CHAPTER 5-5

ECOPHYSIOLOGY OF DEVELOPMENT:
GAMETOPHORES

TABLE OF CONTENTS

Growth ....................................................................................................................... .........................................5-5-2
Stem Growth .................................................................................................................................5-5-2
   Water.................................................................................................................................................5-5-3
   Light .....................................................................................................................................................5-5-4
   Tropisms ..............................................................................................................................................5-5-6
   Photoperiod .......................................................................................................................................5-5-7
   Temperature .......................................................................................................................................5-5-7
   Growth Regulators ..........................................................................................................................5-5-8
Branches and Apical Dominance .................................................................................................5-5-9
   Environmental Factors ...................................................................................................................5-5-11
   Growth Regulators .........................................................................................................................5-5-11
   Nutrients ............................................................................................................................................5-5-14
Leaves ...............................................................................................................................................5-5-14
   Light .....................................................................................................................................................5-5-14
   Water ....................................................................................................................................................5-5-15
   Nutrients .............................................................................................................................................5-5-17
   Growth Regulators ..........................................................................................................................5-5-18
Rhizoids ............................................................................................................................................5-5-19
   Temperature .......................................................................................................................................5-5-19
   Light ....................................................................................................................................................5-5-20
   Tropisms ..............................................................................................................................................5-5-21
   Adhesion .............................................................................................................................................5-5-22
   Growth Regulators ..........................................................................................................................5-5-22
   Wounding ..........................................................................................................................................5-5-22
   Habitat Conditions ..........................................................................................................................5-5-23
Bryophyte Senescence .....................................................................................................................5-5-25
Ecological Interaction ....................................................................................................................5-5-25
Summary .............................................................................................................................................5-5-27
Acknowledgments ............................................................................................................................5-5-27
Literature Cited .................................................................................................................................5-5-27
CHAPTER 5-5
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Figure 1. *Bryum pseudotriquetrum* gametophores, showing leaves, stems, and rhizoids. Photo by Janice Glime.

**Growth**

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.
Chapter 5-5: Ecophysiology of Development: Gametophores

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.

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

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

Figure 6. Sphagnum magellanicum, a species in which stem diameter increases with distance of capitulum from water surface. Photo by Michael Lüth.
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 *Drepanoclados* (Figure 10) has longer internodes in low light (Lodge 1959), making leaves appear to be sparse.

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 duriae* (Figure 12) photosynthesis attenuated at 5400 lux (Glime & Acton 1979); field intensities where *Fontinalis duriae* 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 12. *Fontinalis duriae*, an aquatic species where photosynthesis attenuates at low light levels (5400 lux). Photo by Jan-Peter Frahm.

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.

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.

Branching seems to be under a different set of wavelength 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.

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.

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

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.

**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.
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 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?

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).

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.

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?

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

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.
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**

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). Thus it is important in development of organs, wound healing, identification of "infectious intruders" by the immune system.

**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* 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* and *Conocephalum conicum*, 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 Ca2+ is then available to enter the cell, most likely accounting for the observed increase in Ca2+ there.

As is typical with hormone responses, not all bryophytes respond the same way. Marchantia palmata growth is 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 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, ethylene causes crumpled branches and stem tips (Figure 27; Glime 1983). G. Mogensen (pers. comm.) has seen similar crumpled branches as a common phenomenon in the Arctic. The crumbling 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.

Submersed mosses (Fontinalis, Drepanocladus) 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 27. Effects of ACC (and presumably ethylene) on apical leaves of Fontinalis squamosa. Photo by Janice Glime.

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. 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 Ca2+ that then enters the cell, replacing the positive H+ ions that were just lost. Anions that come with the Ca2+ create a salt within the cell, causing an osmotic gradient. Water follows by osmosis.

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 28). 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 29). 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.

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 28. Effects of apical dominance on growth forms of bryophytes and tracheophytes. Drawings by Janice Glime.](image)

![Figure 29. Branch buds developing near the broken tip of *Fontinalis squamosa*. Photo by Janice Glime.](image)

![Figure 30. New growth from a senescent antheridial splash cup of *Polytrichum ohiense*. Photo by Janice Glime.](image)

![Figure 31. Position of branch buds in bryophytes vs. tracheophytes. Drawing by Janice Glime.](image)

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 58) 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 32) 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.
Environmental Factors

In his work with *Racomitrium lanuginosum*, 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 33), 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 34). 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?

Growth regulators

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 35), *Funaria*]. However, our own studies on *Fontinalis* may offer some insight.
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 36). Stange (1964) demonstrated apical dominance in another acrocarpous moss, *Funaria hygrometrica*.

![Figure 36. *Splachnum ampullaceum*, a moss with known apical dominance due to IAA distribution. Photo by Michael Lüth.](image)

Tremaine and Glime (unpub.) grew *Fontinalis duriaeii* (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, with intermediate growth in the controls (no IAA) (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 duriaeii* (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.

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*, 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). 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 37.

![Figure 37. 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.](image)

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*.

Cytokinins have been shown to enhance IAA-induced ethylene formation (Goodwin & Mercer 1983), which is likely to cause senescence. But in the moss *Anoectangium thomsonii*, Chopra and Rashid (1969) observed that, at any concentration of added kinetin, there was an increase in the number of buds and the rate of bud initiation. However, further shoot development was inhibited. Spiess *et al.* (1972), working with *Pylaisiella selwynii* (Figure 38), 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 38. *Pylaisiella selwynii* on bark, where bud formation depends on cytokinin, but not further development. Photo by Janice Glime.](image)

But this relationship of buds with cytokinin does not seem to apply to all mosses. In the moss *Plagiomnium cuspidatum*, 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*.

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.

Let us re-examine the case of *Plagiomnium cuspidatum* (Figure 39). Although this moss is acrocarpous, it has lateral (plagiotropic) branches in addition to its upright stem (Figure 39). These branches may behave more like branches of pleurocarpous mosses in their response to ethylene, IAA, and cytokinins.

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Although *Fontinalis* 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 40, see also Figure 29), but when my student and I removed the apices from *F. antipyretica var. gigantea* (Figure 41), the removal had no observable effect on branching. Since this variety does little branching normally, it may have been an inappropriate taxon to test.

Berthier (1966) found that maximum apical dominance in *Fontinalis* 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.

![Figure 40. *Fontinalis antipyretica* with wounded tip that now has grown rhizoids and a new branch. Photo by Janice Glime.](image)

![Figure 41. *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.](image)

Figure 39. Upright and plagiotropic growth forms of the moss *Plagiomnium cuspidatum*. Photo by Michael Lüth.

We know that high concentrations of ACC, presumably resulting in ethylene production, inhibit branch development and bud production in *Fontinalis squamosa* and *F. antipyretica* (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*. 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.

Many acrocarpous mosses lose apical dominance when sporophytes are produced, resulting in innovations such as those in *Bryum* (Figure 42) or when antheridia develop as in *Philonotis* (Figure 43). This suggests that the sporophyte or archegonium causes the stem apex to cease producing IAA. We have already seen that in *Polytrichum*, male plants retain their apical dominance and resume growth from the center of the male splash cup when the succeeding year's growth begins (Figure 30).
Figure 42. Innovation (arrow) in *Bryum versicolor*. Photo by Michael Lüth.

Figure 43. *Philonotis fontana* showing multiple branches just below the antheridial head. Photo by Janice Glime.

But why can't branches and stems grow simultaneously? Since both produce leaves that are photosynthetic, where is the tradeoff? Perhaps the experiments of Tremaine and Glime (unpub.) on *Fontinalis duriæ* 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.

**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 *Haplotrichium* (Figure 44) 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 44. *Haplotichium 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 45), 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 45. *Funaria hygrometrica* with archegonia and young sporophytes. Photo by Andrew Spink.

**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 46). However, controls of these phenomena are different, and the reduced leaves on elongated stems in the *Funaria* cultures under cellophane discussed earlier attest to this fact.

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 47).

In some species leaf dimensions and leaf shape are highly plastic and dependent on light and moisture conditions. Hodginott 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 proliger* (Figure 48) 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 49) 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.

**Light**

Water modifies leaf form as well. *Drepanocladius* has longer and proportionally narrower leaves and loses its falcation (curved shape; Figure 51-Figure 51) in water (Lodge 1959). Normally straight *Fontinalis* leaves (Figure 52) become falcate (Figure 53) when grown in air (pers. obs).

**Water**

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5-5-16 Chapter 5-5: Ecophysiology of Development: Gametophores

Figure 51. Modifications in leaf morphology of *Drepanoclados fluitans* due to submergence, in this case causing loss of falcation. Redrawn from Lodge 1959.

Figure 52. *Fontinalis novae-angliae* with normal submerged leaves. Photo by Janice Glime.

Figure 53. Falcate leaves of *Fontinalis novae-angliae* grown on moist paper out of water. Compare these to the straight leaves in Figure 52. 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 *Drepanoclados* leaves increases as salt concentrations increase (Figure 54; Lodge 1959). On the other hand, Voth (1943) found that *Marchantia polymnorpha* had rapid maturity and slightly smaller cells in higher concentrations of salts.

In *Sphagnum*, leaf response differs among species. In *S. papillosum*, the leaf becomes significantly longer when the capitulum is farther from water, but in *S. magellanicum*, there is little difference (Li et al. 1992; Figure 55). *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 56) and more pores per cell (Figure 55 right; Figure 57). Such evidence demonstrates the plasticity of species to respond to the environment and emphasizes the importance for common garden experiments in systematic studies.
Hair points (hair-like extensions of leaf tip) in Racomitrium lanuginosum (Figure 58) 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.

The moss Schistidium apocarpum (Figure 59-Figure 60) varies considerably in the development of hair points, even on the same plant. Schistidium rivulare (Figure 61), which does not produce hair points, probably differs from S. apocarpum in its production of some growth-controlling substance.

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 62) 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.
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*, 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* 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 63), and in others they were more contorted, like stepping on a wadded up ball of paper (Figure 27; Glime & Rohwer 1983).

In *Fontinalis antipyretica*, application of ACC resulted in undulations on both young and old, mature leaves (Figure 63; 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 63). Since *Fontinalis* has been considered as closely related to the Neckeraeaceae, where undulations are characteristic of several species, it suggests that a gene controlling ethylene production or ACC distribution might be responsible for this morphology.

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.

In *Fontinalis antipyretica*, 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 64). In these plants, elongation of outer leaves accounted for all growth of the plant during the 8-week experiment (Glime & Rohwer 1983).

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 53). 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, \textit{Brachelyma} and \textit{Dichelyma} (Figure 66), have falcate leaves and grow most of the year out of the water.

Ethylene seems to have played a major evolutionary role in the 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 \textit{Haplomitrium} (Figure 67), 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 67) 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 68).

Ethylene is known as a senescence hormone, \textit{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 69), as shown by Glime and Rohwer (unpub. data).

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.

\textbf{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.

\textbf{Temperature}

Furness and Grime (1982) demonstrated that switching of developmental processes can be due to different temperature optima. In \textit{Brachythecium rutabulum} (Figure 70) growth is greatest at 20°C, primary branching at 16°C,
and rhizoid production at 12°C. By contrast, in *Fontinalis hypnoides* (Figure 71), rhizoids are produced at 15-20°C (Figure 72-Figure 73), 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 73). 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 70. *Brachythecium rutabulum*, a moss for which 20°C is optimum for growth. Photo by Michael Lüth.](image1)

![Figure 71. *Fontinalis hypnoides*, a species that lives in both streams and lakes. Photo by Janice Glime.](image2)

![Figure 72. Flow and temperature effects on mean number 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.](image3)

![Figure 73. Flow and temperature effects on mean number 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.](image4)

Light

Light can influence both form and production of rhizoids in bryophytes. In *Riccia crystallina* (Figure 74) 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 75) (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.
Chapter 5-5: Ecophysiology of Development: Gametophores

Tropisms

Rhizoids locate their substrate by a combination of gravitropism and phototropism, followed by a thigmotactic response (contact response). Light can play a strong role in determining the direction of rhizoid growth. In *Fontinalis squamosa*, rhizoid growth was strongly photonegative (Figure 77), 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.

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 77. *Funaria hygrometrica*, on the other hand, has positively gravitropic rhizoids 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.

Otto (1976) demonstrated several attributes of the rhizoids of gemmae of *Marchantia polymorpha*. 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.

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* 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.

**Adhesion**

Once a bryophyte makes contact with a solid surface, the tips tend to flatten and branch (Figure 78). 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 79) 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 78. Branched tip of *Fontinalis squamosa* rhizoid in response to contact. Photo by Janice Glime; drawing by Margaret Minahan.](image)

![Figure 79. *Lophocolea cuspidata*, a leafy liverwort that produces an adhesive (sulfated mucopolysaccharide). Photo by Jan-Peter Frahm.](image)

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 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. Others have likewise shown that IAA induces rhizoid production in wounded parts of plants (LaRue 1942; Maravolo and Voth 1966).

**Wounding**

New growth results in most bryophytes as a result of wounding. In *Fontinalis*, 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 80). 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 80. Rhizoids on an explant of *Fontinalis squamosa*, exhibiting spiral growth from the cut stem. Photo by Janice Glime.](image)

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. 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*, I have found that my explants always produce rhizoids at or near the broken lower end of a stem piece, as in Figure 80, 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*](image)}
*Fontinalis duriaeii* (Figure 82) began to adhere to rocks after about 9 weeks and little additional attachment occurred after 14 weeks of contact (Figure 83). 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 *Calliergon cuspidatum*, *Pleurozium schreberi*, and *Brachythecium rutabulum*, 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.

**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 84-Figure 85). 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 81 and Figure 82. 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 (Odu 1978a; Figure 86).

**Figure 81.** *Hygroamblystegium fluviatile* with rhizoids grown in culture. Photo by Janice Glime.

**Figure 82.** *Fontinalis hypnoides* rhizoids produced in culture. Photo by Janice Glime.

**Figure 83.** Model for rhizoid attachment to four rock types (shale, granite, basalt, sandstone – data combined) in *Fontinalis duriaeii* in a natural and an artificial stream. n = 12 for each rock type and each stream. Based on Glime *et al.* (1979).

**Figure 84.** *Cyrtomnium hymenophyllum* demonstrating rhizoids that surround the stem at base. Photo by Michael Lüth.

**Figure 85.** *Bryum* sp. showing rhizoids that surround the stem at base. Photo by Michael Lüth.

Mosses that grow prostrate on hard substrates typically develop rhizoid tufts (Odu 1978a), as seen for *Fontinalis* (Figure 82). 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* 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* and *Calliergon cuspidatum*, 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*, rhizoids of germinating spores formed toward the positive electrode (Chen & Jaffe 1979), suggesting that this polarity may begin at the spore stage.

Based on Odu’s (1978b, 1979) observations, I predicted that the pleurocarpous *Fontinalis* 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 87) (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* contact a surface they branch prolifically and attach (Glime 1987c; Figure 78). This is consistent with observations of Odu and Richards (1976) on the leafy liverwort *Lophocolea cuspidata* and the mosses *Hypnum cupressiforme* var. *cupressiforme* and *Platyhypnidium riparioides* that respond similarly to contact.

Figure 86. Relationship of cell length to rhizoid length in acrocarpous (◊Bryum capillare, △Pohlia nutans, ●Dicranum scoparium) and pleurocarpous (●*Hypnum cupressiforme* var. *cupressiforme*, ■Rhynchostegium confertum, ●Homalothecium sericeum) mosses, showing the greater length typical of acrocarpous mosses. Means are of 50 cells with 10 rhizoids used per species. Redrawn from Odu (1978a).


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 81-Figure 82), but taxa from other wet habitats often lack them. This absence is typified by such genera as *Sphagnum* and *Drepanocladus* s.l. 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 88) 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.

My observations on *Fontinalis hypnoides* (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*, 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.

**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. In mosses such as *Hylocomium splendens* (Figure 89), one might find 4-7 years of live growth atop several more years of senescent or dead plant.

Figure 88. *Drepanoclados 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.

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.

Figure 89. Living plants of *Hylocomium splendens* forming a turf on top of their own senesced branches (arrow). Photo by Michael Lüth.

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* (Kimmerer 1991; Figure 90). 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 90. 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* (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 91). 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!

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 populations, litter of the stinging nettle (Urtica) stimulates growth (Willis 1978). Willis attributes this added growth to moisture and nutrient release, but we cannot rule out the possibility of hormonal interaction as well.

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

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

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

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

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

Growth in bryophytes is both stem and branch growth, making it non-linear, but can also be a weight gain without any elongation. Growth in very low light causes etiolation. Water and light are necessary for growth, with a wide range of light being optimal among the various taxa. A common optimum seems to be around 3500-5500 lux for shade-adapted taxa.

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

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

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

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

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

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

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

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Literature Cited


