawing by Margaret Minahan.

It is interesting that the flattened portion of the rhizoid occurs only at the tips in the pleurocarpous mosses, whereas in the acrocarpous mosses it extends far back from the tip (Odu 1989). Yet few acrocarpous mosses occur on

vertical surfaces, most likely due to the small area available for adhesion compared to the weight of an outward-growing moss. Pleurocarpous mosses, on the other hand, have abundant surface area in contact with the substrate, and rhizoids typically occur throughout.



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

Growth Regulators

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



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

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

Wounding

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



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

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

Numerous experiments show that ethylene levels rise as a result of wounding. In fact, most experiments on plants probably begin with elevated ethylene due to handling by the experimenter. If this is true, what occurs in a moss subjected to continual stress of a fast current? Using artificial streams in the laboratory, Glime and her students (Glime *et al.* 1979) found that rhizoids of several aquatic mosses [*Hygroamblystegium fluviatile* (Figure 99), *Fontinalis duriaei* (Figure 100)] began to adhere to rocks after about 9 weeks and little additional attachment occurred after 14 weeks of contact (Figure 101). In these experiments, pieces of freshly wounded moss were tied to

the rocks to insure contact and maintain their location. Odu (1978b) found a much shorter period of rhizoid growth for *Calliergonella cuspidatum* (Figure 102), *Pleurozium schreberi* (Figure 103), and *Brachythecium rutabulum* (Figure 104), species that grow mostly on soil or in standing water. Their rhizoid growth rates leveled off after about 6 weeks, and after 10 weeks there was no further growth.



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



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

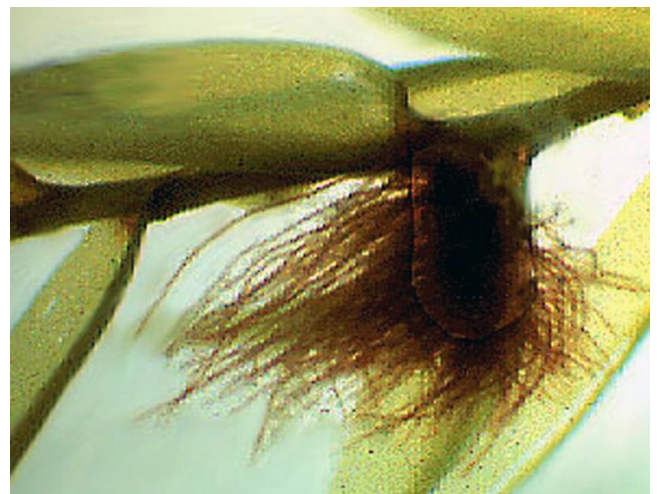


Figure 100. *Fontinalis hypnoides* rhizoids produced in culture. Photo by Janice Glime.

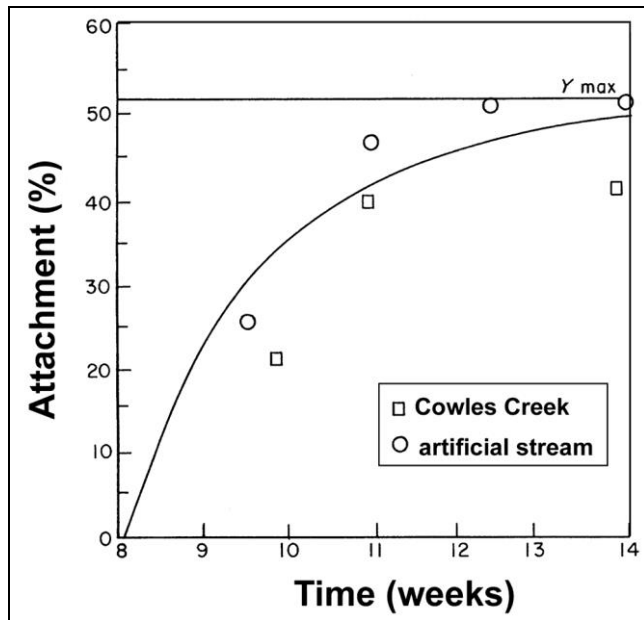


Figure 101. Model for rhizoid attachment to four rock types (shale, granite, basalt, sandstone – data combined) in *Fontinalis duriaei* in a natural and an artificial stream. $n = 12$ for each rock type and each stream. Based on Glime *et al.* 1979.



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



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

Habitat Conditions

Odu (1978a, 1979) has found that acrocarpous mosses produce rhizoids all the way around the stem, but these are

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



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



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



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

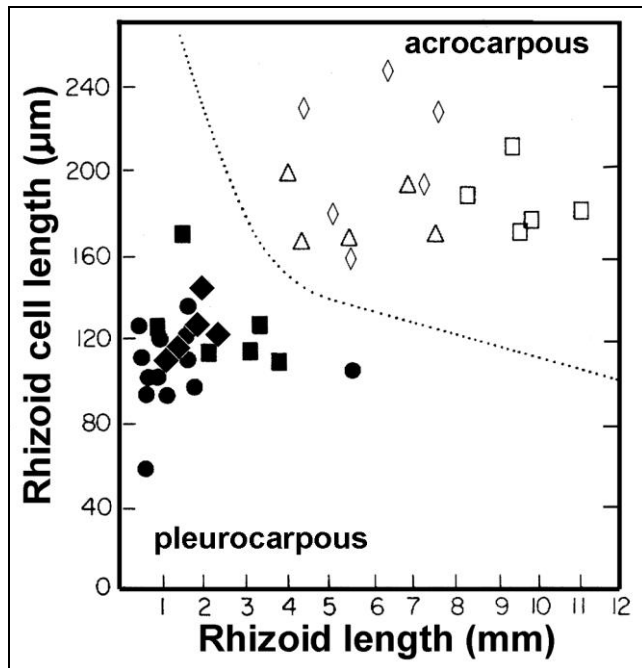


Figure 107. Relationship of cell length to rhizoid length in acrocarpous (\diamond *Bryum capillare*, Δ *Pohlia nutans*, \square *Dicranum scoparium*) and pleurocarpous (\bullet *Hypnum cupressiforme* var. *cupressiforme*, \blacksquare *Rhynchostegium confertum*, \blacklozenge *Homalothecium sericeum*)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

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



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

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

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

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

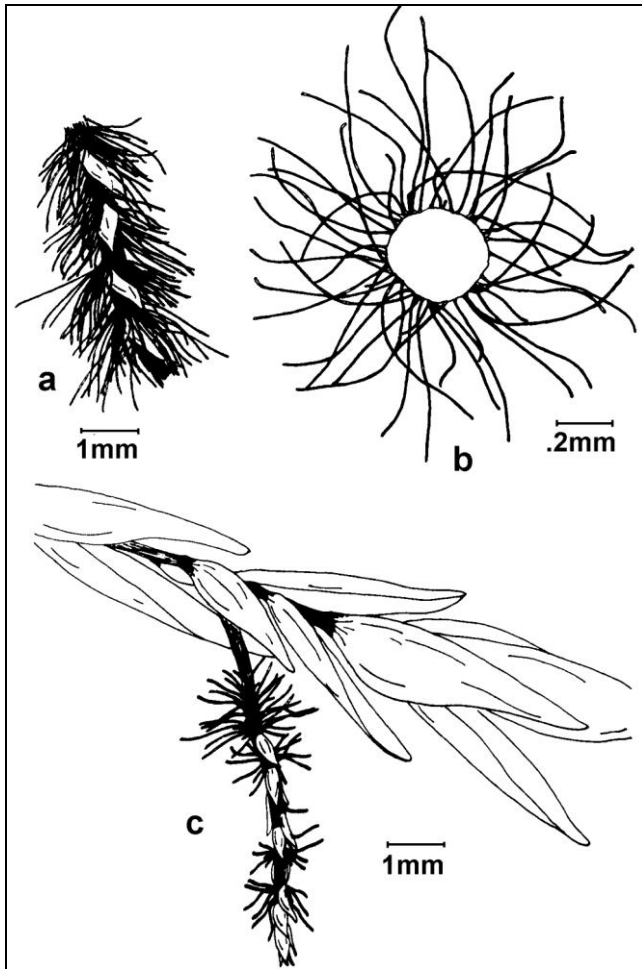


Figure 109. Rhizoids of *Fontinalis* on stoloniferous branches. **a.** *Fontinalis dalecarlica*. **b.** cross section of stoloniferous branch of *Fontinalis dalecarlica*. **c.** *Fontinalis novae-angliae*. From Glime 1980.



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

My observations on *Fontinalis hypnoides* (Figure 100) (Glime 1980) help to explain the control of rhizoid production in the aquatic habitat. The number of rhizoids increased with temperature when cultured at 1, 5, 10, 15, and 20°C. Furthermore, mosses in flowing water produced more rhizoids than those in standing water. The latter observation might be explained by **ethylene** control, since

ethylene is known as an inhibitor of rhizoid elongation in ferns (Miller *et al.* 1970). In our experiments on *F. squamosa* (Figure 31), ACC (ethylene precursor) inhibited rhizoid production with increasing concentrations in cultures on wet filter paper, and the inhibition was more severe in mosses in water (Glime & Rohwer 1983). Since ethylene is not very soluble in water, it could easily accumulate around the moss and be a cause for the retardation of rhizoids in standing water, whereas flowing water would remove the ethylene. On the other hand, this removal action must counteract the increased production of ethylene we might expect to result from the mechanical stress of flowing water. But no one has demonstrated that mechanical stress does indeed induce ethylene production in bryophytes, as it does in tracheophytes. And we can reasonably expect the effective concentrations are different in bryophytes. Just as roots and shoots respond differently in tracheophytes, different parts of bryophytes can respond differently from each other and from parts with similar functions in tracheophytes.



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

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

Conduction

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

Bryophyte Senescence

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

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



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

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



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



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

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

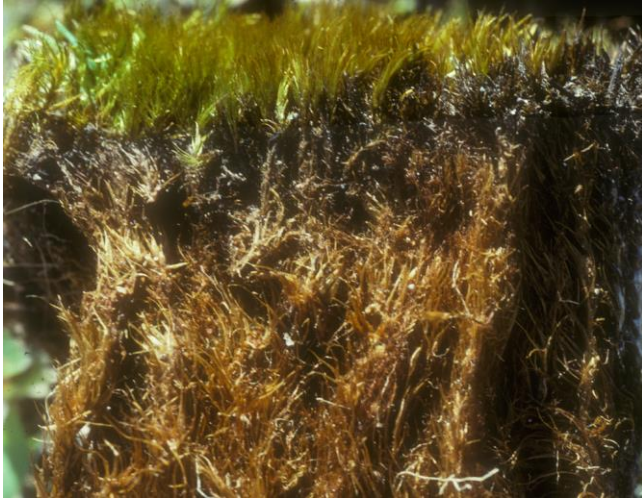


Figure 115. Senescence in lower, brown portion of *Dicranum scoparium*. Photo by Janice Glime.

Ecological Interaction

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

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

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

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

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

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

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

Summary

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

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

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

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

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

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

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

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

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

Acknowledgments

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