CHAPTER 5-4

ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOPHORE BUDS

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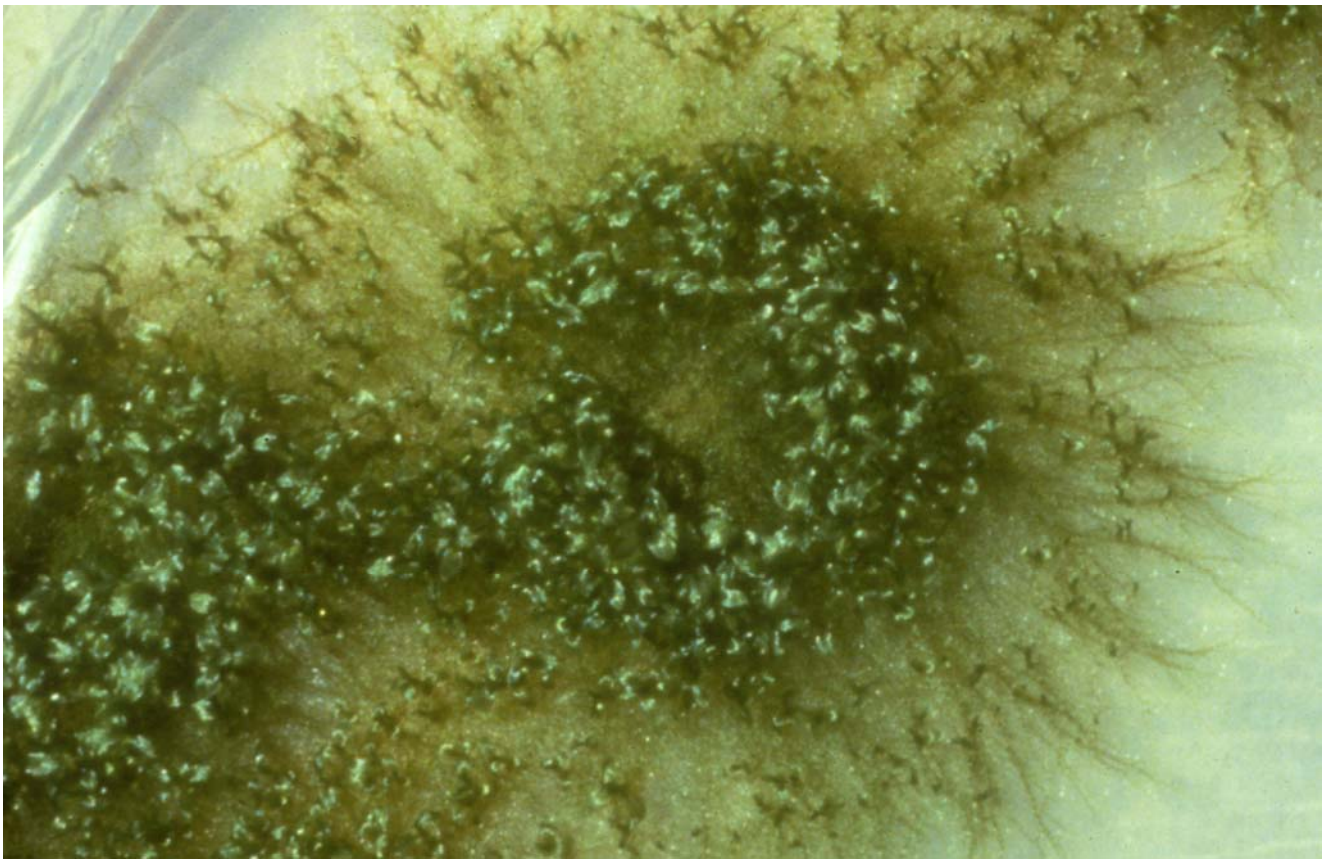

Figure 1. ***Funaria hygrometrica*** with prolific buds forming a doughnut, all from the protonemata produced by one spore. Photo by Janice Glime.

Establishment Success

The next step in the development of mosses and leafy liverworts is the production of gametophore buds – those forerunners of the upright plant, or gametophore (Figure 1-Figure 2). (That suffix, phore, means a supporting structure, and of course, the leafy gametophyte will ultimately bear the gametangia and gametes.) As protonemata grow, they change the environment, providing shade, leaking hormones and other substances, and changing the moisture retention capability of the population. These may contribute to the developmental changes leading to the growth of the leafy plant. We have learned in ***Physcomitrella patens*** (Figure 3) that going that next step to bud formation requires cytokinins, resulting in a rapid influx of calcium. This is followed by bud development on the second sub-apical caulonema cells (Gonneau *et al*. 2001). But application of ABA will inhibit bud formation (Christianson 2000a), suggesting a possible adaptation to drought.

Figure 2. Moss protonema with young bud. Photo by Chris Lobban, with permission.

Figure 3. ***Physcomitrella*** sp. bud with cutting faces, a species for which kinetin induces buds. Photo by Magda Turzańska, with permission.

Spore density may play a role in the establishment success (Hassel & Söderström 1999). In ***Pogonatum dentatum*** (Figure 4), young shoots on a new forest road in northern Sweden represented far less than the number of spores sown. Using planting densities of 1/2 capsule, 1 capsule, and 2 capsules in 10x10 cm plots, Hassel and Söderström found the mean establishment rate after one year was 11, 10, and 12 shoots, respectively; in the second year it was 17, 20, and 22. Apparently other factors were far more important to establishment after germination. When planted in Petri plates on nutrient-rich agar in a growth chamber, this species produced a mean of 712,000 spores per capsule and reached 96.6% germination after 21 days.

Figure 4. ***Pogonatum dentatum***, a moss where spores and sporelings may compete with each other, controlling density. Photo by Michael Lüth, with permission.

Light and Photoperiod

Mitra and coworkers (1959, 1965) found that protonemal buds in ***Pohlia nutans*** (Figure 5-Figure 6) were produced only in white and red light but never in blue or green light, or in darkness. Furthermore, Pringsheim and Pringsheim (1935) found that dark-grown cultures of ***Funaria*** (Figure 1) produced gametophore buds if exposed to white or red light, but not blue or green light, perhaps explaining its lack of success in the forest. Mitra and Allsopp (1959) found that sugar was important in bud formation in ***Pohlia nutans***, but they also concluded that a more specific substance was needed as well. They determined that this unknown substance was one synthesized only in the presence of light, again implicating possible phytochrome mediation.

Figure 5. ***Pohlia nutans*** on Svalbard. Photo by Michael Lüth, with permission.

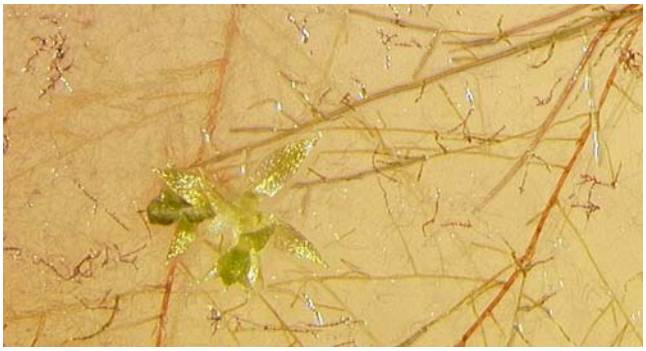

Figure 6. ***Pohlia nutans*** protonemata with buds. Photo courtesy of Sean Robinson.

We also know that in ***Funaria hygrometrica*** (Figure 1) bud initiation is enhanced by red light and reversed by far-red (Simon & Naef 1981). Results in both of these studies are consistent with phytochrome as the light receptor and suggest the possibility of photoperiod control of bud formation. These results could implicate a role for the IAA/cytokinin balance. In fact, Szweykowska (1963), after inducing buds in ***Ceratodon purpureus*** (Figure 7) in the dark with kinetin (a cytokinin), suggested that the kinetin replaced the role of light. This implies that the role of light might be to induce the production of a cytokinin.

Figure 7. ***Ceratodon purpureus***, a species that produces gametophore buds in the dark when grown on medium with kinetin. Photo by Michael Lüth, with permission.

Light intensity is also important in development of the normal form of gametophores. Low light results in etiolated stems (Figure 8). The expanding stems also exhibit a strong phototropism (Figure 9).

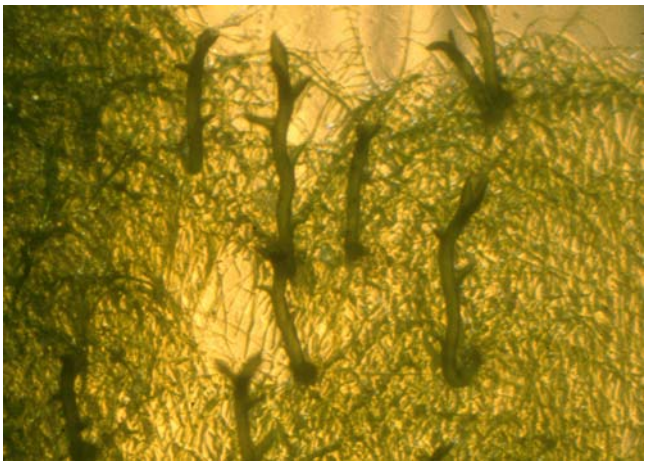

Figure 8. Etiolated stems of ***Funaria hygrometrica*** cultured in low light. Compare the etiolated stems to the compact ones in Figure 1. Photo by Janice Glime.

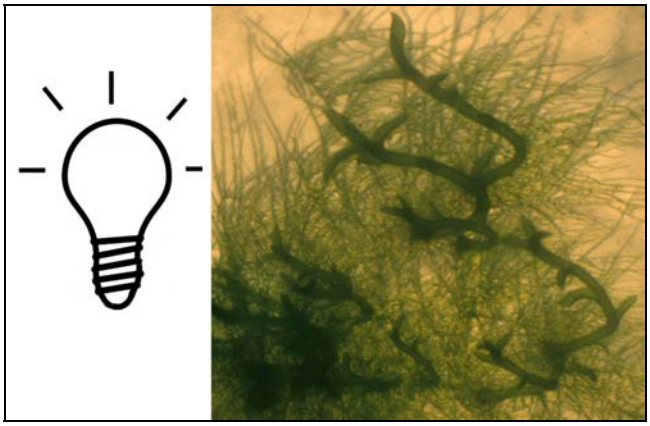

Figure 9. ***Funaria hygrometrica*** in culture exhibiting strong phototropism. The Petri plate is covered with black paper on the right side so light is coming from left side. Photo by Janice Glime.

Growth Regulators

Growth regulators, *i.e.* hormones, work together to initiate and control developmental stages in bryophytes. These may be produced by the bryophyte or by an associated organism. For example, in ***Leptodictyum riparium*** (Figure 10), yeast extract serves as an inhibitory factor for shoot growth, causing death of the protonemal shoot buds Belkengren (1962). On the other hand, protonemal growth continues.

Cytokinins

Bopp (1974) found that all **cytokinins** he tested produced buds on isolated caulonemata. In fact, the response of ***Funaria hygrometrica*** (Figure 12-Figure 13) to cytokinin by producing buds was so reliable that it became the standard bioassay for cytokinin in plant physiology (Christianson 2000b). In ***Tortella humilis*** (Figure 11), buds are induced by kinetin (Bopp 1980). But von Schwartzenberg *et al*. (2007) found that some cytokinins had no effect.

Figure 10. ***Leptodictyum riparium***, a species in which yeast inhibits shoot growth and causes death of protonemal buds. Photo by Michael Lüth, with permission.

Figure 11. ***Tortella humilis***, a species in which protonemal buds are induced by kinetin. Photo by Michael Lüth, with permission.

Bopp (1974) found that when the protonema is removed from the cytokinin it loses its bud-producing ability, except at 2^{o}C. This suggests that the cytokinin is quickly broken down, except at low temperatures, and must be continuously produced by an active caulonema to induce bud formation. On the other hand, we also know that IAA inhibits the development of buds (Reski 1998), so that moving it to a new medium should have been expected to enhance the production of buds. On the other hand, it appears that cytokinins and IAA work together in some cases (Cove & Ashton 1984), suggesting that we should look for a habitat role in the selection for these hormonal behaviors.

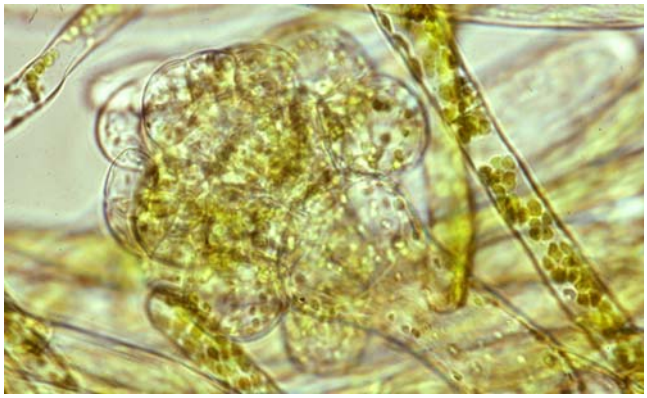

Figure 12. Protonema of ***Funaria hygrometrica*** showing young bud before leaf differentiation. Photo by Janice Glime.

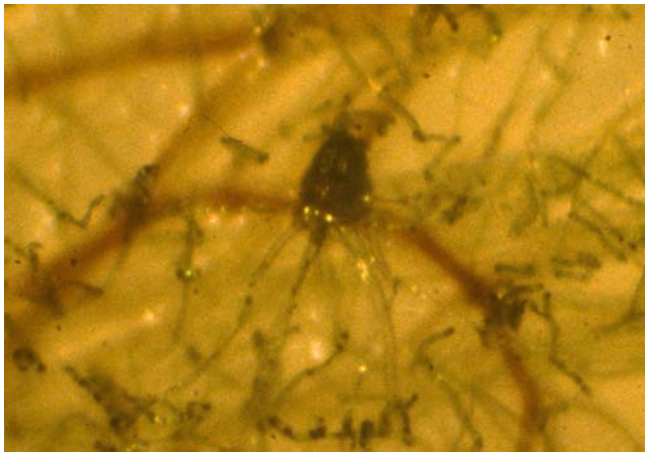

Figure 13. Bud on protonema of ***Funaria hygrometrica*** showing older bud beginning to form leaf shape. Photo by Janice Glime.

Cytokinins have been implicated elsewhere in bud initiation. Szweykowska (1963) found she could get ***Ceratodon purpureus*** (Figure 7) to initiate buds in the dark by adding **kinetin** (a cytokinin), but could get no buds even in light without it, again suggesting an environmental role in bud production.

In ***Hyophila involuta*** (Figure 14), basal medium is insufficient for the induction of buds (Rahbar & Chopra 1982). Even additions of auxins, gibberellic acid, abscisic acid, chelates, vitamin B_{12}, activated charcoal, and coconut milk, and altered hydration, *p*H, temperature, and light intensity and duration do not induce buds. Cytokinins induce multicellular protonemal gemmae. Instead, only the interaction of IAA with kinetin or DMAAP induces normal buds.

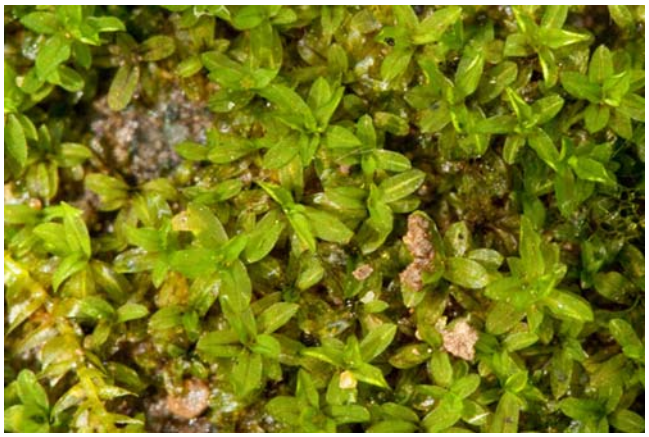

Figure 14. ***Hyophila involuta***, a species that produces protonemal buds on basal medium with no added hormones. Photo by Robert Klips, with permission.

But of course, much of what we know comes from the model system of ***Physcomitrella patens*** (Figure 15). Reski and Abel (1985) demonstrated that the chloronema and caulonema respond to different concentrations of cytokinins. Only the chloronema responds to low concentrations, and only the caulonema responds to high concentrations, with both producing buds in their own appropriate range. Reski and Abel suggested that cytokinins in the environment might induce buds on the chloronemata.

Figure 15. ***Physcomitrella patens*** culture with buds. Photo by Anja Martin in Ralf Reski, Lab through Wikimedia Commons.

In the moss ***Trematodon brevicalyx***, behavior is much like that of ***Hyophila involuta*** (Figure 14) (Chopra & Dingra-Babbar 1984). Protonemata of this species remain bud-free on basal medium and are not induced by the addition of IAA, GA, ABA, chelates, salicylic acid, or alterations in temperature *p*H, agar, sucrose levels, light levels, or photoperiod. These substances do, however, affect the initiation of gemmae and growth rates of the protonema. In this case, only cytokinins (including bryokinin and zeatin) cause bud initiation. And unlike the response of ***Hyophila involuta***, addition of IAA with the kinetin reduced the number of buds considerably.

Bopp and coworkers (1978) found that **caulonema-specific proteins** (CSP) correspond with the ability of the caulonema to respond to cytokinin and produce buds. Isolation of single cells results in the loss of ability to maintain CSP, so regeneration of protonemata occurs. Since a protonema is the first product of regeneration in mosses, it seemed logical that CSP degenerated more rapidly than other protein, causing the reversion to protonemata. However, Bopp *et al*. (1978) showed this to be incorrect. Erichsen *et al*. (1978) found that kinetin is metabolized, primarily to adenine derivatives, immediately upon uptake into the protonema. When adenosine was added, kinetin turnover was reduced. Since adenosine induced bud formation, we can surmise that it is not kinetin, but some product further in a reaction chain that has stimulated bud production.

It appears that this protonemal bud cytokinin system differs from other more familiar branch bud cytokinin systems. Rather, the induction of buds from moss protonemata involves not just one, but two cytokinin-mediated events. The second event controls the number of buds (Christianson & Hornbuckle 1999). Increase in cytokinin subsequently results in the increase in RNA in protonemal bud cells and an increase in the adenine:guanine ratio (Schneider *et al*. 1969). It follows, then, that another factor in controlling bud formation is the DNA replication. In the caulonema, DNA can replicate to 8 copies and even 16 copies in older cells (Knoop 1978). Buds arise irregularly from these older cells, coming instead from the younger apical cells without the DNA duplication (Bopp *et al*. 1980). (Whew! At least we don't end up with 16*n* plants!) We now know that ABA can intervene to prevent the second cytokinin event in shoot bud formation, at least in ***Funaria hygrometrica*** (Figure 13) (Christianson 2000b). Since the ability of ABA to inhibit bud formation is concentration dependent, this

cytokinin inhibition system is useful as a bioassay for ABA as well.

Could these multiple sets of DNA in the protonema contribute to the known bryophyte resistance to radiation damage during a critical life cycle stage? How does the second cytokinin event relate to these subsequent DNA multiplication events in bud formation? There seems to be so much we can learn about cell function from these one-cell-wide protonemata.

The actual cytokinins involved remained elusive, but in 2007, von Schwartzenberg *et al*. experimented with a number of cytokinins, identifying 20 different ones in ***Physcomitrella patens*** (Figure 3, Figure 15). They found that although the cytokinin iPRMP was the most abundant extracellular cytokinin, adding it to wild-type plants had no effect on initiating buds. When they created mutants that over-expressed heterologous cytokinin oxidase/dehydrogenase (CKX), buds were reduced or retarded. Based on their experiments with mutant plants, the researchers suggest that extracellular N6-(Δ2-isopentenyl)adenine (iP) and N6-(Δ2-isopentenyl)adenosine (iPR) are the main cytokinins responsible for inducing buds.

Auxin-Cytokinin Interaction

Results of adding cytokinins seemed to vary among species, and soon other ideas emerged to explain bud initiation. In the moss ***Anoectangium thomsonii*** (**Pottiaceae**; Figure 16) exogenous kinetin and auxin act **synergistically** (complement or help each other) to produce buds (Chopra & Rashid 1969). Burkholder (1959) found that ***Atrichum undulatum*** (Figure 17) remained in the protonema stage in 2% sucrose plus IAA, whereas **arginine** and **glycine** (amino acids) favored leafy shoots. (Recall that Factor H is an arginine derivative.) Sood (1975) tried numerous additives and light regimes in an attempt to induce buds in ***Pogonatum aloides*** (Figure 18); only with a combination of kinetin, IAA, and sucrose could he induce buds. Normal buds grew and produced leafy gametophytes only in a combination of 0.05 ppm IAA, 1 ppm kinetin, and 0.25% sucrose.

Figure 16. ***Anoectangium thomsonii***, a species in which exogenous kinetin and auxin act together to produce buds. Photo by Digital Museum, Hiroshima University, with permission.

Figure 17. ***Atrichum altecristatum*** protonemata and buds. Most of these protonemata are awaiting the right hormonal signal to produce buds. Photo courtesy of Eric Schneider.

Figure 18. ***Pogonatum aloides*** protonemata and young gametophores, indicating that the cytokinin and associated hormone conditions are beginning to be at the right levels. Photo by Michael Lüth, with permission.

Kumra (1985) found that not only cytokinin but also the auxins IAA, **2,4-D** (herbicide that mimics IAA), **NAA** (naphthylacetic acid potassium), and **NOA** (naphthoxyacetic acid, an auxin that inhibits auxin influx into cells) shortened the time to bud initiation and increased the number of buds produced in the moss ***Anisothecium molliculum***. On the other hand, ***Bryum atrovirens*** (Figure 19) produced no buds in culture on a basal medium until auxins were added (Chopra & Vashistha 1990). Antiauxins did not induce buds in ***B. atrovirens***. Furthermore, the auxin concentration influenced the morphology of the leafy plants, with lower concentrations producing more normal-looking plants. The herbicide 2,4-D caused an increase in bud number but did not improve shoot morphology. It appears that in at least some mosses IAA is necessary for bud development.

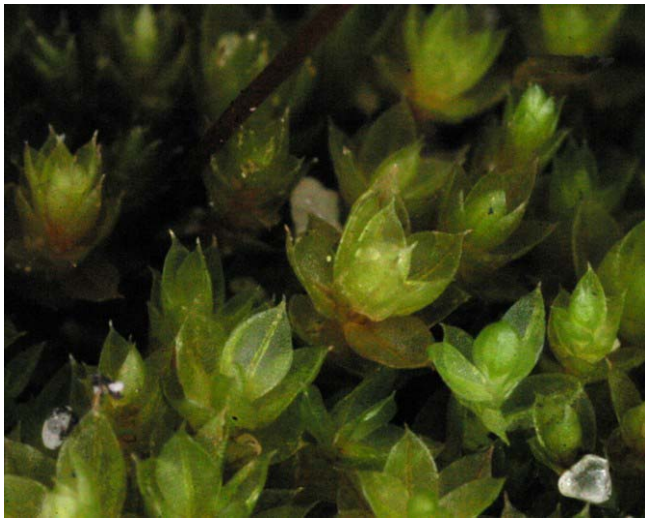

Figure 19. ***Bryum atrovirens***, a species that requires added auxins on basal media to produce buds. Photo by Jan-Peter Frahm, with permission.

In 1968, Bopp showed that **gibberellins** will increase the number of buds and that **IAA** can in some cases cause a similar effect. On the other hand, Sarla and Chopra (1987) found that cultures of ***Bryum pallescens*** (Figure 20) supplemented with 2,4-D, IAA, and NAA failed to produce buds, unlike the response of ***Anisothecium molliculum*** (Chopra & Vashistha 1990), whereas NOA induced at least some buds. Later, Duckett *et al.* (1993) found that cytokinin stimulates bud formation in ***Ephemerum*** (Figure 21-Figure 22), but that IAA instead induces chains of desiccation-tolerant brood cells, similar to those in aging cultures, which are heavily covered with mucilage. This causes one to wonder if in fact the IAA may have induced ethylene production that led to premature aging.

Figure 20. ***Bryum pallescens***, a moss that does not respond to auxins for bud production. Photo by David T. Holyoak, with permission.

Figure 21. The ephemeral moss ***Ephemerum serratum.*** t least one member of this genus responds to cytokinins to produce protonemal buds, but responds to IAA by producing brood cells. Photo by Michael Lüth, with permission.

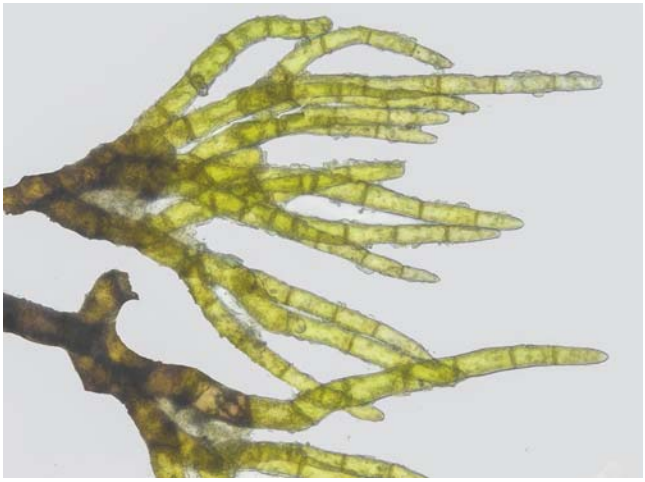

Figure 22. ***Ephemerum spinulosum*** protonema, a species in which cytokinin induces buds, but not IAA. Photo by Dick Haaksma, with permission.

In the aquatic moss ***Palustriella decipiens*** (Figure 23), low concentrations of growth regulators (IAA, kinetin) promoted both gemmae formation and bud induction on protonemata grown from fragments (Ahmed & Lee 2010).

Figure 23. ***Palustriella decipiens***, a species in which buds might are induced on secondary protonemata (from fragments) by low concentrations of IAA or kinetin). Photo by Michael Lüth, with permission.

In ***Physcomitrella patens*** (Figure 3, Figure 15, Imaizumi *et al*. (2002) identified two cryptochrome genes. Using disruptants of these genes, they determined that cryptochromes were involved in many regulatory signals in moss development, including the induction of protonemal side branches and gametophore buds. They also played a role in altering auxin responses, including the expression of auxin-inducible genes. The involvement of blue light in these responses suggest that cryptochrome signals, induced by blue light, may act to repress auxin signals, hence controlling plant development.

Ethylene

Few experiments have examined the role of **ethylene** in bryophytes. It appears that it could play a role in the maturation of protonemata and formation of buds. In experiments on ***Funaria hygrometrica*** (Figure 24), I found that a high concentration of **ACC**, the ethylene **precursor** (previous compound in chemical pathway), induced buds sooner than did lower concentrations or controls with no ACC (Figure 24; Glime unpublished data). This could be an effective signalling device to let the moss know that there were sufficient protonemata to form a colony large enough to sustain moisture and could explain the ability of ***F. hygrometrica*** and other mosses to fill the available space with protonemata before making gametophores. As a gas, ethylene would accumulate and build in concentration around the developing protonemata.

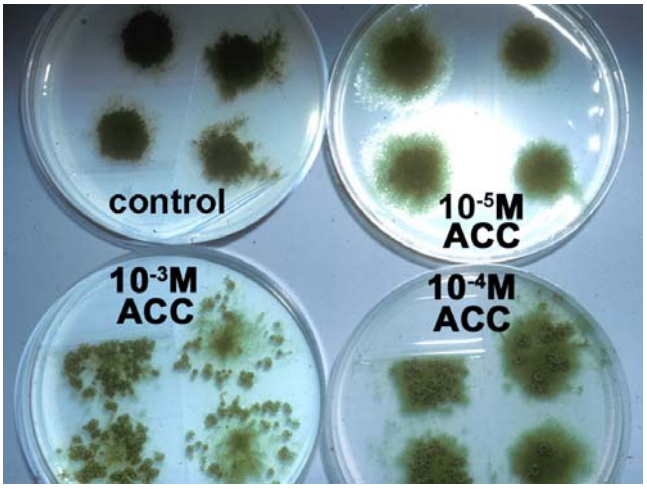

Figure 24. Effects of ACC, the ethylene precursor, on bud formation in ***Funaria hygrometrica***. The highest concentration tested caused the earliest bud formation. Photo by Janice Glime.

But how do all of these factors relate to the ability of the moss to complete its normal life cycle in nature? We can only speculate here, and weak speculation it is. It appears that light quality, and probably duration, plays a role. This could be manifested in a phytochrome-mediated response that stimulates the production of necessary hormones, or in a photosynthetic response that builds stores of sugars, or some balance between these two. Furthermore, the lack of water could reverse the process by causing the protonema to produce ABA, hence preventing the completion of the cytokinin-directed process of bud development.

Moss protonemata seem to differ as widely in their physiology as do their mature gametophores. Cytokinin, IAA, 2,4-D, ethylene, GA, arginine, and glycine have all induced buds in some species. IAA and cytokinin can work synergistically to cause bud formation. But IAA can also inhibit bud formation and in some cases will induce the production of brood cells. ABA can prevent the second cytokinin event, which controls number of buds, and consequently inhibit bud formation. Somehow, all of this ties in with the duplication of DNA, up to 16 sets in some taxa, that seems to keep the distal cells of the protonema from producing many buds. We have no understanding of how these various signals relate to habitat or microclimate.

Interactions with Other Organisms

In the aquatic moss ***Fontinalis squamosa***, development of gametophores is difficult to achieve in culture (Glime & Knoop 1986). Only one plate in 113 produced gametophores after 48 days in a variety of culture conditions. Nevertheless, the other protonemata continued to grow. Interestingly, in the plate with gametophores, more than ten were produced, and these occurred on protonemata that had developed from more than one spore. This suggests that either some necessary condition was supplied in that plate or that an induction factor was produced when one moss began to bud. Since one bud occurred in advance of all the others, it is possible that it induced the others.

The low production of buds in ***Fontinalis squamosa*** cultures (Figure 25) suggests that some critical factor may be supplied by its natural habitat (Glime & Knoop 1986). Support for this need for an exogenous substance comes from the fact that the one culture that produced gametophores was contaminated with fungi. Capsules of ***Fontinalis*** (Figure 26) are usually produced in shallow water or above the water, so this might permit spores to lodge on wet rocks. In this thin water layer, any products produced by fungi, bacteria, and **periphyton** (algae and other microorganisms living on plant; Figure 27) would be in relatively high concentration in the film on the rock. Fungi are known to leak gibberellins, and we have seen that these can increase the production of buds.

Figure 25. ***Fontinalis squamosa*** protonema grown in white light. To reach the bud stage, it seems to require hormones supplied in its environment. Photo by Janice Glime.

Figure 26. ***Fontinalis squamosa*** var ***curnowii*** with capsules, a stage that often occurs above water. Photo by David Holyoak, with permission.

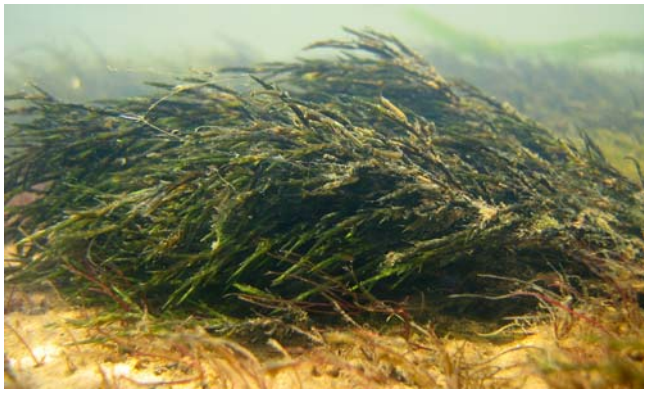

Figure 27. ***Fontinalis novae-angliae*** with extensive detritus that can contribute hormones needed for development. Photo by John Parker, with permission.

Fontinalis (Figure 25-Figure 27) is not the only moss that has shown a response to something from its neighbors. Hornschuh *et al*. (2002) found that the bacterium ***Methylobacterium*** (Figure 28) caused a response similar to that known for cytokinin application to the protonemata, promoting protonemal growth and stimulating bud formation. This bacterium is common on the leaf surfaces of the moss, especially in the grooves between adjacent lamina cells.

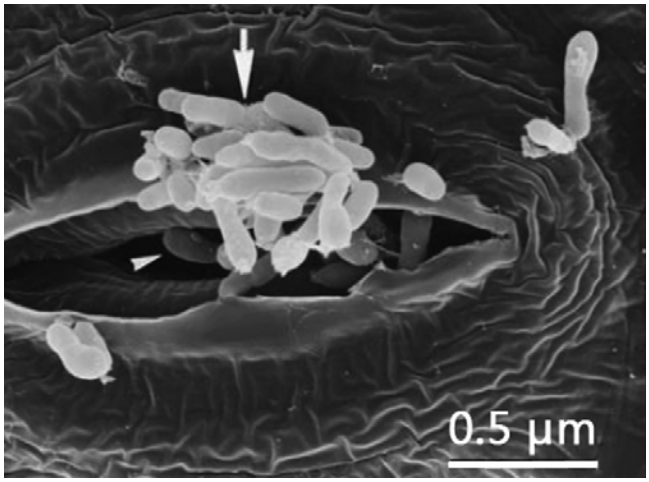

Figure 28. ***Methylobacterium*** sp. in sunflower stoma. Photo by U. Kutschera, through Wikimedia Commons.

Another environmental substance is B_{12}, a vitamin produced by green algae (Chlorophyta) and blue-green bacteria (Cyanobacteria). Spiess and coworkers (1971) have shown that in the presence of the bacterium ***Agrobacterium tumefaciens*** (Figure 29), the moss ***Pylaisiella selwynii*** (Figure 30) forms gametophores, but that little gametophore development is achieved in the absence of the bacteria. Spiess *et al*. (1973) have shown that vitamin B_{12} can probably be supplied by ***Rhizobium*** (Figure 31) or ***Agrobacterium***.

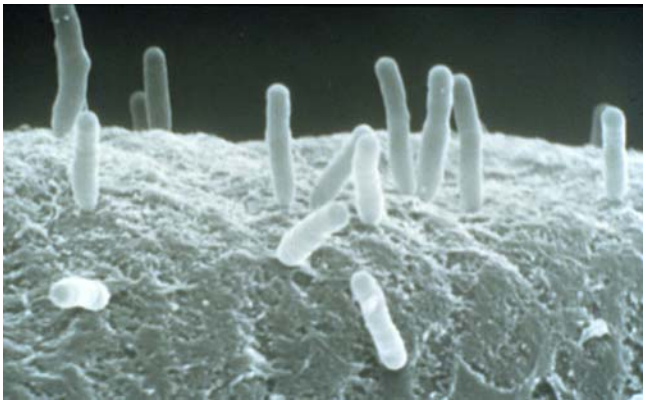

Figure 29. ***Agrobacterium tumefaciens*** on plant tissue. Photo by Martha Hawes, University of Arizona, through NSF public domain.

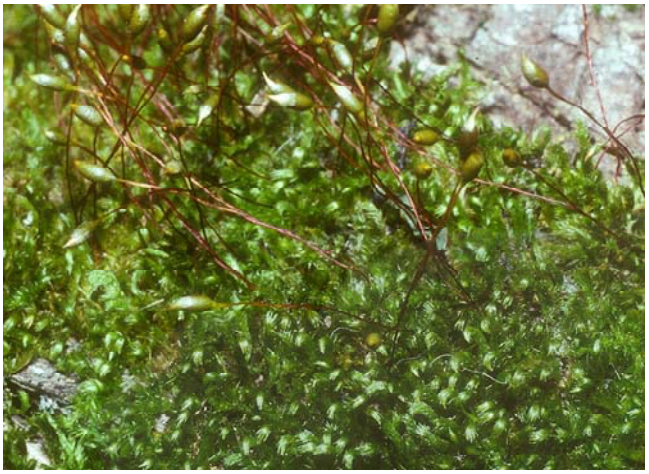

Figure 30. ***Pylaisiella selwynii*** growing on bark. Photo by Janice Glime.

Figure 31. ***Rhizobium*** *leguminosarum* (green). The genus ***Rhizobium*** may supply vitamin B_{12} to the developing protonema, stimulating bud production. Photo through Creative Commons.

Nutrients or Inhibitors?

It appears that the protonema may have different requirements for nutrients than the mature plant, at least in some taxa. Li and Vitt (1994) found that nitrogen in particular might inhibit the establishment of many peatland species. They felt that the different abilities of these taxa to utilize nutrients over the temporal scale of establishment might be a strong determinant of the bryophyte patterns of the mature peatland.

Many heavy metals are needed by plants in minute quantities. They serve in making enzymes and carriers for electrons. But these same metals soon become toxic in greater quantities. Kapur and Chopra (1989) found that many metal ions (cobalt, cadmium, aluminum, lead, nickel, zinc, copper, mercury) inhibit protonemal growth, increase the time for bud initiation, decrease number of buds, and retard the gametophore growth in the moss ***Timmiella anomala*** (Figure 32). At a concentration of 10^{-6} M, nickel increases protonemal growth slightly, but at 10^{-5} M it drastically decreases the number of gametophore buds. Cobalt inhibits protonemal growth but seems to have no effect on bud formation. Phillips and Peterson (1982) likewise found heavy metals to be highly toxic to the protonemata. The most toxic was copper, yet copper in small quantities is essential to formation of chlorophyll. Mercury, cadmium, and zinc were likewise toxic, in that order.

Figure 32. ***Timmiella anomala***, a species in which heavy metals can inhibit bud production. Photo by Michael Lüth, with permission.

Perhaps the most critical nutrient involved in bud formation is calcium. As in germination and protonemal growth, calcium seems to be essential in bud formation. Olarinmoye *et al.* (1981) found this to be true for ***Stereophyllum radiculosum*** (Figure 33), where a minute quantity of calcium is essential. Saunders and Hepler (1982, 1983), in studying *Funaria*, suggested that control of intracellular calcium may be the means of regulating cytokinin. They indicated that increases of intracellular calcium were most likely essential for bud initiation. Calcium is important in gluing cells together, so it is unlikely that much growth could occur without it. This essential nutrient could surely play a role in determining where mosses are able to get established, with some species being better at facilitating uptake when the element is scarce and others being excluded from such habitats.

Figure 33. ***Stereophyllum radiculosum*** on bark. Photo by Scott Zona, with permission.

Little is known about the effects of nutrients on protonemal bud development. Yet what we know suggests they could be of great importance in controlling the establishment of bryophytes. In particular, heavy metals seem to increase the time required for bud formation and decrease the number of buds, suggesting that the bryophytes would be less competitive and may be unable to establish before tracheophytes arrive to outcompete them. In some cases, a nutrient such as nitrogen, essential for all proteins, may inhibit bud formation if present in quantities sufficient for most tracheophytes, perhaps explaining the dominance of ***Sphagnum*** in low-nutrient fens and bogs. Calcium is essential for all stages of development because it is part of the glue that holds the cell walls together, but it may also play a role in regulating cytokinin and therefore regulating production of gametophore buds.

Temperature

Although temperature surely plays a role in protonemal development, its effects seem to be poorly known. Kumra and Chopra (1985), in studying ***Anisothecium molliculum***, found 25ºC to be optimum for bud formation, the same temperature that was optimum for protonemal growth. This temperature, however, would seem a bit high as an optimum for these C_3 plants, but one must consider that the spores must presumably wait to germinate until after danger of frost is gone, or at least infrequent, then must grow a protonema before a bud can form. The bud must then expand into a leafy gametophore (Figure 34). By this time, the rapidly increasing temperatures of spring are giving way to the heat of summer, so there may be no other choice.

A surprising effect of temperature is seen in the epiphytic ***Macromitrium*** (Figure 35). Female protonemata can produce buds at 10ºC, whereas male protonemata require a lower temperature for bud formation (Une 1985). Yet, when one considers the rest of the life cycle, and the

timing of gametangial formation in males and females, this is not surprising at all. Male plants and male gametangia in general seem to be initiated first, therefore requiring initiation at a lower temperature if both males and females are to be mature at the same time.

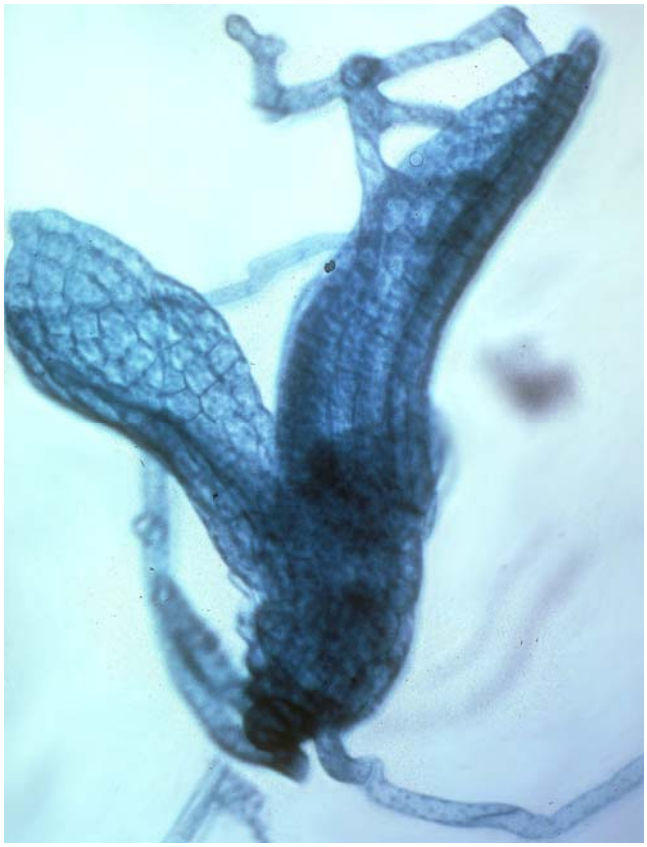

Figure 34. Bud expanding on moss protonema. Photo by Janice Glime.

Figure 35. ***Macromitrium microstomum***, a genus in which the male and female protonemata respond to different temperatures to produce buds. Photo by Tom Thekathyil, with permission.

There appear to be specific nutrient and time requirements among the bryophytes that determine when the gametophore buds will develop (Giordano *et al*. 2002). In the case of ***Pleurochaete squarrosa*** (Figure 36), 8-10 months were needed for buds to form, whereas in ***Funaria hygrometrica*** (Figure 1) and ***Bryum capillare*** (Figure 37), buds formed in young cultures after only a few weeks. Yet it is likely that these time requirements are temperature dependent and will vary among geographic locations.

Figure 36. ***Pleurochaete squarrosa***, a species that requires 8-10 months to form buds on the protonemata. Photo by Barry Stewart, with permission.

Figure 37. ***Bryum capillare*** growing in a crevice, a species that forms gametophore buds in only a few weeks. Photo courtesy of Peggy Edwards.

Using cultures derived from single spores, Chopra and Bhatla (1981) found that normal gametophytes of ***Bryum argenteum*** (Figure 38) could be grown at 25±2°C at 3500 to 4000 lux of continuous light.

Figure 38. ***Bryum argenteum***, a species that will produce upright gametophytes at 25±2°C. Photo by Dick Haaksma, with permission.

Summary

Cytokinins seem to be a common need for initiating gametophore buds in mosses, whereas ABA can inhibit them. Density of protonemata seems also to exercise control over the number of buds in some species, most likely through a hormonal exudate. Wavelength of light can also be important, with white and red light stimulating bud formation in ***Pohlia nutans***, but blue, green, and darkness failing to do so. A red/far red reversal suggests the involvement of phytochromes and perhaps involves IAA. The balance of amino acids can likewise be important. An increase in the adenine:guanine ratio results from an increase in cytokinin, coupled with a replication of DNA up to 16 copies in older cells. Most of the buds, however, arise from the younger apical cells.

Gibberellins can increase the number of buds, but it is not clear if these are supplied by the moss. GA and other growth substances, such as vitamin B_{12}, can be supplied by co-inhabiting organisms – bacteria, fungi, and algae.

Heavy metals are generally toxic and can inhibit development, but some, such as nickel, can enhance it at low concentrations. Temperature surely plays a role, but we seem to know almost nothing about it.

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