CHAPTER 7-1
WATER RELATIONS:
CONDUCTING STRUCTURES

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CHAPTER 7-1

WATER RELATIONS: CONDUCTING STRUCTURES

Movement to Land

The most obvious need for photosynthetic organisms in their move from water to land was the continued need for water. At this time, most photosynthetic organisms still had a dominant gametophyte, and all indications are that the movement onto land carried with it that gametophytic dominance. As life on land progressed through evolution, plants with sophisticated vascular tissue ultimately developed. At the same time, the gametophyte in these highly vascularized tracheophytes (lignified vascular plants) solved its water problems by ultimately being contained within the protection of sporophytic tissues in the seed plants.

This reduction of the gametophyte might necessarily have forced a reduction in conducting tissues because the surrounding sporophytic tissue on the one hand reduced available space and on the other made vascularization much less necessary in the gametophyte. But in gametophyte-dominant bryophytes, survival on land required a means for getting water, and the nutrients carried with it, from one part of the plant to another. Despite their being the first land plants, as Raven (2002) has put it, plant biologists have taken a "top-down" view of land plants, seemingly expecting the bryophytes to have a simpler version of the same system as tracheophytes.

But bryophytes have been around much longer than tracheophytes, and their gametophytes have remained dominant. Hence, should we not expect them to have evolved means of water movement in the gametophyte generation during all these millennia? First of all, consider the desiccation-tolerant tracheophytes. These are almost all small plants (Raven 2002). Many bryophytes are likewise desiccation tolerant, and they too are small.

Bryophytes as Sponges

Sponges, both animal and synthetic, gain and retain water through small chambers and capillary spaces. Bryophytes, due to their small size and tiny leaves, are natural arrays of chambers and capillary spaces. As this story unfolds, you will soon see that bryophytes are indeed sponges, aiding their own water needs and in some cases massively affecting the ecosystem (interacting community & habitat).

All life needs water, and the most severe stress for organisms venturing onto land was undoubtedly just that. But already, algae had developed means of becoming dormant through zygospores when they faced unfavorable circumstances. However, those first land organisms had to find ways to get water to all their internal parts, and often this water was in very limited amounts. For bryophytes, surviving water loss and prolonged periods of drought was a necessity for survival, so it is not surprising that during their 450 million years of evolutionary history (Proctor 2000a) they have perfected physiological mechanisms that
outdistance those of their tracheophyte counterparts (Oliver et al. 2000a). This ability has led plant physiologists to use bryophytes as model systems for the study of desiccation tolerance physiology, even to the extent of attempting to introduce those genes to crop plants (Comis 1992; Oliver et al. 2000b). And this use has made it into the agricultural literature with articles such as "Miracle Moss" (Comis 1992).

It appears that despite the typical relegation of bryophytes to the category of "non-vascular," conduction has played a major role in the phylogenetic history of bryophytes. Hedenäs (1999) examined the importance of various character states on the phylogenetic history of pleurocarpous mosses (typically the ones that grow horizontally) and determined that, based on redundancy analysis, gametophyte variance relates to characters associated with water conduction. Furthermore, one of the most important environmental variables in this phylogeny was the non-wetland to wetland gradient. On the other hand, Proctor (2000b), in “The bryophyte paradox: Tolerance of desiccation, evasion of drought,” points out that a desiccation-tolerant tree is hardly conceivable. Height necessitates highly developed conducting systems that are unnecessary in short plants, and even among the bryophytes, it is the tall *Dawsonia* (Figure 2) and *Polytrichum* (Figure 3-Figure 4) that have conducting systems that almost mimic those of *tracheophytes* (plants having tracheids, *i.e.* the lignified vascular plants).

*Polytrichum* (Figure 3-Figure 4), among the most conductive bryophytes in the northern hemisphere, have been used to generalize about the behavior of soil and airborne minerals in mosses during ecosystem processes. But this moss can behave very differently from most of the other genera that carpet forest floors. Puckett (1988) warns that mosses with internal conduction (as in *Polytrichum*) do not make good monitors. Anderson and Bourdeau (1955) concluded that dew and rain were the main sources of water for bryophytes, excluding the groundwater source so vital for tracheophytes. It is therefore important that ecosystem ecologists, especially those studying water relations and nutrient cycling, have a basic understanding of the variety of ways that bryophytes move water and nutrients.

![Figure 2. *Dawsonia*, one of the tallest and most highly structured of all mosses. Photo by Janice Glime.](image2.jpg)

Ecosystem processes cannot be understood without understanding the role of bryophytes and their water relations. A lack of understanding of bryophyte water relations has led ecologists to conduct inappropriate experiments or draw erroneous conclusions about such topics as nutrient cycling and effects of air-borne pollutants on mosses in general in the ecosystem. Mosses such as *Polytrichum* with capsules 1 Kristian Peters, through Creative Commons.

![Figure 3. *Polytrichum commune* with capsules 1 Kristian Peters, through Creative Commons.](image3.jpg)

Nearly every botany book on the market defines bryophytes as non-vascular plants, distinguishing them in this way from all other embryophytes. In fact, many bryophytes are vascular, but lacking lignin [associated with cellulose in cell walls of sclerenchyma (thick-walled supporting cells), xylem vessels, and tracheids; Hébant 1977] and the variety of perforated and spirally thickened cells typical of xylem. Rather, many bryophytes have unique cells that perform conduction in rather different...
ways from the "true vascular plants." Kawai has published a series of colored photographs (e.g. Figure 1), using specific stains, that illustrate the wide presence and variety of such tissues among many families of mosses (Kawai 1971a, b, c, 1976, 1977a, b, 1978, 1979, 1980a, b, 1981, 1982, 1989, 1991a, b; Kawai & Ikeda 1970; Kawai & Ochi 1987; Kawai et al. 1985, 1986; Ron & Kawai 1990). Hence, it is safer to distinguish the bryophytes as non-lignified plants (still waiting to be disproved) or non-tracheophytes, and the lignified vascular plants as tracheophytes. This puts a slightly new perspective on the way we look at their roles in ecosystems.

When we consider bryophytes, we are tempted to think about wet habitats where mosses grow close to water, basking in the sun of a bog, or cooling off in the spray of a waterfall. Certainly these are habitats where bryophytes are common, but keep thinking. What about those rocks on the cliff or the sand of the dunes (Figure 5)? In fact, can you think of any habitat that has plants but where it is impossible to find mosses? There are not many, and if you visualize some of the rocky habitats in your mind, you realize that these organisms undergo tremendous changes in moisture and temperature, even within a single day, occupying habitats where no vascular plants can survive.

![Figure 5. Aloina ambigua growing in sand. Photo by Michael Lüth, with permission.](image)

If we try to speculate about those first organisms to survive on land, we would probably consider them to be simple organisms with no organized vascular systems. There was no selection pressure for any wasteful vascular tissue while these organisms were living in the water. Water may have been the primary force limiting plants from vast colonization of land. Gray (1985) suggests that it was the ecophysiological tolerance to desiccation, appropriate life cycle strategies, and short vegetative life cycle that permitted widespread colonization during the mid Ordovician (~441-504 million years ago) to the mid Early Silurian (~400-440 million years ago) — strategies that describe bryophytes.

Even with so many diverse habitats occupied by plants today, we still consider the move from water to land to have been a major one. Imagine the changes that were necessary. Consider that the greatest overriding challenge was to keep their cells wet. Land plants responded to this challenge in two ways. Some, the ones we traditionally called vascular plants (the tracheophytes), acquired lignin, developed a complex water transport system, and encased themselves in a waxy, waterproof cuticle. Others, the bryophytes, developed strategies that we are only beginning to understand, including external transport, cell-to-cell transport, and the ability to survive desiccation. In the words of Proctor (2000a), "Bryophytes... evolved desiccation tolerance and represent an alternative strategy of adaptation to life on land, photosynthesizing and growing when water is available, and suspending metabolism when it is not. Limited by mode of life, but also liberated: prominent on hard substrates such as rock and bark, which are impenetrable to roots and untenable to vascular plants. Bryophytes (in species numbers the second biggest group of green land plants) may be seen as mobile phones, notebook computers and diverse other rechargeable battery-powered devices of the plant world – not direct competitors for main-based equivalents, but a lively and sophisticated complement to them."

Bryophytes are adapted to land but restricted in their morphology by a biochemical impasse, i.e. the inability to synthesize lignin (Niklas 1976). Because they lack lignin, they lack the tracheids and vessels of other plants, but have produced instead vascular strands with similar elongate shapes. Nevertheless, they are unable to support a large structure or great mass because they lack the strengthening ability of lignin. Because of their importance in both structure and physiology, water relations seem an appropriate place to start in our consideration of the limits imposed on bryophytes, for without that understanding, we cannot understand their other limitations, nor can we fully evaluate their ecological relationships.

**Conducting Structure**

Conducting structures are not new expressions in bryophytes. Edwards et al. (2003) found at least fourteen types of such structures in mesofossils from a Lochkovian (Lower Devonian) locality in the Welsh Borderland, Shropshire. These are distinguished by variation in the combination of cells in the central strand and the cell wall architecture. The elongate cells may have smooth, uniformly thick or thin walls, walls with smooth projections pointed inward, or bilayered walls. The innermost walls are perforated by pores with the dimensions of plasmodesmata. These perforations are not well organized and some resemble the secondary thickenings most similar to the S-type tracheids of the Rhyniopsida (Figure 6-Figure 7), a primitive tracheophyte with lignified vascular tissue. Edwards and coworkers suggest that the imperforate bilayered examples may have been used in water conduction, cells that exhibited globular residues may have facilitated metabolite movement, and smooth-walled elongate cells seemed to be involved in support. They were unable to identify these mesofossils to genus, but concluded that there was widespread anatomical diversity among these early bryophytes.

Broadly speaking, imperforate bilayered examples may have been involved in water conduction, cells with globular residues with or without pitting involved in metabolite movement, and smooth-walled examples with or without projections involved in support.
Bryophytes have two paths of water movement, often both in the same plant: internal through a central cylinder (endohydric) and external along the surface of the leafy or thallose plant (ectohydric) (Buch et al. 1938). Some thallose liverworts, Polytrichaceae, and Mniaceae represent the endohydric groups (Buch 1945, 1947; Proctor 2000b), but there are many others with at least some internal conduction. Metzgeria furcata (Figure 8), a "thallose" liverwort in the Jungermanniopsida, and others in the Marchantiopsida, have midribs (Figure 9) with enlarged internal cells (Figure 10), but the relative importance of these midrib cells for conduction is largely unknown.

In liverworts, conducting tissues are restricted to the gametophyte, whereas in mosses, they are sometimes also in the sporophyte (Ligrone et al. 2000). Among the liverworts, the Calobryales and Pallaviciniaceae in the Metzgeriales have water-conducting cells with walls perforated by pores derived from plasmodesmata. The hydroids (water-conducting cells) of bryoid mosses are imperforate. In the Polytrichaceae, there is an axial system of microtubules in the leptoids (food-conducting cells) and in the parenchyma cells of the stems and setae of other mosses such as Sphagnum, representing the variety of expression of conducting cells in the bryophytes.
possible. Furthermore, in the lignified vascular plants, it is the sporophyte generation that carries out organized internal conduction, and the gametophyte, with rare exception, does not. By contrast, in bryophytes it is the leafy gametophyte that must obtain and conduct water and nutrients about the plant, although conduction also occurs in the moss sporophyte (Ligrone et al. 2000; see Chapter 5-9).

Although the hornworts (Anthocerotophyta) have been considered by some to be reduced from more advanced plants, water-conducting tissue is unknown in this phylum (Ligrone et al. 2000), although Hébant (1977) reported the presence of cells resembling phloem sieve cells (leptoids?) in Dendroceros (Figure 11). Likewise, few liverworts (Marchantiophyta) have specialized conducting tissues in their gametophytes (Figure 12-Figure 18), and none have them in the sporophyte. Nonetheless, conducting strands have been known since 1901 in the thallose liverwort Pallavicinia lyelli (Figure 19; Tansley & Chick 1901). As in mosses, Pallavicinia conducting strands (Figure 20) closely resemble tracheids, with long cells, tapering ends, and obliquely oriented pits, and they, like xylem cells, are dead at maturity (Richardson 1981).
Unlike the liverworts, as already noted mosses can have conducting cells in both generations (Ligrone et al. 2000). In some liverworts of Calobryales and in Pallaviciniaceae of the Metzgeriales (Figure 19-Figure 20) and the moss Takakia (a primitive moss once thought to be a liverwort; Figure 22), there exist water-conducting cells with perforated walls derived from plasmodesmatal pores (Ligrone et al. 2000), but these do not seem to be organized into a distinctive central strand (group of elongate cells forming central axis of stems and thalli of some bryophytes, usually thin-walled and often colored; Figure 54). Furthermore, the water conducting cells of Takakia (Figure 21-Figure 23) do not seem to be homologous with either the hydroids of other mosses or with those of the Metzgeriales or the leafy liverwort Haplomitrium (Figure 24), lending support to its basal lineage (Ligrone et al. 2000).
Leptomes and Hydromes

Kawai (1991a) describes the moss stem as having a basic structure much like that of tracheophytes with an epidermis surrounding the cortex (Figure 26-Figure 27). This basic structure describes most of the pleurocarpous mosses that move internal substances mostly horizontally.

Among the acrocarpous mosses (those mostly upright mosses with the sporophyte at the stem apex), more complex stems can have a conducting cylinder in the center of the stem. This cylinder connects the base of the stem to the apex, but in most cases it is not connected to the leaves by any sort of leaf trace. The center of this conducting cylinder is comprised of hydroids and stereids, making up the central strand (Figure 28) (Zamski & Trachtenberg 1976). As you can guess from

Dendroligotrichum dendroides (Figure 25, Figure 45, Figure 69) can reach 60 cm height and transports water endohydrically (internally) (Atala & Alfaro 2012). Its water-conducting hydrome follows Murray’s law, i.e. the sum of the radii of the conduits to the third power ($\Sigma r^3$) is maintained across branching of these conduits. This means that the conduction system is optimized for maximal water transport per unit of ‘vascular’ tissue biomass. As the vascular tissue ascends toward the apex, there is acropetal (base to apex) tapering and an increase in conduit number at ascending levels. Since this architecture is similar to that of tracheophytes, Atala and Alfaro reasoned that it had undergone the same selection pressures in its evolution.

Figure 22. Takakia lepidozioides showing rhizomes and stems. Photo from the Herbarium of Hiroshima University, with permission.

Figure 23. Cross section of stem of Takakia lepidozioides showing no evidence of a central strand. Photo with permission from Botany website, UBC.

Figure 24. Haplotrichium gibbsiae showing stems that lack a central strand. Photo by Jan-Peter Frahm, with permission.

Figure 25. Dendroligotrichum dendroides, a moss with non-lignified vascular tissue. Photo by Felipe Osorio-Zúñiga, with permission.
the name, hydroids are water-conducting cells. They are somewhat similar to tracheids but lack any horizontal connections (i.e. no pits) and are not lignified. And as you will see later, their chemistry and development are different from that of tracheids. Hydroids collectively make up the hydrome (also known as hadram or hydrom) (Scheirer 1980).

Figure 26. *Trichodon cylindricus* stem cs showing lack of central strand. Photo by Janice Glime.

Stereids are elongate, thick-walled, slender, and fiber-like cells that add support to the stem, typically arranged in a cylinder around the hydrome. The stereids are collectively known as the strome (Hébant 1977) (also known as sterom; Zamski & Trachtenberg 1976). They can also occur in the leaf costa (midrib-like strand; Figure 57), as will be discussed below, where they also serve as support.

Hébant (1977) describes the living parenchyma cells around the central strand in the Polytrichaceae to be a hydrom sheath, a term originated by Tansley and Chick (1901). This seems like an unnecessary term with only limited usage. However, Hébant reports that both starch grains and oil droplets are frequent in these cells. In *Polytrichum commune* (Figure 3), these cells have accelerated enzyme activity at the same time the protoplasts of the hydroids degenerate. Furthermore, some members of the Polytrichaceae have stereids among the central strand cells. These have acid phosphatase activity in *Dawsonia longifolia* (Figure 2), suggesting they may have a role in the maturation of the hydroids.

Whereas the hydrome is relatively common, the leptome (also known as leptom; Figure 28) is less well known. The simple structure of its cells (leptoids) makes them difficult to distinguish from cortex parenchyma cells in cross section, but in vertical section they can be seen as longer cells surrounding the central strand and somewhat resembling phloem sieve cells (Figure 1, Figure 52). Their function, like that of phloem cells, is for photosynthetic conduction, but they may also transport hormones or other substances. These cells in the Polytrichales (Figure 31) have oblique sieve plates, organized marginal endoplasmic reticulum, and partial nuclear degeneration (Scheirer 1975; Crandall-Stotler 1980).

In mosses like the Mniaceae (Figure 28-Figure 30) and Polytrichaceae (Figure 31), distinguishing the hydroids is fairly easy. However, not all distinctive cells in the center of the stem are hydroids. In other mosses, small to large cells comprise a distinctive central tissue (Figure 27), but we have no experiments to demonstrate their functions in conduction. It was not until 2002 (Ligrone et al. 2002) that immunocytological testing revealed the nature of the central tissue cell walls of 8 mosses and 4 liverworts. Little follow-up work has occurred, hence much of our understanding is still conjecture.

Figure 27. *Molendoa sendtneriana* (acrocarpous; Pottiaceae) stem cross section showing a central tissue that is differentiated. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 28. *Plagiomnium* (Mniaceae) stem cross section illustrating well-developed central strand. Photo by Janice Glime.

Figure 29. *Plagiomnium ellipticum* stem cross section showing central strand with hydroids. Photo by Ralf Wagner (<www.dr-ralf-wagner.de>), with permission.
Figure 30. *Rhizogonium* (*Mniaceae*) stem cross section showing hydroids (stained blue in center). Photo courtesy of Isawo Kawai.

Figure 31. *Polytrichum* stem cross section illustrating well-developed central strand. Photo courtesy of Isawo Kawai.

Consider, for example, the genus *Sphagnum* (Figure 32). Central cells can vary considerably among species (Figure 33-Figure 38) and can be much smaller than the outer layer that comprises the epidermis (Figure 39). Yet these small cells of the central core are not conducting cells (Hébant 1977). Instead, *Sphagnum* typically uses its descending branches as wicks because they form capillary spaces around the stem (Figure 32).

Figure 32. *Sphagnum obtusum* showing descending branches that help to create capillary spaces and the wicking activity for upward movement of water. Photo by Michael Luth, with permission.

Figure 33. *Sphagnum obtusum* stem cross section with larger parenchyma cells in the center, surrounded by smaller thick-walled cells. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 34. Stem cross section of *Sphagnum contortum* with three distinct cell types but no hydroids. Photo by Michael Lüth, with permission.

Figure 35. *Sphagnum* stem cross section with small-celled central core, dark band of cells, and 3-4 layers of outer hyaline cells. Photo from Botany website, University of British Columbia, Canada, with permission.
Schimper (1857) determined that the hyaline outer cells of stems and the hyaline cells of leaves in *Sphagnum* were dead at maturity (Figure 40). Furthermore, they have true perforations strengthened by spiral fibers (Figure 41). Branches are smaller than the stem and typically have a single outer hyaline layer and smaller, often thick-walled cells in the central core (Figure 42-Figure 43).
Schnepf (1973) later found that microtubules are fundamental in the development of the spiral thickenings of \textit{Sphagnum} by lifting the plasmalemma off the wall to form an extraplasmatic space in which wall material is accumulated. The wall area where the pore will form becomes progressively thinner until only the cuticle remains. The cuticle eventually ruptures, making a pore. The protoplasts likewise eventually disappear.

The \textbf{Marchantiophyta} lack water-conducting cells except for two families of leafy and two of thallose liverworts (Ligrone \textit{et al.} 2000, 2002). These conducting cells are formed by protoplasmic degeneration due to acid phosphatases, as in the mosses, but their wall development is different from that of the mosses (Crandall-Stotler 1980). They lack wall hydrolysis but possess numerous plasmodesmata-derived pores on all walls and never develop polyphenolic compounds (Hébant 1978). No food-conducting cells are known among the \textbf{Marchantiophyta} (Figure 44).

Hydroids

The elongated, water-conducting \textbf{hydroids} typically occur in groups of 2-3 in bryophyte stems (Hébant 1970); they are similar to tracheids, but lack lignin and secondary wall thickenings (Taylor 1988). Consequently, hydroids are usually thin-walled (Zamski & Trachtenberg 1976) and lack the helices and other thickenings typical of tracheids. Vanderpoorten and Goffinet (2009) sum up three major differences between hydroids of bryophytes and the tracheids and vessels of tracheophytes: hydroids lack secondary wall patterns; bryophyte lignin-like polymers are not cell-specific as they are in tracheophytes and are more likely to offer protection against microbes; hydroids collapse during water stress, making them highly resistant to cavitation (drop in vascular pressure due to vapor pockets resulting from desiccation) (Ligrone \textit{et al.} 2000). This combination creates a fundamental difference in response to drying, with bryophytes being desiccation tolerant and tracheophytes preventing desiccation by pumping water from the soil, closing stomata, and reducing water loss with a waxy cuticle (Vanderpoorten & Goffinet 2009).

<table>
<thead>
<tr>
<th>Litter type</th>
<th>soluble carb</th>
<th>hemi-cellulose</th>
<th>cellulose</th>
<th>&quot;lignin&quot;</th>
<th>ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pinus resinosa} leaves</td>
<td>35.41</td>
<td>13.44</td>
<td>19.37</td>
<td>23.56</td>
<td>3.68</td>
</tr>
<tr>
<td>angiosperm tree leaves</td>
<td>43.89</td>
<td>11.59</td>
<td>20.43</td>
<td>11.04</td>
<td>6.97</td>
</tr>
</tbody>
</table>

*Not a true lignin in mosses.

Hydroids senesce at maturity and become dead, empty cells, like those of xylem, with slanted end walls that abut on the end wall of the next cell, as in tracheids (Richardson 1981). This change from living cells to empty dead cells is a result of acid phosphatase activity that degenerates the protoplast (Crandall-Stotler 1980). Hydroids of \textbf{Bryophyta} typically lack perforations but sometimes have secondary polyphenolic thickenings on the lateral walls of
Scheirer (1975). Scheirer (1973) used *Dendroligotrichum* (Figure 45) (*Polytrichopsida*) to demonstrate that hydrolysis leaves behind only cellulose remains of the primary walls of end walls of hydroids. Subsequent examination by electron-dense crystals of Prussian blue on the end walls in *Polytrichum commune* (Figure 46) suggests that these end walls are highly permeable (see Figure 47), but that substances are unable to move through the lateral walls (Scheirer & Goldklang 1977).

To understand any relationship between hydroids of bryophytes and tracheids or vessels of tracheophytes, we must understand their structure. We can consider that part of their structural development is similar to that of tracheophytes because they, like xylem cells, are dead at maturity (Richardson 1981). But is their chemical nature similar? It appears that the bryophytes have derived their water conducting cells in a variety of ways.

Hébant (1973a) found that strong activity of acid phosphomonoesterases occurs in the differentiating water-conducting cells of various mosses and at least one liverwort. But a lesser activity is also present in leptome cells and certain parenchyma cells of some *Polytrichales*.

Some chemical labelling tests gave similar results in as divergent taxa as *Takakia* (Figure 21-Figure 23) and *Polytrichum* (Figure 46-Figure 47), but different results in *Mnium* (Figure 70) (Ligrone et al. 2002). And Ligrone and coworkers found labelling of both water-conducting cells and parenchyma cells in *Haploclonia* (Figure 98), but only of water-conducting cells in *Polytrichum*. Ligrone et al. found that the arabinogalactan protein (AGP) antibody labelled the water-conducting cells in all Bryophyta tested (8 species) except the large polytrichaceous moss *Dawsonia* (Figure 48). No labelling occurred in the liverworts (4 species). Hence, it appears that the chemicals present are similar, but that they occur at different places within the plants.

Differences in labelling between the water-conducting cells and the cortical cells appeared to be mostly quantitative in these few species (Ligrone et al. 2002). On
the other hand, electron microscopy revealed clearly distinct differences in the location of the antibodies within the cell walls of these two cell types, suggesting that their presence in a particular location was tissue specific in its regulation. Even within the Polytrichaceae (Figure 45-Figure 48) there is considerable diversity in the immunocytochemistry. In short, the bryophytes have a widely diverse chemistry in their conducting cells, but as such, they differ strongly from those of tracheophytes. Ligrone et al. (2002) consider the presence of several carbohydrate antigens in the cell walls of hydroids to indicate that hydrolysis of non-cellulosic polysaccharides is not part of the maturation process, a strong contrast to that in tracheophytes (see Hébant 1977).

Accompanying these chemical differences are differences in structure. True perforation plates (end walls of vessels) have not been found in Polytrichaceae (Figure 45-Figure 48) (Frey & Richter 1982) or most other mosses (Hébant 1973b). Consequently, Frey and Richter (1982) set out to discover them in mosses. In the dendroid moss Canalohypopterygium tamariscinum (Figure 49), they found structures resembling perforation plates of Ephedra (Gnetophyta), although they were not numerous and were restricted in location to branching areas. Perhaps this type of vascular structure permits them to be dendroid, lacking the close structure of leaves along the stem needed for capillary action. Smith (1964) had already demonstrated perforations in the conducting elements of the liverwort Symphyogyna circinata (Figure 50). Furthermore, pits are known, particularly in end walls, from Haplomitrium (Figure 98) [considered to be basal to leafy liverworts (Crandall-Stotler & Stotler 2000)] and Takakia (Figure 21-Figure 23) (now classified as a primitive moss in the Takakiopsida), as confirmed by electron microscope. Although hydroids do not seem to contain true lignin, as do tracheophyte xylem cells, they do contain a polyphenolic cell wall component that functions similarly to lignin (Pressel et al. 2010). This compound protects the wall from hydrolytic attack and aids in internal transport of water. In Rhacocarpus purpurascens (Figure 51), Edelman et al. (1998) found walls composed of "mainly lignin, hemicellulose (H-bonded to cellulose in plant cell walls), and cellulose in a ratio of ca. 9:8:5." Although the resonance spectrum indicated various characteristics typical of lignin, some specific peaks associated with known lignin compounds were missing. Thus the question remains, is this true lignin?

![Figure 49. Canalohypopterygium tamariscinum. Photo by Pieter Pelser, with online permission for educational use.](image)

![Figure 50. Symphyogyna circinata. Photo by Filipe Osorio, with permission.](image)

![Figure 51. Rhacocarpus purpurascens, a moss that produces a cell wall substance similar to lignin. Photo by Michael Lüth, with permission.](image)

**Leptoids**

Leptoids (Figure 52) are very similar to phloem sieve cells, and in fact, Behnke (1975) calls them just that. Taylor (1988) considers that in some cases they are nearly identical to protophloem cells of certain tracheophytes. They, along with parenchyma cells, comprise the leptome (=leptom) (Hébant 1970, 1974; Behnke 1975; Figure 28). We know that they are typical in the Polytrichaceae, but have also been found in Sphagnum, Hookeriaceae, Neckeraeceae, and Orthotrichaceae (Ligrone & Duckett 1994, 1998; Duckett & Ligrone 2003). Except in the setae of a few species (Hébant 1974), leptoids have not been found in the arthrodontous mosses (considered more advanced) and are unknown in liverworts. It is likely that they are much more common than we realize because in cross section without stain they appear no different from the unspecialized parenchyma cells.

![Figure 52. Leptoids.](image)
Figure 52. Cross section of Polytrichum juniperinum and longitudinal section of Atrichum undulatum stem to illustrate parts of central strand (leptoids and hydroids) and stem structures. Drawings by Margaret Minahan, modified from Hébant (1977).

In the moss family Polytrichaceae (Figure 52, Figure 54), leptoids have an axial system of microtubules with polarized cytoplasmic organization (Ligrone et al. 2000). In other mosses, including Sphagnum (Figure 32-Figure 43), such organization may also occur in stem and seta parenchyma cells. Even rhizoids and caulonemata of mosses and liverworts and thallus parenchyma cells of liverworts may have a similar organization for transporting nutrients symplastically (through cells, inside the membrane) for longer distances. But, as will be seen later in this chapter, these food and water conducting cells are fundamentally different from the phloem sieve cells and tracheids of tracheophytes. Nevertheless, Ligrone et al. (2002) found that the cell wall and tissue complexity of bryophytes are "on a par with higher plants."

The leptoids are distinct in vertical section by their elongate shape and slightly oblique end walls (Figure 55) (Behnke 1975). At maturity, the nucleus degenerates, as in phloem sieve cells (Richardson 1981), but protoplasm remains. In Polytrichum (Figure 52), the leptoids are not connected end-to-end by sieve plates or pores as in tracheophytes, but by numerous plasmodesmata. However, Cortella and coworkers (1994) considered the thin areas of central strand parenchyma cells to be primary pit fields in Hookeria lucens (Figure 53) stems and suggest that these cells have a conducting function.

Even the development of leptoids seems similar to that of phloem sieve cells. During leptoid maturation in Polytrichaceae, ribosomes (centers of protein synthesis) disintegrate and nuclei become smaller and inactive, although they do not dissolve completely as in tracheophytes; mitochondria persist. The parenchyma cells contain starch-storing chloroplasts. As in their tracheophyte counterparts, leptoids move carbohydrates and other substances away from the apex.

Figure 53. Hookeria lucens. Photo by Jiří Kameniček, with permission.

Figure 54. Polytrichastrum formosum stem cross section showing central hydroids (with orange walls in center) and considerable differentiation in the cells of the central strand. Leptoids are present outside the central strand and are not discernible in cross sectional view. Photo from Botany website, University of British Columbia, Canada, with permission.

Figure 55. Hypnum sp., a pleurocarpous moss, stem longitudinal section. Note the long cell with what appear to be broken side walls, a disintegrating diagonal cross wall, and a partially missing protoplast. This appears to be a leptoid, but we need conduction tests to verify it. Photo courtesy of Isawo Kawai.

Rhizome

The rhizome (underground, horizontal stem connecting upright plants), on the other hand, has
**hypodermal** and **radial strands** but lacks connecting traces and a sterome. The **hypodermis** (Figure 56), also present in some stems, consists of one to several layers of distinct cells just beneath the epidermis and may be thick-walled or colored.

Long-distance transport brings its own set of problems. These plants can undergo transpiration, causing them to lose water (Raven 2003). In some liverworts and many mosses, but not hornworts, there are dead cells in the tissues. These may function in long-distance **apoplastic** (outside cell membranes) water transport. **Symplastic** transport, on the other hand, seems to have a high resistance to flow, emphasizing the importance of apoplastic movement.

**Leaves**

In most tracheophytes, the leaf is a critical structure in creating the movement of water from the roots to the tops of tall plants. This movement, known as the **transpiration stream**, requires the loss of water from the leaf, creating a vapor pressure deficit that brings water upward like someone sucking on a straw. But bryophytes typically do things quite differently, as we shall see in a later subchapter. They typically take in water from above, not below, hence requiring a new look at the role of leaves in water movement. It appears that the greatest need is not to move water to the leaves, but rather to move substances made in the leaves to other parts of the plants.

**Costa**

Within the leaf, water may move cell to cell among the **lamina** cells (Figure 57), but many leaves have a **costa** (Figure 57-Figure 58) that is often accompanied by supporting **stereid** cells (Figure 59). Unlike the midrib of ferns and seed plants, the costa does not branch and rebranch to deliver water or other substances to or from cells of the leaf lamina (Figure 58), although in some taxa, for example **Hygrohypnum** (Figure 60), it may have one or more branches. Nevertheless, the costa has elongate cells that we might expect to facilitate a more rapid movement of water within the leaf (Figure 58), but does it?

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**Figure 56.** *Polytrichum* stem cross section showing **hypodermis**. Photo courtesy of Isao Kawai.

**Figure 57.** Cross section of moss leaf blade showing arrangement of broad portion (lamina), costa, and supporting stereids. Large cells in costa serve for conduction. Photo by Janice Glime.

**Figure 58.** *Crumia latifolia* leaf showing elongate costa cells and nearly isodiamic lamina cells. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

**Figure 59.** *Crumia latifolia* leaf cross section showing enlarged costa with many stereids supporting the conducting cells. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.
On closer examination of the costa, we find that those elongate cells are living cells with oblique end walls, thin cell walls, and living protoplasm (Hébant 1977)! These are not hydroids, but are leptoids. Hence, it appears that in addition to its supporting role, the costa can have the role of conducting substances from the leaf toward the stem. (We will see shortly how this system connects to the leaf traces in the stem.) It appears that the costa should not have a role in conduction of water.

**Sphagnum**

*Sphagnum* (Figure 61) has the most unusual water system in its leaves of any bryophyte. Its leaves have two types of cells, and rarely a border in addition. These two types are the water-holding, colorless, dead hyaline cells and the green chlorophyllose (photosynthetic) cells (Figure 62-Figure 63). The hyaline cells serve as water reservoirs for the photosynthetic cells. Their walls have true perforations and are strengthened by spiral thickenings, suggesting the structure of tracheophyte vessels (Figure 62-Figure 63) (Hébant 1977). The pores (perforations) begin with a thinning of an area of the cell wall and presence of a thin membrane. Eventually these rupture to create the pore, using the process already described above for the hyaline cells of *Sphagnum* stems.

Leafy Liverworts

Leafy liverwort leaves never have a costa (Figure 64), leaving us to assume that transport of water and other substances in the leaves, if needed, is cell-to-cell transport through ordinary leaf parenchyma cells. But in this group (Jungermanniopsida), leaves are never more than one cell thick, giving all cells direct exposure to water from the atmosphere or other surroundings.
We might also ask the role of **underleaves** (Figure 65) in this group. These may be non-existent (*e.g.* *Jamesoniella*, Figure 66) to quite large (*e.g.* *Porella*, Figure 67). Underleaves may be an evolutionary left over with no function, but their persistence suggests they may offer some advantages in water retention. They create capillary spaces on the under side of the stem and thus may aid in water retention. This space may also aid water uptake by holding water, but in many cases this would require that the stem (Figure 68) absorb the water. It would be interesting to experiment with different types of underleaves to see how they affect water uptake, especially by the stem, and how long they are able to hold a water reservoir.

![Figure 65. *Calypogeia integristipula* demonstration of underleaves. Photo by Michael Lüth, with permission.](image1)

![Figure 66. *Jamesoniella undulifolia* showing absence of underleaves (arrow). Photo by David T. Holyoak, with permission.](image2)

![Figure 67. *Porella platyphylla* showing its large underleaf and lobule. Photo by Paul Davison, with permission.](image3)

Another water reservoir in a number of leafy liverworts is the **lobule** (Figure 67). This structure, present in *Frullania, Porella, Lejeuneaceae*, and others can create a small reservoir of water suitable for small aquatic invertebrates such as rotifers and Protozoa to carry out their entire life cycle. These are discussed further in Chapter 7-4.

**Leaf Traces**

Conduction from stems into leaves is typically through the parenchyma cells of the stem cortex, as will be described in a later sub-chapter. True **leaf traces** (conducting cells connecting the leaf costa to the hydrome; Figure 69) exist in some *Polytrichales*, but in other cases they do not quite reach that far. In the *Mniaceae* and *Splachnaceae* there are **false leaf traces** (Figure 70) that extend into the cortex from the leaf but do not connect with the central strand of the stem (Figure 71) (Hébant 1977). In *Funaria hygrometrica*, some specimens have true leaf traces that reach the central strand, and others do not.

Hébant (1969) found that in *Polytrichum* (Figure 4), the true leaf traces extend from the leaf costa toward the central strand, but they become reduced near the central strand. Nevertheless, Hébant (1969) found that 7-8 hydroids of each leaf trace could connect to the central strand in grassland *Polytrichum commune* (Figure 46). This connection, however, seems to be related to water availability. In bog populations, only three hydroids form
the connection. For specimens grown under water, no leaf traces connected to the central strand.

But wait! Many kinds of leaves have a costa, the rib that extends part way or all the way down the center of the leaf. But the costa cells are fairly wide cells, albeit elongated, and contain a living protoplast (Hébant 1977). The end walls are oblique and have numerous plasmodesmata. They are in fact leptoids, not hydroids, and do not seem to have an important water conducting function in many mosses, if any. Rather, they conduct photosynthate and other substances from the leaf to the stem. These materials are thus deposited in the stem tissue. Could these actually connect with leptoids in the stem, permitting transport to stem tips or to rhizomes? In fact, in Polytrichum commune they do connect to the leptoids of the stem axis. Why then are there hydroids in the leaf traces? What do they connect? Is there any correlation between having a costa with leptoids and a stem with a central strand? Do all leaf leptoids connect with stem leptoids? So little we know...

Sporophyte Conduction

In tracheophytes, it is the sporophyte that has the vascular tissue, and in the setae of mosses, one might find conducting tissues (a central strand) even when it is absent in the gametophyte. This should not be too surprising since the gametophyte is much better adapted to absorbing water from the atmosphere than the cuticle-endowed sporophyte. It is most likely necessary for a number of substances to be transported from the gametophyte into the sporophyte as it develops. And as we might expect, these conducting strands in setae are best developed in the Polytrichaceae (Hébant 1977), a family in which the peristome exhibits the more primitive character of nematodontous teeth.

Is perhaps no coincidence that a species with a vascularized stem also has a vascular seta. This seems to be the case in Plagiomnium undulatum (Figure 72).
On the other hand, leptoids can occur in the setae of some arthrodontous mosses even when they are absent in the gametophytes. Nevertheless, leptoids of setae, unlike those of tracheophytes, show less differentiation than in their gametophytic counterparts. In the setae of the Polytrichaceae, leptoids are not intermixed with specialized parenchyma cells and apparently lack enlarged plasmodesmata in their end walls, as seen in gametophytes of some taxa (Hébant 1974). To add interest to the picture, the leptoids are present in forms that are transitional between the parenchyma cells and the fully differentiated leptoid cells (Hébant 1974).

Meager evidence exists for the presence of leptoids in setae of other genera. Among these are Funaria, Meesia, and Splachnum (Hébant 1977). In Tortula muralis (Figure 73), Favali and Gianni (1973) have claimed that the leptoids are intermixed with the parenchyma cells in the seta and a similar claim was put forth by Bassi and Favali (1973) for Mnium orthorrhynchum, but Hébant (1977) was unable to find any convincing evidence that this was true in either case.

Stem sections indicate that at least a central strand is present in the acrocarpous mosses Dicranum scoparium (an arthrodontous moss; Figure 75-Figure 76) and Tetraptis pellucida (a nematodontous moss; Figure 77-Figure 78). The presence of leptoids cannot be determined from these views, nor can the function of the central strand. Cross sections of these setae can be compared with stems of the same species in Figure 73-Figure 78.

Figure 73. Tortula muralis seta cross section showing modified cells in center of seta. Photo from Botany website, University of British Columbia, Canada, with permission.

Figure 74. Tortula muralis or plinthobia stem cs. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 75. Dicranum scoparium seta cross section showing broken center with modified cells similar to those of stem (Figure 76). Leptoids do not seem to be visible. Photo from Botany website, UBC, with permission.

Figure 76. Dicranum scoparium stem cross section showing differentiated central tissue with hydroids, but representing a genus where leptoids are often absent. There appear to be hydroids that are breaking up, possibly surrounded by a narrow band of leptoids. Photo from Botany website, University of British Columbia, Canada, with permission.
Hébant (1977) pointed out that no electron microscope study existed on the histology of the conducting tissue of the capsule. He could offer little on its organization, stating that the conducting strand terminates shortly after it enters the capsule. In *Funaria hygrometrica* and *Polytrichum commune* the hydroids terminate within the capsule as a small ampulla, but such an ampulla is absent in *Dawsonia*, *Dendroligotrichum*, and *Fissidens*.

### Adaptation and Evolution

The hydroids and leptoids present interesting evolutionary implications, since it appears that they are primitive characters that are lost in more advanced bryophyte taxa (Hébant 1970; Behnke 1975). Unlike most tracheophytes, the mosses retain conducting cells in both generations, but the haploid generation is the first to lose leptoids evolutionarily, as in *Funaria* (Behnke 1975), a moss that still has a central strand in the stem (Malcolm & Malcolm 2006) and leptoids in its setae (Hébant 1977).

### Being Acrocarpous

Some acrocarpous mosses may lack a central strand. For example, *Leptodontium flexifolium* (Figure 79–Figure 80) grows on acid substrata but lacks the central strand (Figure 80), but it has a leaf costa (Figure 79). Even the ubiquitous *Ceratodon purpureus* (Figure 81), a moss that occurs on substrata from roadsides and exposed rocks to pools in the Antarctic, lacks a central strand (Figure 82), and likewise has a costa (Figure 83–Figure 84). Other taxa that frequently become dry, like *Grimmia* species (Figure 85) also often lack specialized cells in the center of the stem (Hébant 1977).
Figure 81. *Ceratodon purpureus* leaves. Photo by Don Loarie, through Creative Commons.

Figure 82. *Ceratodon purpureus* stem, a moss with a wide range of habitats from dry fields to Antarctic pools, yet it lacks hydroids. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 83. *Ceratodon purpureus* showing distinct costa. Photo by Malcolm Storey, through Creative Commons.

Figure 84. *Ceratodon purpureus* leaf cross section showing costa and involute margins. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 85. *Grimmia pulvinata* stem cross section showing little differentiation in the central cells of the cortex. Are these hydroids? This genus can have hydroids or lack them. The tissues flaking away from the stem are leaf cross sections. Photo from Botany website, UBC, with permission.

**Being Pleurocarpous**

Pleurocarpous taxa that grow close to the ground may have less need for hydroids when all their leaves are more or less equally placed to gain water, as can be seen in *Calliergonella lindbergii* (=*Hypnum lindbergii*; Figure 86). Pleurocarpous mosses (Figure 86-Figure 89) have fewer problems in getting wet and sharing water among cells because they grow horizontally, compared to the need for upright mosses to distribute water, especially tall ones that grow alone, like *Dawsonia* spp. (Figure 2). On the other hand, these mosses may have evolved the loss of hydroids before our extant species existed and have not regained their hydroids, as might be the case for *Hylocomium splendens* (Figure 88-Figure 89), a moss that grows in fairly open wefts, but lacks a central strand. Nevertheless, it would seem that the pleurocarpous mosses still need to transport photosynthate and hormones, among other things. Hence, we should expect leptoids in many, if not all, pleurocarpous mosses. Unfortunately, it is hard to find information on leptoids in these taxa. The same need, and lack of information, could be said for leafy liverworts.

Figure 86. *Calliergonella lindbergii* forming a thick mat. Photo by Michael Lüth, with permission.
Figure 87. *Calliergonella lindbergii*, a pleurocarpous moss, stem cross section showing a small area of differentiated central tissue. Photo by Hermann Schachner, through Creative Commons.

Figure 88. *Hylocomium splendens*, showing its open growth habit that will permit easy escape of water. This moss grows in an almost dendroid pattern, but together with many stems that form wefts. Photo by Dale Vitt, with permission.

Figure 89. *Hylocomium splendens*, a pleurocarpous moss, stem cross section showing absence of any hydroids or central strand. Photo from Botany website, University of British Columbia, Canada, with permission.

In *Climacium* (Figure 90), the stem has very reduced strands of conducting tissue (Hébant 1977). This moss stands upright like a small tree. The stem is nearly naked, making external upward transport limited. Hence this moss must rely on water that lands on the leaves. Instead of specialized water conducting cells, *Climacium* species have good supporting tissues in their stems, permitting the stem to support the leafy tree-like portion.

Figure 90. *Climacium dendroides* showing the nearly naked supporting stem. Photo by Keith Bowman, with permission.

Aquatic

It should be no surprise that aquatic taxa like *Fontinalis* (Figure 91-Figure 93) lack hydroids. Likewise, in *Touwia* (Figure 94), a pleurocarpous moss in the Neckeraeae, there is no cross-sectional evidence of a central strand (Figure 95). Rather, like *Fontinalis*, this streambed moss has many thick-walled cortex cells that help to protect the stem from breakage in stream flow. Its leaves likewise have a thick *costa* (Figure 96) that can resist the ravages of flow. But even in such epiphytic taxa as *Neckera crispa* conducting cells are lacking, suggesting an evolutionary loss early in this branch. Taxa like *Touwia* with a strong costa but no conducting cells in the stem suggest that the costa cells that are elongate in a leaf where other cells are shorter may serve a function more important than conduction – that of supporting tissue, and may sometimes serve both functions. It is likely that they also have regenerative ability.
Figure 91. *Fontinalis squamosa* SEM image of stem cross section, showing the absence of specialized cells in the center of the stem. Photo by Janice Glime.

Figure 92. *Fontinalis dalecarlica* stem cross section showing absence of hydroids. Note the thick-walled outer cortical cells that give this stem the strength needed to survive in the rapidly flowing water of mountain streams. Photo by Janice Glime.

Figure 93. Longitudinal section of stem of *Fontinalis gracilis* showing elongated, thin-walled cells of the cortex. The cells at the arrows appear to be particularly long. Could they be leptoids? Photo by Isawo Kawai, with permission.

Figure 94. *Touwia laticostata* (?) branches showing leaves with thick costa. Note the remaining costae on the lower branch after it suffered abrasion. Photo courtesy of Andi Cairns.

Figure 95. The moss *Touwia laticostata* (?) stem (lacking discernible hydroids) and leaves with thick costa. Photo courtesy of Andi Cairns.

Figure 96. *Touwia laticostata* (?) leaf showing thick costa. Photo courtesy of Andi Cairns.

**Using a Partner**

**Epiphyllous** bryophytes have an unusual habitat on their host leaves. Water usually does not stay and is even repelled by the host leaf surface. *Radula flaccida* (Figure 97) has at least partially solved the problem by producing rhizoids that penetrate the host leaf cuticle and epidermal cells, extending into the tissues of the host (Berrie & Eze...
Berrie and Eze found that both water and dissolved phosphorus salts can be obtained from the host leaf. Hence, it appears that the liverwort is at least partially a parasite (Hébant 1977).

Throughout the kingdoms we see examples where two organisms share responsibilities in their mutual survival. Among these partners, the fungi seem to have perfected the strategy, making it possible for plants to greatly increase their available surface area without expending the effort to build the needed tissues. Such is the case for some bryophytes, a partnership for which we have limited understanding. Among those with such a relationship is the genus *Haplomitrium* (Figure 98) (Carafa et al. 2003). *Haplomitrium* secretes mucilage (Figure 99) from its underground rhizomes, forming an environment that harbors fungal hyphae. In *H. gibbsiae* (Figure 98), the fungus is restricted to the epidermal cells where it forms lumps, but in *H. ovalifolium* (Figure 98) it also infects the adjacent cortical cells, forming lumps. Through such partnerships, these species can gain access to both deeper and wider sources of nutrients in the sol substrate.

In tracheophytes, this partnership strategy has been used by a number of hemiparasites that partner with a fungus that partners with a tree or shrub. This arrangement permits them to gain carbohydrate energy from the photosynthesizing canopy while living in the darker environment under its protective cover. Our knowledge of bryophyte partnerships is still too primitive to ascertain how important this relationship is in permitting many bryophytes to subsist in such low light conditions.

**Summary**

Movement onto land required means of obtaining and retaining water. Bryophytes, reputedly the first colonizers, often are not the nonvascular plants we once thought them to be. They often possess hydroids, surrounded by stereids, that conduct water and together comprise the hydrome. Hydroids lack lignin and spiral thickenings, distinguishing them from tracheids and vessels of tracheophytes. Leptoids that conduct sugars, arranged as in tracheophytes, with the water-conducting cells surrounded by the sugar-conducting cells, are less well known because they are distinguishable in longitudinal section. In a few mosses, these stem conducting tissues connect by leaf traces to the leaves. Bryophytes usually have a thin cuticle, but it seems to lack wax in most cases. Rhizoids, although anchoring the plants as do roots, typically do not serve in obtaining water, but exceptions exist. Acrocarpous species more commonly have a central conducting strand, whereas pleurocarpous mosses remain close to the substrate and a central strand may not be useful.

Bryophytes function like sponges in the ecosystem by holding water and maintaining moisture in the soil below. But they also absorb water like a sponge, using capillary spaces. At times when water is limiting, the bryophytes are able to survive through their exceptional desiccation tolerance.

Mosses may have a costa (rib similar to a midrib) in the leaf, but it does not branch to reach all the cells (as in most tracheophytes) and may not always serve a conduction role. This is connected to the stem vascular strands only in the Polytrichaceae. Thallose liverworts may have a midrib to transport water and other substances, but leafy liverworts have no evidence of water-conducting cells in the stem and no costa in the leaf.

Even sporophytes have elongated cells in the seta. In younger sporophytes these may be important in conduction of nutrients to the developing capsule. Aquatic species presumably do not need conduction since they are bathed in water. But they still need to move solutes and especially sugars from leaves to other locations. Some bryophytes have mycorrhizal associates that help take in water and...
minerals. Others are connected by rhizomes that permit them to "scavenge" by obtaining photosynthate from connected stems that are in more favorable positions.

Acknowledgments

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CHAPTER 7-2

WATER RELATIONS: MOVEMENT

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CHAPTER 7-2
WATER RELATIONS: MOVEMENT

Figure 1. Grimmia nutans supporting drops of water that will eventually be absorbed into the moss through the leaf surface. Photo by Michael Lüth, with permission.

Water Movement

Early experiments with dyes demonstrated that in mosses water is able to move in conducting tissue of the central cylinder, leaf traces, and the costa (Zacherl 1956), depending on capillary spaces, as it does in tracheophytes (Table 1). Bopp and Stehle (1957) confirmed not only these internal pathways, but that movement also occurs from cell to cell (symplastic) in the cortex of the lower part of the stem, as well as on the outer surfaces of leaves and stems (Figure 1). But it is more likely that most of the movement across the cortex and internal leaf is through the free space of the cell walls where it does not have to cross cell membranes until it reaches its destination (Proctor 1984). Such apoplastic (outside cell membrane or in free space) movement across the cortex is known even in Polytrichum juniperinum (Figure 2) (Trachtenberg & Zamski 1979), where a central strand and leaf traces are available to facilitate movement of water.

Table 1. Relationship of bryophyte structures, size of space, and capillary rise. From Proctor (1982), based on Slatyer (1967).

<table>
<thead>
<tr>
<th>Radius of meniscus</th>
<th>Ht of capillary rise</th>
<th>Bryophyte structures in similar size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mm</td>
<td>1.5 cm</td>
<td>Large, concave leaves; spaces among shoots</td>
</tr>
<tr>
<td>100 µm</td>
<td>15 cm</td>
<td>Spaces between leaves, paraphyllia</td>
</tr>
<tr>
<td>10 µm</td>
<td>1.5 m</td>
<td>Space within sheathing leaf base, tomentum, hyalocyst of Sphagnum &amp; Leucobryum</td>
</tr>
<tr>
<td>1 µm</td>
<td>15 m</td>
<td>Interstices between leaf-surface papillae</td>
</tr>
<tr>
<td>100 nm</td>
<td>150 m</td>
<td>Spaces between cell-walls?</td>
</tr>
<tr>
<td>10 nm</td>
<td>1.5 km</td>
<td>Spaces between cell-wall microfibrils</td>
</tr>
<tr>
<td>1 nm</td>
<td>15 km</td>
<td>Glucose molecule</td>
</tr>
</tbody>
</table>

As in tracheophytes, water movement in both endohydric and ectohydric mosses is facilitated by tension forces (Zamski & Trachtenberg 1976), but unlike the case in tracheophytes, water moves in both directions in a source-sink fashion dependent upon availability (Bowen...
This bi-directional movement applies not only to external movement, but to the hydrome as well. For bryophytes, the first water availability most commonly does not start with the soil, but with the tips of stems and leaves by way of rain, fog, or dew.

Early observations showed that in general external conduction is much more rapid than internal conduction (Bowen 1933a, b, c; Clee 1939). This most likely relates to frictional resistance in the small internal routes. On the other hand, we should expect water to rise higher in small internal capillary spaces (Table 1). What seems strange, however, is that the utility of internal conduction in at least some bryophytes can change with age toward greater use of external conduction. Mizushima (1980) found that in older stems of Entodon rubicundus no internal conduction could be detected at 75% atmospheric humidity, but in younger stems, a slow internal conduction could be detected in the central strand. Both young and old stems exhibited external conduction, travelling up to 1 cm in 12 hours. This loss of internal conduction in older plants may support the contention of Kawai (1991), among others, that mosses may have been derived from vascular plants by reduction.

One item of curiosity is that not all bryophytes have vacuoles (Oliver & Bewley 1984). Surely this plays some role in their ability to hold water, and most likely affects nutrient placement and protection from toxic substances as well, but no one seems to have looked at this role in bryophyte physiology (Bates 2000).

**Ectohydric**

Ectohydric mosses (almost all mosses) rely primarily on external transport of water and can absorb water over the entire plant surface (Figure 3). These taxa generally have no water repellent layers, or these are restricted to such locations as the apices of papillae, and they are easily wetted (Proctor 1982, 1984). Movement is due to capillarity and the relationships are complex. As the moss becomes hydrated, its capillarity changes due to expansion of leaves, untwisting, and other forms of movement and gyration (Deloire et al. 1979). They benefit from a large surface area relative to their volume (Proctor 1984) due to numerous leaves and often such structures as paraphyllia (reduced leaflike structures on the stem or branches of some pleurocarpous mosses) and tomentum (felt-like covering of abundant rhizoids on stem).
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Figure 5. *Zygodon viridissimus*, a moss in which the apical cell of the leaf lacks wax, permitting water entry. Photo by David Holyoak, with permission.

The ectohydric habit depends on entry of water through the moss surface and permits a moss to respond to dew and fog by absorbing water directly, even though rooted plants may never receive a drop of it. Such bryophytes can live in high elevations and on deserts that receive less than 25 cm rainfall per year, obtaining water that cannot be measured by conventional precipitation methods. Most tuft-forming (acrocarpous) mosses are (partially) endohydric, whereas most mat and carpet formers (pleurocarpous mosses) are ectohydric (Richardson 1981). In addition, some upright mosses such as *Sphagnum* (Figure 6) and *Andreaea* (Figure 7) are ectohydric. Schipperges and Rydin (1998) clearly showed this by clipping the capitula from the stem; these clipped capitula were unable to recover from desiccation, whereas unclipped capitula became rehydrated. But Even *Sphagnum* has highly specialized cells in the stem that have all the traits of a bryophyte type of conducting cell (Ligrone & Duckett 1998).

In ectohydric bryophytes, the uppermost leaves and shoot apices have the most rapid conduction of water, so that lower leaves are often supplied last (Zacherl 1956). Zacherl believed that no internal conduction was possible in the absence of a central strand. This apical movement may be beneficial in conserving water when water is scarce and only the leaves at the tips of the stems are receiving enough light for photosynthesis. These also are the leaves most exposed to fog and dew.

Using dyes and *Dicranum scoparium* (Figure 8) as a model subject, Bowen (1933c) demonstrated that external conduction was "exceptionally rapid" and internal conduction slow. Mägdefrau (1935), using the same species, determined internal conduction to be only about 1/3 the total conduction – not bad for a bryophyte. Klepper (1963) found that under conditions of desiccation, the protoplasts of this species become dense and evacuolate, undoubtedly developing considerable imbibitional pressure (due to adsorption of water by colloidal particles, much as seeds do. This would cause them to readily take in water when it becomes available.

Once the water enters the plant the distinction between endohydric and ectohydric no longer matters. Although the initial movement of water is clearly ectohydric in most dry mosses, once it has entered the moss it has the opportunity to move apoplastically to reach places where it is needed.
for cellular metabolism. It is interesting that endohydric bryophytes can be facultatively ectohydric. Bayfield (1973) found that Polytrichum commune (Figure 9) was ectohydric under moderate moisture flux, but under high evaporative flux (i.e. dry air) it was predominantly endohydric.

Figure 9. Polytrichum commune, a moss that is ectohydric under moderate moisture flux but endohydric under dry air. Photo by George Shepherd, through Creative Commons.

Despite all the laboratory experiments on conduction, we still have little concept of the relative importance of the two pathways over a large time scale in nature. Certainly, as demonstrated in Polytrichum (Figure 9), the relationship changes as the moss dries. Is it not likely that internal movement of water from older to younger parts then predominates, keeping the photosynthetic and growing apical tissue wet as long as possible? Surely the same apoplastic routes available to Polytrichum are available to all mosses. The natural transpirational stream that carries water from the shoot apices to the atmosphere could be expected to play a similar role to that found in tracheophytes and maintain upward movement (or outward in pleurocarpous mosses) through capillary spaces as long as water was available and internal tension did not exceed that resulting from transpirational loss.

What quantities do the various mosses move from moss mat to atmosphere and how much is moved from the soil to the moss mat? Do the mosses provide an overall net gain to the soil by preventing rapid loss to the atmosphere following rainfall? Do they retain water that would otherwise be lost as runoff, contributing it slowly to the soil and plant roots beneath? Or is their major contribution that of depriving the soil of water during showers of short duration? There is no mass balance equation that includes the role of bryophytes in the overall water budget in any ecosystem.

**Endohydric**

Endohydric mosses, including Polytrichum (Figure 2, Figure 9, Figure 13), Mnium s.l. (Figure 10, Figure 17), and Bryum (Figure 3), generally have surfaces that contain a water-resistant cuticle (Lorch 1931; Buch 1945; Bayfield 1973; Proctor 1979a), thus reducing their ability to take in water through their leaves. In some of these, that cuticle is endowed with a wax similar to that found in tracheophytes (Proctor 1979b; Haas 1982). However, this waxy coating of a moss leaf offers only a low water diffusion resistance similar to that of tracheophyte mesophyll (Nobel 1977; Proctor 1980) and may be more important in repelling water to permit a higher CO₂ diffusion into the leaf (Proctor 1984). Among ectohydric mosses, waxy cuticles seem to be either generally lacking or very thin. Mosses like Polytrichum and many members of the Marchantiales are actually water repellent, thus requiring half an hour or more to take up water (Proctor 1984). These endohydric bryophytes utilize, in the case of mosses, the system of non-lignified hydroids and leptoids to conduct water and sugars, respectively.

Figure 10. Mnium spinosum with water droplets on its leaves. This moss is very slow to absorb water due to its water-resistant cuticle. Photo by Michael Lüth, with permission.

But it appears that even these endohydric mosses rely on ectohydric transport. Instead of moving water inside the moss at the first opportunity during its external vertical rise, it is the tips of the plants that exhibit primary water absorption (Brown 1982). Water travels upward through the capillary spaces created by the leaves. Mosses like Polytrichum may facilitate this apical absorption by preventing any significant absorption by the cuticularized lower and more mature leaves.

In these predominantly endohydric mosses, rhizoids may serve functions of conduction much as do roots and root hairs. It appears that endohydric mosses such as Polytrichum (Figure 2, Figure 9, Figure 13), Dawsonia (Figure 11), and Climacium (Figure 12) transport water from the substrate beneath to their tips before moving it through an internal conducting system, sometimes called the central strand. Although Polytrichum commune (Figure 9) has demonstrated the ability to transport water externally along its stems, Mägdefrau (1938) contended that the major conduction is internal through the central strand. However, Trachtenberg and Zamski (1979) determined that despite the ability of rhizoids to absorb and transmit water, the major absorption is still through the aerial gametophyte, due to its greater efficiency. Because of the extensive development of conduction cells in Polytrichum (Figure 13), where central hydroids are surrounded by a cylinder of leptoids, Hébant (1970) considers this and other mosses to have similarities to the xylem and phloem of primitive vascular plants.
there is no connection between the central strand and the costa, and in some cases there is no costa at all. Furthermore, Colbert (1979) showed that there is no connection between the central strand of the stem and that of the branches in Climacium americanum (Figure 14), C. dendroides (Figure 12), Rhytidiadelphus triquetrus (Figure 15), and Rhytidium rugosum (Figure 16).

But how does the water reach the leaves in the endohydric mosses? Zacherl (1956) used fluorescent dyes to show that in Polytrichum (Figure 2, Figure 9, Figure 13), the costa (midrib-like structure) links with the central strand, forming true leaf traces. In many taxa, however,

Figure 11. *Dawsonia polytrichoides*, a moss with good internal conduction. Photo by Niels Klazenga, with permission.

Figure 12. *Climacium dendroides*, a moss with external conduction from base to tip. Photo by Jan-Peter Frahm, with permission.

Figure 13. Cross section of a *Polytrichum* stem showing green hydroids of the central strand in center and larger leptoids surrounding them. Photo by Isawo Kawai, with permission.

Figure 14. *Climacium americanum*, a moss with a central strand with no connection to the leaf. Photo by Bob Klips, with permission.

Figure 15. *Rhytidiadelphus triquetrus*, a moss with a central strand that does not connect to the leaves. Photo by Janice Glime.

Figure 16. *Rhytidium rugosum*, a moss with a central strand that does not connect to the leaves. Photo by Michael Lüth, with permission.
Nevertheless, in the absence of those connections the extension of the costa into the stem cortex still can function to complete internal transport across normal cortical cells (Zacherl 1956). For example, in Mnium (Figure 10) the costa does not link directly with the central strand of the stem, but ends blindly in the ground tissue, forming false leaf traces (Figure 17). The ends of the costae (Figure 18) act as wicks, transferring liquids across the ground tissue from the central strand and into the leaf costa, most likely using a diffusion gradient across the cortex.

![Figure 17. Cross section of Mnium stem showing false leaf traces. Photo by Janice Glime.](image)

The cortex behaves as capillary tubes and draws the water across the stem parenchyma to the leaf, much as water traversing the roots of tracheophytes. Trachtenberg and Zamski (1979) demonstrated, using PbS and Pb-EDTA (which accumulates Pb ions in tissues in proportion to the amount of water passing through), that the water actually moves in the capillary spaces of the cell walls – apoplastic conduction. Beckett (1997), using pressure volume isotherms, determined that cryptogams, including bryophytes, contain significant amounts of intercellular water when fully hydrated, whereas flowering plants do not. It is this extracellular pathway that permits water to move from leaf surfaces inward and into stems, where it can be conducted in the hydrome as well as apoplastically.

It is interesting that the uppermost leaves are the first ones to receive water internally (Zacherl 1956), just as in the ectohydric mosses.

Trachtenberg and Zamski (1979) further learned that the sterome, assumed to be supporting tissue, can provide an alternative pathway for water conduction. That its mass was much greater than needed for support had already been noted by Lorch (1931). Furthermore, xerophytic mosses have a very large sterome (Goebel 1915) that is used for conducting and holding water. In mosses such as Fabroniaceae and Orthotrichaceae that lack a hydrome, the sterome is large (Van der Wijk 1932) and seems to supply this function. In fact, Trachtenberg and Zamski (1979) suggest that the transport from hydrome to leaves in Mnium (Figure 17) may take place through stereids. They support their hypothesis by demonstrating that the lead chelate solution applied to the leaves penetrates the sterome. They suggest that the most probable means of translocation of water from leaves into the stem is through the dense mass of stereids in leaves and leaf bases to the central cells of leaves and leaf traces to the hydrome. But only in the Polytrichaceae does there seem to be a connection between the leaf traces and both the leaf and hydrome. Rather, the apoplastic route through cell walls in the stem cortex is a more likely route in most cases.

Mixohydric bryophytes are those in which both endohydric and ectohydric methods are important. Many of the species in this group are small, acrocarpous mosses of loams or clays. These soils dry out frequently, but their fine texture permits them to maintain a moist top layer for a period of time after rain. Hébant (1977) contends that truly mixohydric mosses are not very abundant, implying that the ectohydric pathway is far more important in most. However, in reality, most (perhaps all) mosses are mixohydric in that they have both internal and external means of conduction to at least some degree.

Nocturnal

For many mosses, nighttime is the only period of rehydration. This is especially true for desert mosses such as Syntrichia ruralis (Figure 19). Csintalan et al. (2000) found that this moss obtained sufficient water through nighttime dew to accomplish 1.5 hours of net photosynthetic gain immediately after dawn. They suggested that such early morning periods might permit regular molecular repair due to desiccation damage during prolonged dry periods.

![Figure 19. Syntrichia ruralis. Photo by Michael Lüth, with permission.](image)
showed a nocturnal gain in mass due to vapor from the forest floor. As the forest floor cooled at night, distillation occurred with moisture condensing on the moss surface. The cooling temperatures and moisture provided by the forest floor was sufficient to cause the moss tips to reach dew point. This seems to be most evident in late summer when the lower organic layers have warmed the most and the surface temperature is thus relatively lower at night, causing the condensation. When a vapor barrier was used to prevent ground water from rising, no mass gain was in evidence.

Mechanisms of Water Movement

Bopp and Stehle (1957) found that a mechanism similar to the diffusion pressure deficit seen in higher plants worked in moving water up the moss. By using fluorescent dyes, Bopp and Stehle showed that water moved up the leafy gametophyte both internally and externally, but that dye went quickly to the foot of the sporophyte imbedded in the gametophyte, then moved up the seta through the central strand. In mosses with the calyptra removed, the flow rate increased, suggesting that transpiration loss may perform a function of pulling water, similar to that found in tracheophyte guard cells, suggesting that this area may be one of transpirational water loss.

Although bryophytes lack leaf stomata, they do lose water through their wax-free leaves. For example, the transpirational loss rates of the moss cover in the lichen tundra (16-20% of total precipitation) is not unlike that from the ericaceous shrubs of the tundra heath (24-26%) or the alpine dwarf shrub heath (16-20%), whereas tracheophytes in a wet meadow can have 135% loss (Larcher 1983 – data from many authors).

Using mosses from five different habitats ranging from wet to dry, Bowen (1933a,b,c) determined that the water ascends the mosses as capillary films between the leaves and stem, being absorbed at the stem and branch apices by the younger cells with unthickened walls. From there it diffuses through internal tissues laterally, then downward, not upward as in tracheophytes. Conduction from the base through the central strand is slow in cut stems but much slower when the stem base is still intact (Bowen 1933a,b,c). In the latter case, water must penetrate the thick walls of the rhizoids and stem/rhizome. And at least some of the species have cuticles on the rhizoids!

Once water reaches the central strand, it travels there preferentially (Hébant 1977). Internal ascending water travels through the narrow, elongated, thin-walled cells (presumably hydroids). In addition to apical absorption, leaves and stem epidermis absorb some of the water, albeit less readily due to cell-wall thickening and cuticles.

The capacity of both internal and external water conduction seems to diminish as the moisture of the habitat increases (Bowen 1933a, b, c). Mägdefrau (1935) contends that at 90% humidity, Polytrichum (Figure 2, Figure 9, Figure 13) can maintain its turgor with internal conduction only, but at 70% both internal and external conduction are necessary. For all other families of mosses, with the possible exception of the Mniumaceae, a significant amount of external conduction seems necessary.

Vitt (1990) suggests that those mosses that must endure a greater range of fluctuations in water availability may be more plastic in their responses. At least among the boreal mosses, the ectohydric, drought-tolerant Hylocomium splendens exhibits highly variable growth over its North American range, but the endohydric, less drought-tolerant Polytrichum strictum (Figure 21) exhibits more constant growth throughout its range (Vitt 1990).
the central strand may be more concentrated and thus more easily discerned. Rather, Bowen argues that water, and hence dye, accumulate in the central strand, whereas their movement across the cortex is transitory only. Thus, when water appears to have reached a certain height in the stem from internal movement through the hydrom, it may in fact have arrived there from the aerial surfaces across the cortex. Using 12 plants of *Polytrichum commune* (Figure 9) and blocking the entry of water into the hydrome from the cut surface with wax, she was able to demonstrate rapid movement externally, up to 42 cm in one hour, reaching a maximum of 96 cm in 24 hours (Bowen 1931). When basal leaves were removed (and the wounds sealed) and the cut stems were not blocked, she demonstrated considerably less movement internally through the hydrome. Using only three plants, the greatest rise internally was only 12 cm.

It is interesting that the dependence on endohydric gametophytic conduction seems to have diminished in the evolution of bryophytes, with the creeping (pleurocarpous) taxa exhibiting less developed conducting systems. Instead, the ectohydric habit is well-developed. Yet, no pattern exists (Hébant 1977). Even the xerophytic *Orthotrichum* (Figure 22) lacks a central strand, although despite its acrocarpous appearance it is technically pleurocarpous and thus related to taxa that have apparently lost the central strand.

**To summarize,** water is known to move from one bryophyte part to another by four pathways: hydroids, free spaces in cell walls, cell to cell, and externally.

Figure 23. *Funaria hygrometrica.* Photo by Michael Lüth, with permission.

Table 2. Effectiveness of internal conduction compared to total in mosses after 24 hours in 70% relative humidity. Conduction measurements are grams water/0.2 grams dry mass; % is percent of internal compared to total rate. From Mägdefrau (1938).

<table>
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<tr>
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<tr>
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<td>2.24</td>
<td>3.32</td>
<td>67</td>
</tr>
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</table>

**Transport to Sporophyte**

The seta, lacking leaves, must necessarily conduct water internally. Conduction from the gametophyte to the sporophyte tissue seems to be governed by several factors, as observed in *Funaria hygrometrica* (Figure 23) (Bopp & Stehle 1957). The sporophyte receives its water from the haustorial foot that is imbedded deeply into the central strand of the gametophyte.

In *Dicranum undulatum* (Figure 24), it appears that the embryo has a role in development of the conducting strand in the gametophyte stem, as no conducting strands were present below archegonia that had not been fertilized (Roth 1969). Hébant and Berthier (1972) made similar observations on *Polytrichastrum alpinum* (Figure 25). This underscores the apparent importance of the transfer of water from the gametophyte central strand to the sporophyte. There are no plasmodesmatal connections between the gametophyte and the foot of the seta (Hébant 1977). However, the **transfer cells** have extensive wall ingrowths (labyrinth, Figure 26) that greatly increase the surface area of the plasma membrane, thus increasing transport (Hébant 1977). In *Funaria hygrometrica* (Figure 23) fluorescent dyes showed that the jacket around this foot...
was separated by a narrow intercellular space which became colored before the central strand (Bopp & Stehle 1957). This capillary space moved the liquid quickly to the central strand of the sporophyte. When comparing species that had no transfer cells, Bopp and Weniger (1971) found that uptake by the sporophyte was greatly reduced.

Figure 24. *Dicranum undulatum*, a moss where conducting strands seem to develop only in stems under archegonia with embryos. Photo by Jan-Peter Frahm, with permission.

Figure 25. *Polytrichastrum alpinum*, a moss where conducting strands seem to develop only in stems under archegonia with embryos. Photo by Michael Lüth, with permission.

Hébant (1977) describes detailed studies of a variety of mosses in which the very tip of the sporophyte foot directly penetrates the central strand of the gametophyte. At the end of the foot, an appendage of mostly necrotic cells is the only separation of the conducting cells between the two generations. Conduction in the sporophyte was increased when the calyptra was removed; the apparently non-closing stomata of the capsule may contribute to transpirational water loss.

As can be observed in *Physcomitrium immersum (=Physcomitrium cyathicarpum)*, both generations have transfer cells at the junction, and the foot epidermal cells are rich in organelles (Lal & Chauhan 1981), especially mitochondria (Hébant 1977), suggesting there might be considerable active transport between the two generations.

Figure 26. Transfer cell between gametophyte and sporophyte showing wall labyrinth. Computer-drawn from photo in Lal and Chauhan (1981).

Summary

We have seen that bryophytes have remarkable abilities to gain, retain, and recover from loss of water. They gain it in their cells both through external (ectohydric) capillary movement and internal (endohydric) transport. Endohydric movement is accomplished either cell-by-cell or through designated elongate cells. Nutrients and water are transferred to the sporophyte through the foot, using special transfer cells with labyrinth walls.

Acknowledgments

This chapter has benefitted from the help of Beth Scafone and Medora Burke-Scoll, who helped me tow the line in explaining things without leaving too much to one's imagination, but at the same time not repeating myself.

Literature Cited


# CHAPTER 7-3

## WATER RELATIONS: PLANT STRATEGIES

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CHAPTER 7-3
WATER RELATIONS: PLANT STRATEGIES

Figure 1. *Tortula muralis* with water drops collected on the leaf awns and setae. Note their collection around the young capsules. Photo courtesy of Peggy Edwards.

**Water Strategies**

Water strategies in bryophytes have been approached in a variety of ways, from splashing gemmae (Brodie 1951) to uptake (Proctor 1981; Rice & Schneider 2004; Rice 2012; Jonas & Dolan 2012; Sand-Jensen & Hammer 2012) to transport (Hébant 1977; Mulder et al. 2001; Proctor & Tuba 2002; Pressel 2006; Rice 2012) to moving nutrients (Buch 1945, 1947; Proctor 1981) to retention (Kennedy 1993; Mulder et al. 2001; Rixen & Mulder 2005).

Mosses often appear to be completely dead, only to be revived by water. Angela Newton (pers. comm., Bryonet) reported that epiphytic mosses in the lowland tropical rainforests, where mosses may appear dead after severe desiccation, recover quickly with actively growing apical and axillary buds on completely dried out plants and even on plants scorched by fire (see Figure 2)! Leaf and stem tissues from xerophytic mosses can regenerate after as many as 19 years of desiccation (Table 1); some spores remain viable after 70 years (Malta 1921). As surmised by Oliver *et al.* (2005), desiccation tolerance appears to be a primitive trait that permitted plants to invade land. Desiccation tolerance (DT) is the ability of an organism or structure to tolerate and survive after equilibrating to a relative humidity (RH) of ≤50% (Alpert & Oliver 2002; Koster *et al.* 2010). An understanding of this tolerance in modern bryophytes is fundamental to a general understanding of desiccation tolerance.

Figure 2. *Polytrichum* showing fresh, green growth one week after a forest fire that scorched adjacent plants in Baraga, Michigan, USA. Photo by Janice Glime.
Most people who are not very familiar with bryophyte physiology consider them to be plants that require damp or wet habitats. But compared to tracheophytes (non-bryophytes; plants with lignified vascular tissue), bryophytes are the ones adapted to drying conditions (Vitt et al. 2014). Even in bryophytes that seem to lack physiological desiccation tolerance, morphological or life history adaptations permit them to survive periodic drought.

In fact, of the known 18,000 species of bryophytes (Shaw & Goffinet 2000), 210 have been documented as desiccation tolerant (Wood 2007), but most have never been tested. Among seed plants, fewer than 1% of those tested are desiccation tolerant (Proctor & Pence 2002). Even in tracheophytes requiring one to several days to resume physiological activity whereas bryophytes (and lichens) typically require an hour or less (Proctor & Tuba 2002).

Both constitutive (always present; fully desiccation tolerant) and inducible [produced when drying conditions occur; previously known as modified desiccation-tolerant (Oliver et al. 1998)] desiccation tolerance exist among plants (Stark et al. 2013) and these will be discussed in Chapter 7-6 of this volume.

Cellular structure remains intact in desiccation, but upon rapid uptake of water it can be disrupted. However, in bryophytes the cellular integrity returns rapidly. Photosynthetic activity recovers quickly, perhaps due to protection of the chlorophyll (Tuba 1984; 1985). During desiccation, there seems to be no gene activity, but gene expression occurs rapidly following rehydration. Among these activities is the production of a number of proteins called rehydrins. These seem to be involved in stabilizing and reconstituting membranes that have been damaged by dehydration. Oliver et al. (2005) suggest that vegetative desiccation tolerance, at least in bryophytes, has changed little from early land invaders and may be using a mechanism that was first used in spores.

Even though many bryophytes tolerate high degrees of desiccation (Dilks & Proctor 1974; Nörr 1974; Dhindsa & Bewley 1976), water content and availability are important for potential accumulation of photosynthates (Alpert 1979). Patidar (1988) found that in Asterella angusta the moisture content is the most important determinant of thallus (flattened, nonvascular plant body) size. In Plagiochasma appendiculatum (Figure 3), optimum growth occurs at 60% moisture, whereas branching and growth are able to occur from 10-100% moisture (Vishvakarma & Kaul 1988)! Reboulia hemisphaerica (Figure 4), on the other hand, requires 70-80% moisture for optimum growth, with growth and branching occurring from 40 to 90%. In other words, no matter how desiccation tolerant a plant might be, it requires water to grow. Representative water contents of bryophyte plants from a wide range of field habitats in the temperate zone, Great Britain, are given in Table 2.
Table 2. Percent water content (compared to dry mass) of bryophytes at full turgor, not including free external water. Values represent means of two measurements. Table based on Dilks and Proctor (1979); those marked with * from Skre et al. (1983) include new growth and 1-year-old growth; those with + from Proctor (2000).

<table>
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</tr>
<tr>
<td>Syntrichia intermedia</td>
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</tr>
<tr>
<td>Homalothecium sericeum</td>
<td>223</td>
</tr>
<tr>
<td>Pseudoscleropodium purum</td>
<td>207</td>
</tr>
<tr>
<td>Thuidium tamariscinum</td>
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<tr>
<td>Dicranum majus</td>
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<tr>
<td>Leptodon smithii</td>
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<tr>
<td>Rhytididiadelphus loreus</td>
<td>165</td>
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<tr>
<td>Pleurochaete squarroso</td>
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<tr>
<td>Neckera complanata</td>
<td>162</td>
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<tr>
<td>Racontum lanuginosum</td>
<td>142</td>
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<tr>
<td>Anomodon viticulosus</td>
<td>141</td>
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<tr>
<td>Polytrichum commune*</td>
<td>95-125</td>
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<tr>
<td><strong>Liverworts</strong></td>
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<tr>
<td>Pellia epiphylla</td>
<td>1180</td>
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<tr>
<td>Conocephalum conicum</td>
<td>871</td>
</tr>
<tr>
<td>Porella platyphylla</td>
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</tr>
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<td>Plagiochila spinulosa</td>
<td>222</td>
</tr>
<tr>
<td>Bazzania trilobata</td>
<td>210</td>
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</table>

Mosses grown in fully hydrated conditions afforded by saturated air enjoy optimal growth and development (Davy 1927). They exhibit more rapid development, more stem branching, more numerous rhizoids, smaller leaves, and smaller and fewer cells with larger chloroplasts than mosses existing at less than full saturation. Even at the scale of a single boulder, bryophytes distribute themselves according to their ability to achieve photosynthetic gain. When examining bryophytes that occupied various microsites on exposed granitic boulders, Alpert and Oechel (1987) found that those species that occurred in microsites with lower water availability were able to attain maximum net photosynthetic gain at a lower water content and to recover better from prolonged desiccation than those taxa in less xeric (dry) microsites. Alpert (1985, 2000) supports the hypothesis that the reason even xerophytic mosses (those adapted to dry habitats) are limited in their ecological distribution is that they often are unable to maintain positive carbon balance during repeated cycles of wetting and drying. Alpert and Oechel (1985) demonstrated this with Grimmia laevigata (Figure 5-Figure 6), a desiccation-tolerant plant that was unable to maintain this balance under a natural, highly xeric regime of wetting and drying in certain microhabitats on exposed granitic boulders in California chaparral. Thus, there is an "inherent trade-off between desiccation tolerance and growth rate."

Water content in a bryophyte ranges widely throughout the year. For example, Klepper (1963) measured 23.8-258% in Dicranum scoparium (Figure 7), Romose (1940) 10-950% in Homalothecium sericeum (Figure 8), Morton (1977) 19-214% in Pseudoscleropodium purum (Figure 9), and 58-307% in Dicranum bonjeanii (Figure 10). Whereas many mosses benefit from high water content, too much water is not good for photosynthesis. Water on the surface blocks CO₂, and most likely high internal water content also interferes with physiological processes (Proctor 2000). Dilks and Proctor (1979) found optima as low as 200% water content and as high as 1500% among the same bryophytes shown in Table 2. Respiration seems to peak around 200% for most of these taxa.
Richardson (1981) divides mosses into three water strategies by habitat: **aquatic**, **mesophytic** (living in continually moist habitats), and **poikilohydric** (organism dries as its habitat dries and resumes normal metabolic activity after rehydration; Figure 6). Unlike most other plants, water content of predominantly poikilohydric bryophyte species is highly related to environmental conditions and weakly regulated by their internal and morphological structures. This strategy permits them to colonize such xeric environments as boulders and tree trunks. In these environments, mosses enjoy release from competition by higher plants, but must still survive the low light intensity created by the trees above.

Some bryophytes do appear to be able to survive in absence of precipitation. In caves in Poland, only 18.1% of the species occurred in very wet places or where there was dripping water, whereas in places that were continuously dry(!), 25% occurred (Jedrzejko & Ziober 1992). Certainly in those dry places atmospheric humidity must have provided the needed water for these very **hygroscopic** (readily absorbing water from air), **ectohydric** (relying mainly on water transport along external surface of plant by capillarity) bryophytes. Shaun Russell (pers. comm.) found that in montane areas of Africa with virtually no rainfall, fog collected on bryophyte surfaces, providing sufficient water for them to survive. A similar phenomenon occurs in geothermal areas (Figure 11).

Figure 11. *Campylopus holomitrius* with water droplets captured from the “steam” emitted by geothermal vents in New Zealand. Photo by Janice Glime.
The drought-tolerant *Pseudocrossidium crinitum* (=*Barbula aurea*; Figure 12) seems to have compensated for its low water availability by having relatively low levels of light compensation and saturation responses for photosynthesis (Rundel & Lange 1980). Such low levels would permit the moss to carry out photosynthesis early in the morning when dew is available and before high evaporation stress occurs. These aerial sources of water are of little use to tracheophytes that must take water in by their roots, not their leaves.

As one would expect, degree of drought tolerance is generally greatest in plants from dry habitats (Clausen 1952, 1964; Johnson & Kokila 1970; Dilks & Proctor 1974). Seki and Yamaguchi (1985) suggest that on some islands with strong summer winds, Shannon diversity decreases as saturation deficits increase. Richardson (1981) claims that aquatic mosses and those that grow in humid forests are damaged quickly by drought. But even such high humidity plants as *Hookeria lucens* (Figure 13) are able to survive desiccation for days (Horst Tremp, Bryonet).

Glime (1971) found that two aquatic mosses (*Fontinalis* spp.; Figure 14) were able to survive on the stream bank out of water (Figure 15) for up to one year and still grow when rehydrated. However, those dried in the laboratory were apparently dead after only 55 hours, perhaps due to rapid drying. Steere (1976) found that *F. squamosa* (Figure 16) from Alaska could not survive a week of air drying. One reason for apparent differences here is that it is difficult to determine when a moss is dead, and even though all leaves may be dead, the stem may still harbor life. *Fontinalis* is subject to annual emergence when stream level drops (Figure 14), and perhaps slow drying on the stream bank permitted it to become dormant and to preserve sufficient energy to repair its membranes upon rehydration. The rapid drying of a laboratory, with unnaturally low humidity and no acclimation period, may have prevented the necessary physiological changes that could permit it to survive. It is well known that it takes longer for the cellular physiology to return to normal in a rapidly dried bryophyte than in a slowly dried one (Oliver & Bewley 1984).
A similar pattern of submersion and desiccation is endured by *Hydropogon fontinaloides* in the Amazon basin (Mägdefrau 1973). It hangs from trees during the dry season, but during the wet season it floats in water. The behavior of *Cratoneuron filicinum* (mistakenly published as *Hygrohypnum luridum*; Figure 17), typically a stream margin species, may explain the lab results. This moss was unable to synthesize protein when rehydrated after it was dried quickly over silica gel for one hour (Bewley 1974). However, it was able to tolerate drying down to 33% of its fresh mass when dried slowly, and slow drying for 5 hours to 66% of its fresh mass had no detrimental effects on protein synthesis.

![Image](image7.png)

Figure 17. *Cratoneuron filicinum*, a moss that is unable to synthesize protein when rehydrated if it dries too quickly. Photo by Teplov through Arctoa, with permission.

Just what endows bryophytes with the ability to inhabit arid microsites that are totally inhospitable for tracheophytes? As you can see from the foregoing examples, we can divide these adaptations into anatomical/structural, growth/life form, and physiological adaptations.

Life cycle adaptations, a major means for an immobilized organism to cope with changing weather and seasons, have been addressed in Chapter 4-6. Schofield (1981) points out that spore germination patterns, protonemal structure, life span, and methods of propagation are all related to the habitat characteristics. Water availability is important to all of these aspects. Multicellular spores are more common in habitats where the spores are subject to desiccation. Protonematal specialization is common in deeply shaded habitats, whereas in mesic habitats the protonemal cells are elongate, *i.e.*, having typical protonemal structure. In the leafy *gametophore* (upright leafy plant), characters such as leaf shape, leaf arrangement and orientation, leaf anatomy, stem cortical cells, hydroids, leptoids, branch arrangement, presence of rhizoids, and presence of paraphyllia all affect water movement. Such small features as surface ornamentation of leaves, stems, and rhizoids affect water absorption and retention and influence habitat specificity.

### Water Cycle Role in Ecosystem

In areas where bryophytes dominate the forest floor, their role in the water cycle can be extraordinary. This is especially true where permafrost prevails (Henry Santeford, pers. comm.). Bryophytes hold melt water until they become saturated. At that point in time, all new melt water is suddenly released and can cause flooding. It is important in some areas to be able to predict this flooding regime for the safety of both animals and humans. Hence, we need to understand both holding capacity and evaporation rates of water from the bryophytes.

Penman (1948) helps us to understand evaporation as it might apply to bryophytes. Although he compared evaporation from bare soil, grass, and open water, the principles apply. He suggests that we need to combine two theoretical approaches to evaporation, an aerodynamic basin in which evaporation is due to turbulent transport of vapor by eddy diffusion, and an energy basis in which evaporation is a way of degrading incoming radiation. This approach eliminates the problem of measuring surface temperature and overcomes the problem of estimating effects as if one is measuring evaporation from a lake surface. Using this method, supported by empirical data, indicates that evaporation from grass follows a seasonal cycle relative to that evaporating from open water, a phenomenon Penman attributes to the change in photoperiod.

In other circumstances, bryophytes may prevent the underlying roots from getting water (Beth Scafone, unpubl. data). If there is a quick rain shower, bryophytes act as sponges, trapping the water before it reaches the soil. On the other hand, bryophytes can reduce evaporation of water from the soil following heavy rain, thus permitting the roots to grow for longer periods of time.

### Structural Adaptations

When I moved to the Upper Peninsula of Michigan, I was struck by the fact that only red oaks were able to live here. A simple difference in one structure made their survival possible in a cold region with long winters – narrow vessels. Large vessels in the other North American species cavitate and the water is unable to reconnect on an appropriate time schedule that permits the oaks to complete their life cycle and gain sufficient energy to continue the establishment of the species.

Many studies have demonstrated the importance of anatomy in determining the mechanical properties of plant tissues, including bryophytes (Hébant 1977; Rossi et al. 1998; Niklas et al. 2006; Frenzke et al. 2011; Atala & Alfaro 2012; Vincent 2012). Getting water and nutrients into and out of a plant is size dependent. Adaptations vary even within a species due to its plasticity in responding to the environment (Sarafis 1971; Buryóvá & Shaw 2005). As discussed by Raven and Handley (1987), for plankton organisms, any size above ~50 µm diameter restricts the growth rate because of the greater restriction of uptake by the boundary layer. Thus, for macroscopic aquatic photosynthetic organisms, even favorable velocity of water and plant morphology cannot reduce the boundary layer restrictions on nutrient uptake enough to compensate for the decrease in uptake rate. Movement of nutrients within the plant involves *cyclosis* (cytoplasmic streaming) in algae, phloem and xylem in tracheophytes, and in bryophytes it often involves both cyclosis and transport through leptoids and hydroids.
Raven and Handley (1987) consider that the energy cost for transporting nutrients would be higher for those organisms using cyclosis than for those using vascular tissue. They also consider that there is a penalty for height in tracheophytes, causing reduced specific growth rate under both resource-saturated and resource-limited conditions. Coupled with this penalty is reduced resource use efficiency with increased plant height. The reason for these penalties is the need for greater supporting tissue and a greater percentage of the tissue dedicated to vascular tissue. But there is also some compensation. Taller plants can capture more light energy and reach more nutrients and water in the soil. Smaller plants, on the other hand, have a potentially higher specific growth rate under these same resource-limited or resource-saturated conditions. The lack of need for supporting structures, requiring breadth, permits smaller plants such as bryophytes to have all or nearly all of their tissues as photosynthetic tissues. Raven and Handley left us with the challenge to discover the differences in transport needs and solutions resulting from these different morphologies.

In the desert moss *Syntrichia caninervis* (Figure 18), conservation of moisture is paramount, surpassed only by the need for rapid uptake. It can therefore serve as a model for adaptations against desiccation. Its growth form is tufted and its leaves are folded upward and twisted around the stem when dry (Zheng *et al*. 2010). The leaf cells are endowed on both the upper and lower surfaces with C-shaped papillae that may have a role in deflecting sunlight to protect the DNA and chlorophyll during dry periods or to reduce the temperature. The leaf costa extends beyond the leaf to form an awn that has forked teeth and is able to capture moisture from the atmosphere. The protonemal cells are small and have thick walls; their cytoplasm is highly concentrated with only a small vacuole.

**Scales**

Exposed surfaces of scales and thallus are often reddish-purple due to the flavonoid pigment *phlobaphene*, formed by oxidation of tannic compounds (McConaha 1939), but the role of phlobaphene is not clear. It is possibly a defense against would-be toxins from tannic compounds that contact the ventral surface. (There is evidence that tannins are toxic to bryophytes, not within their cell walls, but when they are able to act on cell constituents.) Since all these ventral surfaces are wettable, these cells are more susceptible to damage by such toxins.

**Thallose Liverworts**

The structure of most thallose liverworts is so different from that of mosses or leafy liverworts that their water relations warrant separate consideration. They are adapted for predominantly ventral uptake. Thallose liverworts like *Conocephalum conicum* (Figure 19) and *Cyathodium cavernarum* (Figure 20) use ventral appendages (*scales*; Figure 19) to provide capillary spaces that conduct water externally on the underside of the thallus. Marchantialian species use specialized capillary systems on the ventral surface of the thallus to conduct water in either direction. Cell walls of the scales contain *tannins* (McConaha 1939), perhaps acting as an antibiotic.

**Figure 19.** *Conocephalum conicum* showing ventral scale (purple) that provides capillary spaces for external water movement. Photo by Ralf Wagner at <www.dr-ralf-wagner.de>, with permission.

**Figure 20.** Scales of *Cyathodium cavernarum*. Photo courtesy of Noris Salazar Allen.

Kürschner (2004) surmised that the anthocyanin pigments in ventral scales protected the ventral surface from the sun when the thallus was rolled up, as it typically does in many species of *Riccia*. Furthermore, even hyaline scales of *Riccia* and *Oxymitra* (Figure 21) can reduce desiccation. Some species, especially of *Riccia*, sink into the soil surface as the soil dries, reappearing only after precipitation or heavy dew.
This high degree of wettability provides a greater possibility for water entry in thalllose forms. For example, in *Marchantia* (Figure 22-Figure 23), water is conducted along the midrib as well as in interstitial spaces between the blade and scales, distributing water throughout the surface of the thallus. Water movement in *Marchantia* is relatively slow, at 0.4 mm per sec, improving slightly in *Lunularia* (Figure 44-Figure 45) and *Reboulia* (Figure 4), to approximately 0.5 mm per sec, despite their less highly developed capillary systems. However, McConaha (1939) found that movement from base to apex in *Conocephalum conicum* (Figure 24) generally takes only about 20-30 seconds, roughly 1 mm per sec. Despite its slowness, McConaha found this external movement to be much faster than would be possible by internal conduction.

**Ballooning of Epidermis**

In some species of *Riccia* the epidermis is balloon-like and may contribute to protection from desiccation (Kürschner 2004). However, I wonder if those cells don't provide a greater role in focussing the light into the thallus to the chlorophyllous cells when the thallus is hydrated. Kürschner suggested that the chimney-like, hyaline air chambers of *Exormotheca* (Figure 25-Figure 27) may also have a function in protecting the underlying tissue during dehydration.
Rhizoids

Ventral structures seem to be important in this group. In marchantialian liverworts, two types of rhizoids (Figure 29-Figure 28) provide somewhat different functions. The smooth-walled rhizoids (Figure 28) are alive (Duckett & Ligrone 2003) and emerge from beneath the ventral scales (Figure 30), providing contact with the substrate, whereas the tuberculate (pegged) rhizoids (Figure 28) are dead (Duckett & Ligrone 2003) and form a capillary system parallel to the thallus beneath each scale (McConaha 1941). The pegged rhizoids begin growth at right angles to the thallus but change their orientation to follow that of the scales. The pegs, extending into the pegged rhizoid cell, prevent the collapse of the cell when dehydrated, thus maintaining its capillary role (Duckett & Ligrone 2003). When the archegoniophore (stalk supporting female reproductive organs) forms, the pegged rhizoids are wrapped within the archegoniophore by the folded thallus (Figure 31) and function in internal water conduction (Duckett & Ligrone 2003). The presence of the pegs also prevents the collapse of this stalk when the thalli dehydrate (Duckett & Ligrone 2003). These rhizoids have an outer layer of pectic material (like apples).
Previously treated mostly as a taxonomic anomaly, it appears that these two types of rhizoids have distinctly different functions (Duckett et al. 2013). The smooth rhizoids are alive, functioning in nutrition, anchorage, and as entry locations and conduits for fungal symbionts. Their role for fungal entry does indeed require that these rhizoids be alive. The pegged rhizoids, on the other hand, are dead at maturity, a condition first noted by Kamerling (1897), and function as water conduits through these empty tubes. Kny (1890) was the first to suggest that pegs prevent the rhizoid walls from collapse with water loss. Duckett et al. (2013), however, were the first to test this hypothesis. The pegs, along with elasticity, seem to provide the ability of the rhizoids to maintain their functional integrity by preventing their collapse when they are dry. This ability is essential to their function in conduction, a role demonstrated by Bowen (1935) and others (McConaha 1939, 1941), who used dyes to show conduction by capillarity and transpiration. The famous German morphologist Goebel (1905) observed that it is the liverworts with high transpiration rates that also have the most highly developed pegged rhizoids. This is in contrast with those of hygrophilous (water-loving) taxa such as Dumortiera (Figure 32-Figure 33) and Cyathodium (Figure 34).

Figure 32. Dumortiera hirsuta, a hygrophilous species showing fringe of hairs on thallus and archegonial heads, but lacking the pegged rhizoids and scales of the dry habitat species. Photo by Jan-Peter Frahm, with permission.

Figure 33. Dumortiera hirsuta thallus showing rhizoids on the ventral surface. Photo by Chris Lobban, with permission.

Among the leafy liverworts, thallose liverworts, and ferns, living rhizoids die and collapse upon dehydration, an irreversible response (Pressel 2007). Duckett et al. (2013) concluded that smooth rhizoids of liverworts grow at the apex, a character they share with root hairs, fungal hyphae, and moss protonemata. Furthermore, the smooth rhizoids exhibit considerable endoreduplication of Golgi bodies similar to that in moss caulonemata. They reach lengths that commonly are greater than 20 mm, sometimes reaching 30 mm in Marchantia polymorpha (Figure 23), making them the longest cells in liverworts. Duckett and coworkers suggest that this combination of characters may explain their inability to regenerate when damaged (Pressel et al. 2008a; Duckett et al. 2013).

Figure 34. Cyathodium tuberosum, a simple thallus lacking the complex scales and two types of rhizoids. Photo by Jan-Peter Frahm, with permission.

On the other hand, rhizoids of mosses (and protonemata) can recover from desiccation except at the apical cell (Pressel 2007; Rowntree et al. 2007; Pressel et al. 2008b). The pegged rhizoids furthermore are devoid of air bubbles when desiccated, a further indication that they are highly resistant to cavitation (formation of a space; collapse of cells), as are the hydroids of mosses (Ligrone et al. 2000).

Duckett et al. (2013) identified pegged rhizoids in 26 species of thallose liverworts and absence in 5. They compared the diameters of the smooth and pegged rhizoids and measured the time required for dyes to reach the archegonial heads in the taxa compared to time required in several mosses. The rates ranged 30-150 mm h$^{-1}$ in the thallose liverworts, 28-14 mm h$^{-1}$ in mosses, and 127-141 mm h$^{-1}$ in ferns.

Duckett et al. (2013) point out that these pegged rhizoids fulfill the three criteria for conduction defined by Raven (1993): dead at maturity, specialized walls, preferential conduction of water. Duckett and coworkers added a fourth criterion, the ability to maintain functional integrity through periods of dehydration, as in moss hydroids (Ligrone et al. 2000). This maintenance of functional integrity becomes a problem, because unlike trees and stems, there is no adjacent cell to help in rehydration.

Xerophytic liverworts such as Riccia (Figure 35), Reboulia (Figure 4), Targionia (Figure 36), Asterella (Figure 37), and Lunularia (Figure 44-Figure 45) have both tuberculate (pegged) and smooth rhizoids, scale
leaves, and well-defined assimilatory and storage zones, whereas moisture-loving *Dumortiera* (Figure 32), *Cyathodium* (Figure 34), *Pallavicinia* (Figure 38) (Daniels 1998), *Monoclea* (Figure 39), *Neohodgsonia* (Figure 40), and some aquatic *Riccia* species (Figure 41-Figure 42) (Duckett & Ligrone 2003) lack these complex structures. Even in *Marchantia* (Figure 22-Figure 23), with its strong midrib, water moves externally along the midrib and in the spaces between the scales, providing a film of water throughout the thallus (McConaha 1941).

Figure 35. *Riccia sorocarpa* thallus section showing ventral rhizoids, dorsal midribs, and internal photosynthetic layer. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 36. *Targionia hypophylla*, a thallose liverwort with pegged and smooth rhizoids and scales. Photo by Des Callaghan, with permission.

Figure 37. *Asterella saccata* showing scales around the thallus margin. Photo by Jan-Peter Frahm, with permission.

Figure 38. *Pallavicinia lyellii*, a simple thallus lacking the complex scales and two types of rhizoids. Photo by Des Callaghan, with permission.

Figure 39. *Monoclea* cf. *gottschei* with the salamander *Oedipina gracilis*. This liverwort is a simple thallus lacking the complex scales and two types of rhizoids, but clearly having a waxy surface. Photo by William Leonard, with permission.

Figure 40. *Neohodgsonia mirabilis* with archegonial heads in New Zealand, a simple thallus lacking the complex scales and two types of rhizoids. Photo by Odontites, through Creative Commons.
Chapter 7-3: Water Relations: Plant Strategies

Fungal Partners

Despite their roles in anchorage (smooth rhizoids) and conduction (pegged rhizoids), it seems that smooth rhizoids have a major role in the endosymbiosis (internal partnership) of fungi (Pressel et al. 2010, 2012; Duckett et al. 2013). Our understanding of the value of this partnership is meager. In other plants, fungi serve to increase absorptive surface area and often tap into the roots of a tracheophyte, transferring carbohydrates from plants that reach the canopy to plants that are in the low light beneath them. Such a role remains unknown in the bryophytes, but I am confident that we shall discover that at least some species have this advantage. After all, these partners have been around much longer than the flowering plants, known as hemiparasites, that have succeeded in developing this life style. In the leafy liverworts, infections occur exclusively through rhizoids with the Ascomycete Rhizoscyphus ericae as partner (Read et al. 2000; Pressel et al. 2008a, c). This is a widespread fungus that lacks host specificity. Fungal partnerships are discussed in more detail below under "Mosses and Leafy Liverworts."

Main Thallus Structure

Midribs: Seeing the midribs in valleys (Figure 35, Figure 43) makes me think that water is directed from the thallus surface to the midrib in some species. I have to wonder if water is absorbed more easily there. Once the water enters the midrib cells, it can be carried to more distant parts of the thallus more quickly than by travelling through other, shorter thallus cells.

The rib of Lunularia cruciata (Figure 44-Figure 45) has parenchymatous cells in which plasmodesma-derived pores are grouped in small, sparse fields, particularly on transverse oblique walls (Giordano et al. 1989). This suggests that the rib cells may be able to transport water and other substances through the ends of cells. These longer cells should, then, move water faster than crossing the many walls of the hyaline parenchyma (Figure 46). Giordano and coworkers suggested that the reticulate cells may serve a water-holding role and facilitate lateral distribution by both symplastic (within protoplasm) and apoplastic (outside the protoplasm, in intercellular spaces) conduction of substances arriving by way of the rib. They suggest this mechanism may be present in all members of Marchantiales with this thallus construction.

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Rolling Thallus: Midribs may also facilitate rolling of the thallus by creating a crease through the middle of the plant (compare Figure 43 to Figure 47). Rolling conserves water, but at the same time it exposes the ventral surface where scales help to conserve water (Figure 47). Such rolling is common in species of *Riccia* (Figure 69-Figure 70), often supplemented with hairs that cover the thallus.

Internal Conduction: The liverwort *Lunularia cruciata* (Figure 44-Figure 45) may exemplify a means for water movement between cells in thalloid liverworts (Giordan et al. 1989). The thallus has reticulate (like network) hyaline (colorless) parenchyma (thin-walled) cells (Figure 45) with between wall thickenings, large primary pit fields (thin area in walls of many cells in which one or more pits usually develop) with numerous pores derived from plasmodesmata (narrow threads of cytoplasm that pass through cell walls of adjacent cells and allow communication between cells) on unthickened areas of walls.

**Spongy Thallus**

The spongy thallus of *Riccia cavernosa* looks like it should have an important adaptive value. The basal layer gives rise to a layer of irregular vertical column of chlorophyllseous cells overtopped by colorless epidermal cells (*Riccia cavernosa* 2012). While these might seem to have functions similar to those of *Exormotheca*, instead some of these epidermal and chlorophyllseous cells collapse, creating large air spaces at several levels in the tissue of the thallus. The light that reflects from these cavities has a glistening appearance. But does this sequence of events provide any advantage to the plant? At first glance, it looks very much like a sponge, but at a much smaller scale.

The basal pad of isodiametric cells gives rise to a layer of irregular vertical columns of chloroplast containing cells, topped by colorless oval epidermal cells (*Riccia cavernosa* 2012). But early in development, large air spaces develop at several levels in this tissue due to the collapse of some of the epidermal and chlorenchyma cells. The light reflecting inside these cavities gives the thallus a characteristic glistening appearance.

**Cuticle**

For many years we considered the cuticle to be absent in bryophytes, with scattered references referring to them, but with no definitive data on their surface chemistry. However, not only are waxy cuticles present, but waxes are present on the leaves and thalli, albeit in less noticeable quantity than in tracheophytes. In fact, Brockington et al. (2013) considered the cuticle in the thallose liverwort *Marchantia polymorpha* (Figure 22) to be "an overlooked innovation in land plants."

Knowing that *Marchantia polymorpha* has a cuticle, one should not be surprised that the shiny thallose liverwort *Monoclea gottschei* (Figure 39) and the hornwort *Notothylas orbicularis* (Figure 48) have an osmiophilic layer (refers to lipid-containing bodies; a cuticle) with structural resemblance to that of early developmental stage tracheophyte cuticles (Cook & Graham 1998).
What emerged as more interesting in this study is that not all of these bryophyte versions are created equal. In the liverwort *Monoclea gottschei* (Figure 39) the layer is nodular, in the hornwort *Notothylas orbicularis* (Figure 48) it can be either nodular or sheetlike, and in *Sphagnum fimbriatum* (Figure 49) it is sheetlike with regular ridges that run parallel to the edges of the "thalli" (Cook & Graham 1998). It appears that cuticle is ancient, and Cook and Graham suggest that it may have arisen before the charophycean algae and bryophytes diverged from their common ancestor.

The thallose liverwort *Plagiochasma rupestre* (Figure 60) has a non-wettable thallus endowed with hydrophobic wax globules, preventing it from absorbing water through its surface (Kürschner 2004). Instead, it uses the pegged rhizoids for water uptake, a phenomenon that may be common to all members of Marchantiaceae. This dorsal surface wax may have an important role in preventing water logging in the underlying air chambers.

The presence of a cuticle on the upper surface of a thallose liverwort raises the question of water absorption in these species. One might surmise that it occurs through the ventral surface, facilitated by the scales and rhizoids. Presumably there is no cuticle on that surface, but that does not yet seem to have been demonstrated.

In the Polytrichaceae, waxes may serve a different function. Rather than keeping water in, the leaves of these species must keep water out to permit maximum photosynthesis (Figure 50) (Clayton-Greene et al. 1985). Their lamellae provide extra photosynthetic tissue, but water can become trapped there due to the capillary spaces. This protection from water is further enhanced by the rolled margins (Figure 51) of species like *Polytrichum juniperinum* (Figure 52).

**Pores**

The wonderful ventral efficiency of rhizoids and scales is often challenged by a dorsal surface that does little to conserve water. In fact, this dorsal surface water loss may facilitate the movement of water and nutrients through the plant, as it does in leafy tracheophytes. The pores on the dorsal surface function much as do the stomata of tracheophyte leaves in losing water (Figure 46, Figure 58). Maier-Maercker (1982) found that *Conocephalum conicum* (Figure 24) loses water through transpiration from these dorsal thallus pores (Figure 53), accumulating radioactively labelled ions in the cells surrounding the air pores.

The single-layered leaves of mosses and leafy liverworts preclude the presence of stomata there as they would provide only a hole through the leaf, hardly a useful character. Even multicellular layers of moss leaves have no use for stomata because there is no chamber where the gases may gather. But thallose liverworts meet those two requisites – multiple cell layers (Figure 54) and chambers internally (Figure 55). Furthermore, as mentioned above for *Marchantia polymorpha* (Figure 22), the thallus has a cuticle that can at least to some degree repel water. Hence we might presume that it likewise is somewhat resistant to gas exchange, creating a problem for photosynthesis.
Raven (2002) provided evidence that stomata evolved from pores of an epidermis over plant organs at least three cell layers thick, with intercellular gas chambers, and with a cuticle. In this anatomical arrangement, the presence of pores most likely confers an adaptive advantage for photosynthesis.

In some cases, thalloid liverworts seem to lose great quantities of water, 2-3 times that of leaves of the weeping birch tree *Betula pendula* (McConaha 1941). Under extreme conditions, they transpire equivalent to their total content of water in an hour. One reason for this rapid transpiration rate is the areolation of the thallus that creates a large surface area where water can be lost. The pores in these thalli (Figure 56), permitting contact between outside air and internal moisture, have only limited ability to close, thus being a major source of water loss. McConaha (1941) claims that the ventral specializations compensate for the losses from dorsal areolation and pores. Proctor (1980) found that these areolate thalli have internal resistances similar to those of mesophytic leaves of flowering plants (Proctor 1980). As in the flowering plants, the water loss is correlated with pore size and density.

But the photosynthetic cells project into these chambers beneath the pores (Figure 55), and flooded cells cannot exchange gases freely. It should be no surprise then that the openings themselves prevent the entrance of water into the chamber. Their small size (Figure 56) contributes to this. In the absence of a wetting agent, the cohesive forces of water make the aggregation of water molecules too large to enter the holes. This smallness of the hole is further enhanced by the presence of a cuticular ridge (Figure 56-Figure 59) that not only narrows the entrance, but that also repels the water.
Schönherr and Ziegler (1975) found that of the fourteen thallose liverwort species they studied, twelve of them have such hydrophobic (repelling water) ridges, and that cutin is present in these ridges. Furthermore, the researchers considered the pores in these species to be "perfect" in keeping water out of the thallus. **Plagiochasma rupestre** (Figure 60) and *P. peruvianum*, on the other hand, lack such ridges and liquids are able to enter the thallus through the pores. While the waxes and small size of the holes keep water out, the water in vapor form within the thallus is able to escape through the pores, along with oxygen, while CO₂ enters. Therefore, the openings must maximize carbon gain per unit water loss (Raven 2002).

While these pores are an advantage for a hydrated, photosynthesizing thallus, they are a liability for a drying thallus due to the loss of water vapor. But at least some of the liverworts seem to be able to partially control the opening. This is accomplished by curving of the stack of cells surrounding the opening, creating partial closure. In *Preissia* (Figure 61—Figure 62, Figure 30), the barrel-shaped pores (Figure 62) change shape to accomplish control of water loss (Lepp 2008). When turgid with water, the cells at the bottom of the barrel keep the pore open, but when the conditions are dry, the cells lose their turgor and collapse, narrowing the opening at the bottom of this barrel. Those in *Marchantia* (Figure 55—Figure 57) behave similarly (Raven et al. 2005).

**Archegoniophores and Antheridiophores**

In thallose liverworts, the horizontal orientation cannot serve as a model for water movement in the vertical archegoniophore. As determined by Duckett et al. (2013), the archegoniophore has more efficient water movement than the stems of mosses. They attribute this to the efficiency of movement through the rhizoids and capillary

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Figure 58. Pore opening in thallus of *Cyathodium cavernarum*. Photo courtesy of Noris Salazar Allen.

Figure 59. *Conocephalum conicum* pore longitudinal section showing the cuticular ridge. Photo by Ralf Wagner at <www.dr-ralf-wagner.de>, with permission.

Figure 60. *Plagiochasma rupestre*, a thallose liverwort with no cuticular ridge on its pores, but with a waxy cuticle on the thallus. Photo by Michael Lüth, with permission.

Figure 61. *Preissia quadrata* thallus wowing pores (light-colored dots). Photo by Jan-Peter Frahm, with permission.

Figure 62. *Preissia quadrata* thallus showing pores with cuticular ridges. Photo by Kristian Peters, with permission.
spaces among them, as well as the hydrophobic nature of the thallus surface surrounding these rhizoids (Figure 63).

Figure 63. *Marchantia polymorpha* antherial head showing location of rhizoids within the antheridiophore. Photo from Botany website of the University of British Columbia, BC, Canada, with permission.

The importance of this rhizoid-thallus combination for the archegoniophore is suggested by comparing its response to drought with that in mosses. For example, *Marchantia* (Figure 64) archegoniophores and heads can remain fully hydrated for several hours in full sunlight at 20-30°C while the shoots of neighboring *Polytrichum commune* (Figure 65) with their bases in standing water become wilted and must have added rainwater to recover (Duckett et al. 2013), suggesting an efficient system of transport in the archegoniophore.

Figure 64. *Marchantia polymorpha* archegoniophore and archegonial head. Note rhizoids along stalk (archegoniophore) where they emerge from the folded thallus that makes the stalk. Photo by George Shepherd, through Creative Commons.

Figure 65. *Polytrichum commune*, a moss that wilts in full sun despite its wet substrate and colonial habit. Photo by Michael Lüth, with permission.

Duckett et al. (2013) suggest that the length of the archegoniophore is limited to a maximum of 7-10 cm (in *Conocephalum*; Figure 66) because of the problems of air bubbles in the mucilaginous matrix surrounding the pegged rhizoids in the grooves of the archegoniophore, a condition analogous to an embolism in the vessels of tracheophytes (see Canny 2001 a, b).

Figure 66. *Conocephalum conicum* with tall archegoniophore. Photo by Adolf Ceska, with permission.

Antheridiophores provide yet a different mechanism (Duckett & Pressel 2009). Present only in the genus *Marchantia*, they present an antherial head (Figure 67) on a stalk that is much shorter than that of the mature archegoniophore, rarely exceeding 30 mm. Rather than being hydrophobic, the heads are highly hydrophilic and absorb raindrops much like a sponge. Whereas upward flow occurs in the stalk during dry periods, downward flow carries the motile sperm toward the archegonia on immature (shorter) archegoniophores (Figure 68) during rainfall.

Figure 67. *Marchantia polymorpha* antherial head showing location of rhizoids within the antheridiophore. Photo from Botany website of the University of British Columbia, BC, Canada, with permission.
Figure 67. *Marchantia polymorpha* with antheridial heads where water is absorbed like a sponge. Photo by Rudolf Macek, with permission.

Figure 68. *Marchantia polymorpha* young archegoniophores that receive sperm from temporarily taller antheridiophores. Photo by Rudolf Macek, with permission.

**Dormancy**

Volk (1984) found that when *Riccia* (Figure 69-Figure 70) has less than 150 mm of rainfall per year, it requires other means to survive, and it seems that dehydration/dormancy is the solution (Figure 69-Figure 70). Some thallose *Riccia* species are able to survive up to 7 years in this dehydrated state, enduring temperatures up to 80°C. The annual species compensate for this water loss by producing huge numbers of spores, taking advantage of their ornamentation for distribution by animals.

Figure 69. *Riccia sorocarpa* in a fresh, active state. <www.aphotofauna.com>, with permission.

Figure 70. *Riccia sorocarpa* in a dry, dormant state. Photo by Michael Lüth, with permission.

**Mosses and Leafy Liverworts**

We typically think first about structural adaptations for water retention, so we will start there. Sarafis (1971) considered that *Polytrichum commune* (Figure 65) had four ways of controlling water loss:

1. Community level – gregariousness
2. Plant level – leaf density & size, plant height
3. Organ level – leaf movement and inrolling
4. Molecular level – wax on leaf surface

These all relate to structure, but internal structure and cellular level physiology are additionally important.

Bayfield (1973) considered that water loss in endohydric *Polytrichum commune* to be controlled by its leaf arrangement changes. This was accompanied by changes in water potential deficit of the shoots, with conduction being primarily internal under high evaporative flux and external under moderate flux. The ectohydric *Racomitrium lanuginosum*, by contrast, has little control over its water loss.

After examining 439 taxa of pleurocarpous mosses, Hedenäs (2001) reported that most differences in taxonomic character states between environments relate to two functions: 1) water conduction and retention; 2) dispersal. Those characters that seem important for water relations relate to stem central strand, leaf orientation, leaf costa type, alar cells, paraphyllia, and pseudoparaphyllia. But if acrocarpous mosses (upright mosses with terminal sporophytes) had been included, surely many more characters might be added, as it is mostly acrocarpous mosses that occupy the most xeric of habitats.

One feature of structural adaptations is that many are plastic (Buryová & Shaw 2005). For example, conducting strands disappear in the liverworts *Moerckia flotoviana* (Figure 71) and *Haplomitrium hookeri* (Figure 72-Figure 73) under high humidity or liquid culture (Hébant 1977). Hair points (colorless, hairlike extensions at leaf tip) of *Schistidium apocarpum* (Figure 75) likewise disappear in humid conditions (Figure 75).
Rhizoids are less well developed or absent in wet conditions (Smith 1988), even in the same species. In *Andreaea blyttii* (Figure 76), increased moisture results in longer, wider leaves that are more curved with longer cells in the basal margin, wider *costae* (midrib of leaf), and longer stems, but with a decrease in number of leaves per stem (Heegaard 1997). Even in typically aquatic taxa such as *Drepanocladus* (*sensu lato*), leaves become longer, and falcation (leaf curvature) is lost in submersed leaves (Figure 77) compared to those grown out of water (Figure 78), and the reduced light results in greater internode distances (distance between leaf insertions) (Lodge 1959). A similar response is seen in *Fontinalis* (Figure 79). It is interesting that increases in salt concentration increase cell length in this genus. Plasticity itself is an important adaptation.
Proctor (2010) reminds us that adaptations are subject to phylogenetic constraints and that entire clades may represent adaptations to desiccation. All plants must obtain water and CO$_2$, but their multiple ways that this can be achieved.

**Growth Form**

Growth form is important both for obtaining and retaining water. For example, *Grimmia pulvinata* (Figure 80) forms cushions. In this moss, and most likely others with this growth form, size matters. As the clump grows larger, the surface to volume ratio decreases as the larger cushion is accompanied by greater height in the center. This reduces exposed area for gas exchange, but it also reduces the portion exposed to the atmosphere for water loss. In *Grimmia pulvinata* the larger cushions have lower area-based evapotranspiration rates due to a higher boundary-layer resistance, but the relative water storage capacity per dry weight does not change (Zotz *et al.* 2000). Consequently, the hydrated period is considerably longer in larger cushions. And as predicted, the CO$_2$ exchange rate decreases with increasing size of the cushion, with both net photosynthesis and dark respiration decreasing.

Living in clumps affects the ability to gain and to retain moisture. The cushion growth form decreases the surface to volume ratio, thus reducing surface water loss. Clump shape is important in this relationship, particularly in ameliorating wind effects (Proctor 1981; Zotz *et al.* 2000; Rice *et al.* 2001; Rice & Schneider 2004; Rice 2012). But for this system to work, the surface must be as smooth as possible. Greater roughness, resulting from protruding shoots, increases the turbulent air flow, thus increasing evaporation (Proctor 1981; Rice & Schneider 2004). A consequent advantage to the cushion growth form and its retention of water is the slowing the drying rate (Sand-Jensen & Hammer 2012).

Fortunately, this is a self-regulating condition. As a shoot emerges from the surface, the greater exposure and greater evaporation cause its growth to attenuate. Even herbivores might contribute to this evening, choosing the protruding branch because it is easier to munch on. Hence, the surrounding mosses are able to catch up in length, returning the clump to its smooth structure. Thus, moisture limitations create a more matted clump with a smoother surface, limiting turbulent flow and wind penetration into the clump (Longton 1979; Guerra *et al.* 1992; Nakatsubo 1994).

Nakatsubo (1994) examined the importance of the growth form of sub-alpine mosses in controlling their evaporative water loss. The xerophytic species were comprised of large cushions and compact mats. Mesophytic species from the coniferous forest floor were represented by smooth mats, wefts, and tall turfs. The evaporation rate per dry weight was much less in the xerophytic species than in the mesophytic species. However, when compared on a basal area, the evaporation rates were similar. One advantage of the xerophytic species was their ability to increase weight per basal area without increasing roughness.
The density of the clumps seem to be a plastic character that can be modified by the environment (Gimingham & Birse 1957; LaFarge-England 1996; Bates 1998; Rossi et al. 2001). For example, the endohydric *Polytrichum juniperinum* var. *alpestre* (Figure 81), when in humid habitats, forms a looser clump structure and greater roughness than when in drier habitats (Birse 1957).

Figure 81. *Polytrichum juniperinum* var. *alpestre* showing contacting leaves of adjoining shoots. Photo by Des Callaghan, with permission.

Elumeeva et al. (2011) set out to determine the important characters that maintained moisture in sub-Arctic bryophytes. They found that individual shoot properties seemed to have little effect on colony water retention capacity. That is, leaf cell wall properties, water retention capacity, and desiccation rate of shoots made little difference in the water relations of the colony. Rather, the colony desiccation rate was determined by the density of the water-saturated colony. The desiccation rate of the individual shoot had a marginally significant negative effect on the colony rate.

In *Polytrichastrum formosum*, the plants are relatively tall and arranged in loose clumps. Nevertheless, the leaves of adjoining shoots touch, permitting water drops to be trapped by the resulting web. Drop size is an important consideration in conducting experiments using artificial rain. Raindrops usually range 0.5 mm (light rain) to 5 mm (heavy rain) (Best 1950; Brandt 1989; Yakubu et al. 2016). Using this size range, Zającowska et al. (2016) determined that clumps of *P. formosum* were able to retain almost 60% of the applied water. When water was applied to the tips, water ran down the shoots and continued to run down until about 2 minutes after the water application ceased. When a drop lands on a leaf, it is more likely to be trapped by the leaf axil or by a leaf. Thus these clumps are benefitted by the catchment web formed by the overlapping leaves.

With these clump advantages, we might ask why so many mosses use other growth forms that are less compact. But moisture is not the only need for the mosses. A compact nature reduces light penetration, reduces CO₂ diffusion into the clump, and increases shoot-to-shoot competition for nutrients (Bates 1989; Rice 2012).

Mulder et al. (2001) explored the role of species richness on biomass, then compared it when these communities were exposed to experimental drought. They found that under drought conditions biomass increased with greater species richness. They determined that the interaction was facilitative rather than niche complementarity or sampling effects. Survivorship increased for almost all species as richness increased, with the least drought-resistant species receiving the most benefit in biomass. Rixen and Mulder (2005) found similar results in the Arctic tundra.

**Stems and Branches**

Most stem and branch arrangements relate to growth form or life form (see Chapter 4-5 of this volume). However, in some cases there is internal or structural modification, exemplifying the plasticity of some bryophytes. For example, *Philonotis fontana* (Figure 82-Figure 83) exhibits variation among populations in leaf dimensions, whereas their cell dimensions show little response to differences in water regime or light level (Buryová & Shaw 2005).

Figure 82. *Philonotis fontana*, a species whose leaf dimensions vary with habitat. Photo by Des Callaghan, with permission.

Figure 83. *Philonotis fontana* leaf lamina showing cells. These cells vary little in dimensions in different water or light regimes. Photo by Kristian Peters, through Creative Commons.

Overlapping leaves from neighboring shoots helps in the support of the mosses in a clump. In stems, the alternating layers of stiff and soft structures, such as those of the *Polytrichacea*, the strength benefits from the periodic component materials (Vincent 2012) that occur...
in many biological structures and provide stronger mechanical features (Dunlop et al. 2011; Fratzl et al. 2016). This layering provides the stem with both supportive strength and flexibility that prevents breakage. In Polytrichastrum and other members of the Polytrichaceae, the thicker cell walls surrounding the stem provide a higher bending strength (Niklas 1992), much like a paper straw compared with a paper lollipop stick. We also know that a lollipop stick made with twisted paper threads is stronger than a solid, non-twisted one, and that many trees likewise gain strength this way. This possibility needs to be explored in bryophytes.

Schröder (1886) considered the ability of moss stems to resprout from a dormant stem to be one method for withstanding prolonged drought. I had a similar experience with the aquatic moss Fontinalis dalecarlica (Figure 84). In this case, the moss was boiled for 14 hours a day for two weeks in the lab, then returned to the stream. One year later, new growth was present on this moss that was still attached to the numbered rock used in the boiling treatment. Such ability of stem tips to recover from environmental stresses have been largely overlooked.

Figure 84. Fontinalis dalecarlica with from Fox Run, NH, where a plant similar to this produced a green leaf one year after being boiled for 14 hours a day for two weeks. Photo by Janice Glime.

Sphagnum: Li and coworkers (1992) examined the responses of two closely related Sphagnum hummock species, S. magellanicum (Figure 85) and S. papillosum (Figure 86), to distance from water surface and related these responses to structural and physiological adaptations of the two species. They found that both species increase growth in length as water becomes more available, i.e. as the distance from water level decreases. Likewise, dry mass is maximal under wet conditions, with new branches being a major mass contributor, especially in S. papillosum. Furthermore, while experimenting with effects of distance from water on S. magellanicum and S. papillosum, Li and coworkers found that dry conditions result in wider stems (Figure 87), with thicker hyaline layers (Figure 88-Figure 89), than stems with apical capitula near the water surface (Figure 90), presumably increasing both absorption and water-holding ability.

Sphagnum has pores in its stem (Figure 90), in most species, and has very rapid movement of water externally up the stem by capillary action, adapting it for its annual cycle of being stranded well above water level. Some species of Sphagnum have special retort cells (Figure 91) on the stems for absorbing water (Figure 91).

Figure 85. Sphagnum magellanicum, a hummock species with efficient water movement. Photo by Michael Lüth, with permission.

Figure 86. Sphagnum papillosum, a species with inefficient water movement. Photo by David Holyoak, with permission.

Figure 87. Effect of water level (water availability) on stem diameter in Sphagnum magellanicum, a more desiccation-resistant species, and S. papillosum, a more desiccation-tolerant species. Wet denotes 0 cm initial distance of capitulum from water; dry denotes 10 cm initial distance. Bars represent standard errors; stem diameter in dry treatment is significantly greater (Figure 88) in both species. From Li et al. 1992.
Daniels (1989) found that while there is little differentiation between spreading and pendant branches (Figure 92–Figure 93) among Sphagnum plants growing in pools, hummock plants have more closely spaced fascicles (groups of branches), comparatively short spreading branches, and thin, closely appressed pendant branches (Figure 93). Pendant branches help to preserve stem water and maintain the wick effect as water level drops. Daniels determined that leaves of pendant branches on submerged plants photosynthesize actively, while those of hummock plants do not. He found that the two species growing in wet hollows (Sphagnum cuspidatum) or as wet carpets (Sphagnum recurvum) had the highest percentage of unbranched stems. The low hummock species Sphagnum papillosum, on the other hand, had up to six capitula (terminal clump of branches) per stem; the two species growing in the high-humidity, shaded wet woodland exhibited intermediate degrees of branching.

Sphagnum magellanicum has greater ability to move and hold water than does S. papillosum (Li et al. 1992). Therefore, when they grow together in the same hummock, S. magellanicum will not only stay wet longer, but if it is dominant it will keep S. papillosum wet (Figure 94). However, it will fail to do so if S. papillosum is dominant (Figure 94). This is further supported by lab experiments in which S. magellanicum moved water farther externally in 20 hours than did S. papillosum (Figure 95; Figure 96).
Figure 93. Spreading branches and pendant branches on two hummock Sphagnum species. **Left:** S. magellanicum. **Right:** S. papillosum. Photos courtesy of Yenhung Li.

Figure 94. Predominately Sphagnum papillosum (olive colored) lower on the hummock (left side of picture) causes both species to be dry, whereas predominately S. magellanicum (red) higher on the hummock (right side of picture) causes both species to be wet. Photo by Janice Glime.

Figure 95. Comparison of distance travelled by dye in two Sphagnum species from lower (S. papillosum) and higher (S. magellanicum) in the hummock after 20 hours. Group refers to those kept together at field density with half of each species. From Li et al. 1992.

**Central Strand**

In addition to the structural adaptations of stems and branches already described, the vascular system itself may be modified. The central strand (Figure 97) is typically composed of hydroids that are elongated and impose fewer cell end walls through which water must travel. Hébant (1973) found that variation occurred in the vascular elements, particularly in length and diameter, degree of inclination of end walls, and structure of the walls themselves. For example, whereas walls of hydroids are usually thin, they can be very thick, as in the swollen walls of hydroids in the setae of Dicranum scoparium (Figure 98) or the lateral walls of hydroids in the gametophyte central strand of the Polytrichales (Figure 99). But insufficient data exist to relate these variations to adaptive function.

Figure 96. Comparison of upward transport in a low (left) and high (right) hummock species of Sphagnum. Movement of water is indicated by purple dye. Photo courtesy of Yenhung Li.

Figure 97. Stem cross section of Rhizogonium showing narrow cells of central strand. Photo courtesy of Isawo Kawai.

Using several references for comparison, Hébant (1977) showed that the number of hydroids within the Polytrichum commune central strand (Figure 99) can vary with habitat, following an apparent moisture gradient. In a pseudo-alpine grassland he reports 900 hydroids in the central strand, peat bog 400, cultivated in artificial peat 280, and cultivated under water 70. There is no clear indication as to how these numbers affect the rate of conduction, but one would presume that more hydroids conduct more water.
Although in general, leafy liverworts lack conducting tissues in both leaves and stems (Crandall-Stotler 2014), *Haplomitrium* seems to be an exception. At least it possesses a differentiated central strand (Figure 100). But there seem to be no experiments to demonstrate that this actually serves as conducting tissue.

**Rhizoids and Tomentum**

Rhizoids and tomentum (layer of matted woolly down on surface of plant; Figure 101) are adapted for water uptake. Pressel and Duckett (2011) found that rhizoids of all representatives they tested in *Polytrichales, Dicranales,* and *Bryales* (Figure 102) were hydrophilic (tendency to be wetted by water). For example, there is a sharp contrast between the leaves with a waxy cuticle in *Bartramiaceae* (Figure 103) and the highly hydrophilic tomentum-forming rhizoids (Figure 103) with papillae.

Mosses with dense rhizoids or tomentum (Figure 101-Figure 103) seem to be well equipped to retain and conduct water by capillary action. Smith (1988) found that *Bryum pseudotriquetrum* (= *Bryum algens*; Figure 102), with a dense rhizoidal tomentum (Figure 102), held significantly more water than colonies with sparse rhizoids. But the tomentose form lost water more rapidly per unit dry mass than did the ones with sparse rhizoids. Could this be attributed mostly to loss of water from the tomentum? In *Schistidium antarcticum* (Figure 104), the xeric form has less densely packed shoots and thicker cell walls that maintain lower water content than the high-water-holding-capacity hydric turf form. Mosses in Smith’s study took several times longer to drop to minimal water contents than did lichens in the same conditions.
In acrocarpous mosses, rhizoids are produced all the way around the base of the stem, serving on the lower parts for anchorage, and in mosses like the *Polytrichaceae*, for limited conduction (Odu 1978). Rhizoids further up the stem provide capillary spaces that can both store water and facilitate movement. In pleurocarpous mosses, rhizoids appear only on the side of the stem (Figure 105) toward the substrate (Odu 1978), except in the case of those in flowing water (Glime 1987). In *Fontinalis* (Figure 106), where rhizoids have a critical function in anchorage, and this aquatic moss may encounter its substrate in any direction from the stem, the individual rhizoids grow in a spiral (Figure 107) until they encounter the substrate, then form multiple branches (Figure 108) in a small space and cement themselves to the substrate, presumably offering no function of water movement (Glime 1987).

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**Figure 102.** *Bryum pseudotriquetrum* (*Bryales*) showing dense rhizoidal tomentum along stem. Photo by Misha Ignatov, with permission.

**Figure 103.** *Breutelia chrysocoma* (*Bartramiaceae*) showing rhizoidal tomentum. Photo by Michael Lüth, with permission.

**Figure 104.** *Schistidium antarctici*, a moss that becomes morphologically modified by moisture conditions. Photo courtesy of Rod Seppelt.

**Figure 105.** *Hygroamblystegium fluviatile* rhizoids on one side of stem. Photo by Janice Glime.

**Figure 106.** *Fontinalis novae-angliae* cemented to the rock by its rhizoids. Photo by Janice Glime.

**Figure 107.** *Fontinalis squamosa* rhizoids growing in a spiral where they are suspended above the substrate. Photo by Janice Glime.

**Figure 108.** *Fontinalis squamosa* rhizoid tips branching. Photo by Janice Glime.
Higuchi and Imura (1987) cultured three moss species to determine the effects of submersion on the rhizoid characters. The thickness, surface decorations, and positions where the rhizoids arise appear to be stable in altered moisture conditions, but in *Macromitrium gymnostomum* the mucilage that is present in terrestrial cultures is lost in water culture. Rhizoids generally are not produced on submersed mosses in standing water (Odu 1978), perhaps because ethylene, which inhibits their development, cannot escape easily. This conserves energy, because it would seem that they are needed neither for anchorage nor absorption and conduction.

Surprisingly, Trachtenberg and Zamski (1979) found a cuticle on the rhizoids of *Polytrichum juniperinum*, (Figure 109) sharply contrasting with roots and root hairs of tracheophytes, which serve as absorbing organs and have no waxy cuticle. This suggests that they may play little role in water uptake, but rather prevent water loss to the substratum. This raises questions about how widespread this cuticle is on rhizoids of other taxa and how it affects the capillary action they might otherwise afford. Perhaps they play only a role in conservation of water and not in its uptake. Or are these cuticles designed to provide capillary spaces that hold water around the rhizoids and facilitate uptake?

![Figure 109. Polytrichum juniperinum males, a moss that has a cuticle on its rhizoids. Photo by Jan-Peter Frahm, with permission.](image)

Figure 109. *Polytrichum juniperinum* males, a moss that has a cuticle on its rhizoids. Photo by Jan-Peter Frahm, with permission.

It is noteworthy that both leafy liverworts *Haplomitrium* (Figure 72-Figure 73) and *Treubia* (Figure 110) lack rhizoids (Figure 111) (Duckett *et al.* 2013). These unusual liverworts have leaves in three equal ranks and use underground stems (*rhizomes*) for anchorage and for fungal associations. All other liverworts produce *unicellular* (having only one cell) rhizoids (Figure 112). But only the thallose liverworts produce two types. Mosses, on the other hand, have multicellular rhizoids that branch (Figure 113).

![Figure 110. Treubia lacunosa dorsal view. Photo by Jan-Peter Frahm, with permission.](image)

Figure 110. *Treubia lacunosa* dorsal view. Photo by Jan-Peter Frahm, with permission.

![Figure 111. Treubia lacunosa with sporophyte, showing absence of rhizoids. Photo courtesy of Jeff Duckett & Silvia Pressel.](image)

Figure 111. *Treubia lacunosa* with sporophyte, showing absence of rhizoids. Photo courtesy of Jeff Duckett & Silvia Pressel.

![Figure 112. Cephalozia sp. rhizoids showing that they are one-celled. Photo by Jan Fott, with permission.](image)

Figure 112. *Cephalozia* sp. rhizoids showing that they are one-celled. Photo by Jan Fott, with permission.
Mucilage

Stem apices are protected by mucilage secreted by specialized hairs (Berthier et al. 1974). This mucilage seems to play a strong role in protecting the actively dividing tissue, permitting fragments to survive long periods of desiccation until they are able to grow again, and most likely playing a role in water retention, especially for the critical apical cells.

In liverworts and the moss Takakia (Figure 115) there are slime papillae (Figure 114) that may serve a water absorption/retention function as well. The leafy liverwort Haplomitrium (Figure 116) produces extensive mucilage on its rhizomes (Figure 116-Figure 117). It is interesting that these slime papillae appear in the green alga Coleochaete (Figure 118), the genus that seems most closely related to embryophytes, causing one to wonder if they may have been a prerequisite for land adaptation in early plants.
Figure 117. *Haplomitrium gibbsiae* rhizome with mucous. Photo courtesy of Jeff Duckett and Silvia Pressel.

Figure 118. *Coleochaete* thallus, an extant green alga that has the most characters in common with bryophytes. Photo by Yuji Tsukii, with permission.

The thallose liverwort *Conocephalum conicum* (Figure 19, Figure 24, Figure 53) has mucilage ducts in its thallus. Clee (1943) suggests that these may aid in water retention.

**Capillary Spaces**

Although several adaptations to holding water seem to exist [porose leaf cells, ridges, folds, sheathing leaf bases (Figure 119), rhizoids, tomentum], Proctor (1979) contends that most of the water is held in the larger capillary spaces between the moss shoots. Small amounts of dew that accumulate at the moss tip (Figure 120), *i.e.* the growing region, may be critical to survival (Lange 1969; Kappen *et al.* 1979). Hair points that wrap around the succeeding leaves above (Figure 121) help to deflect light and reduce evaporative loss by creating a diversion for air currents. Proctor (1980) experimented by removing hair points and found that when present they reduced water loss by 35% in *Grimmia pulvinata* (Figure 80) and *Syntrichia montana* (=*S. intermedia*; Figure 122). Thus far, it has been difficult to demonstrate that papillae afford any such advantage (Frey & Kürschner 1991). Nevertheless, in leaves they can act as a rapid capillary water movement system (Proctor 1979; Longton 1988; Pressel & Duckett 2011).
The leafy liverwort *Trichocolea* (Figure 123-Figure 124) is highly adapted to take advantage of capillary spaces. Its leaves are highly dissected and *paraphyllia* (leaflike appendages between the leaves; Figure 125) are abundant, permitting this species to act like a sponge. Zehr (1979) observed that it experienced only short-term vapor deficits in its moist habitat and thus was able to grow anytime temperatures were above freezing. Paraphyllia such as those in *Hylocomium splendens* (Figure 126-Figure 127) and *Thuidium tamariscinum* (Figure 128) create capillary spaces much like a tomentum. Other mosses such as *Mniaceae* utilize *paraphyses* (Figure 129) among the archegonia and antheridia to conserve water, using the same capillary principle.
Fungal Partners

Fungal partners associated with roots have been termed **mycorrhizae**, and the same term is applied to fungi that serve as fungal partners to bryophytes. The existence of these relationships has been overlooked until recently, although we have recognized for quite some time that many bryophytes had fungi associated with them. Hence, our knowledge of their importance to the bryophyte is meager. It is likely that they serve a similar role to that in tree roots in scavenging a wide area for water, minerals, and perhaps organic nutrients. But I would also consider it likely that at least some of them play a role similar to that in the **hemiparasites** such as Indian pipe (*Monotropa uniflora*). That is, for those bryophytes living in dense shade, they could find a third partner that has more access to light – a leafy tracheophyte – that provides photosynthate that can be transferred from the tracheophyte, by way of the fungus, to the bryophyte. I am afraid I can see no substance that is likely to be produced by the bryophyte that is useful to the tracheophyte, making the bryophyte also a hemiparasite. Nevertheless, such a 3-way linkage remains to be demonstrated.

In an attempt to unravel the evolution of the fungal symbioses of bryophytes, Pressel *et al.* (2010) examined the ancient basal bryophytes *Treubia* (Figure 110-Figure 111) and *Haplomitrium* (Figure 116-Figure 117. In these liverworts they found intracellular fungal lumps, intercellular hyphae, and thick-walled spores. Unlike the well known glomerophytes found as symbionts in thalloid liverworts and lower tracheophytes, these were more ancient fungi (Figure 130-Figure 131).

In leafy liverwort families sister to the **Schistochilaceae**, the ascomycete fungus *Rhizoscyphus ericace* occurs in the rhizoids (Pressel *et al.* 2010). This fungus has a wide range of hosts, including flowering plants in the Ericales (includes blueberries and heath
plants) and an Antarctic species of the leafy liverwort _Cephaloziella_. Figure 132 shows a member of the _Ascomycota_ inhabiting _Mylia anomala_. In the _Basidiomycota_, the genus _Sebacina_ (Figure 133) is associated with leafy liverworts, but this fungus is host specific. Neither of these liverwort fungi seems to digest its host, whereas the _Basidiomycota_ in the thallose liverworts of _Aneuraceae_ have regular colonization and digestion cycles. The hornworts also demonstrate mycorrhizal relationships with fungi, but thus far there is no evidence that such a mycorrhizal relationship exists in mosses.

We are at a very early stage in our understanding of mycorrhizae in bryophytes. In tracheophytes these associations permit the host plant to gain water and nutrients from a much wider area by accepting these from a fungus that has grown over a wide area, as much as 15 hectares, weighing 10,000 kg (Smith _et al._ 1992). Their role in bryophytes is less clear, but the ancient origin of this association suggests that by now it could be quite sophisticated and beneficial.

**Protonema**

The protonema stage of mosses is a delicate threadlike stage in which every cell is surrounded by air with the potential for creating desiccation. But is it really so delicate?  

In experiments, Pressel and Duckett (2010) demonstrated that protonemata can survive slow drying but not fast drying. This suggests that during slow drying there is time to manufacture something that protects the cells from the effects of desiccation. Indeed, pre-treatment with abscisic acid permits the protonemata to survive fast drying as well. During slow dehydration the cells undergo profound changes, including vacuolar fragmentation, reorganization of endomembrane domains, changes in cell wall thickness, changes in plastid morphology, changes in mitochondrial morphology, and a controlled dismantling of the cytoskeleton. During fast drying, these events do not occur or are incomplete. The abscisic acid permits the rapidly drying cells to partially mimic their behavior during slow drying, permitting them to survive.

**Leafy Liverwort Gemmae**

Liverworts have leaf gemmae that are usually small structures along the leaf margins. Germination on the leaf is not desirable, so it is no surprise that they have a means of preventing it. This prevention may relate to their hydrophobic surface (Duckett & Ligrone 1995). In _Odontoschisma denudatum_ (Figure 134), the wall chemistry changes during maturation, with an increase in electron-opacity.
aborted sporophytes outnumbers that of mature sporophytes. Similarly, in the boreal forest moss *Pleurozium schreberi* (Figure 137) 38% of the sporophytes aborted (Longton & Greene 1969).

**Sporophyte**

When we examine mature sporophytes with their capsules and spores, we don’t give a second thought to the dangers of drying out. But we are misled by this resistant mature sporophyte. Rather, based on studies of field-collected gametophytes of *Microbryum starckeanum* (Figure 135) and *Tortula inermis* (Figure 136) (both species of dry habitats) with immature sporophytes, McLetchie and coworkers found that the sporophyte generation is more sensitive to desiccation and thermal stress than is the leafy gametophyte (McLetchie & Stark 2006; Stark *et al.* 2007). This may of course differ in species with a different phenology in different environmental conditions, but it bears questioning our perception of the importance of desiccation during sporophyte development. This need for desiccation tolerance of the sporophyte may be especially important for species like those of *Polytrichum* that require as much as 20 months for sporophyte development and span an entire year of weather conditions (Arnell 1905; Longton 1972).

**Calyptra Protection**

If we imagine the hairy calyptrae of such mosses as *Polytrichum* (Figure 138), we must ask ourselves how the calyptra avoids absorbing water and holding it against the capsule, creating water logging, or contrarily, draws water from the capsule due to capillary spaces created by the hairs. In other words, why doesn’t it behave like a bath towel? To answer this question, we will look at calyptra development, timing, structure, and its ultimate role.

It appears that the embryonic sporophytes are the stage most susceptible to desiccation stress (Stark 2002, 2005). Nevertheless, some desert mosses have embryonic sporophytes that can tolerate desiccation for long periods, most likely benefitting from desiccation hardening (development of resistance to desiccation) (Stark *et al.* 2014). Several examples exist from non-desert mosses, although the tie to desiccation is unclear. In the boreal forest moss *Hylocomium splendens* (Figure 126-Figure 127) (Callaghan *et al.* 1978) and desert moss *Syntrichia caninervis* (Figure 18) (Stark *et al.* 2000), the number of
The calyptra develops from the archegonium, which expands as the embryo develops. In some cases, the calyptra falls early in capsule expansion, but in others, such as *Polytrichum*, it may remain until the spores are shed. One might then question the role of the calyptra in protecting the embryo through to development of spores. Budke *et al.* (2012) demonstrated that the maternal calyptra provides protection of early post-embryonic sporophytes against desiccation, but that later development of the capsule may incur cuticle development that protects as the capsule emerges from the calyptra. This demonstrates that the calyptra cannot be considered a vestigial structure, but rather that it is essential in preventing desiccation. Haig (2013) agrees that the presence of the calyptra delays the onset of transpiration. Hence, it is prudent to examine the calyptra characters that may provide this desiccation protection.

**Cuticle:** We have already discussed the presence of a 4-layered cuticle for the duration of the calyptra in *Funaria hygrometrica*. Budke *et al.* (2011, 2012, 2013) demonstrated that the cuticle on this calyptra conferred significant protection to the developing sporophyte.

**Hairs:** There appear to be two kinds of hairs on calyptrae, "true" hairs and undeveloped archegonia. In *Fontinalis*, the calyptral hairs develop from aborted archegonia whose eggs (Figure 139) were presumably not fertilized (Glime unpubl.). This results in a small number of hairs near the base of the calyptra.

The hairs on the calyptrae in taxa such as *Polytrichum* (Figure 138) and *Orthotrichum* (Figure 140) could function to prevent desiccation during early development or to deter herbivory both early and late in development, but earlier in development they could also serve important functions for the archegonium, helping to conserve moisture to protect the egg or other uses we haven't considered. I haven't followed the development in taxa other than *Fontinalis* (Figure 139), but the hairs seem too large and numerous in most taxa to be just a lingering of the archegonia or associated paraphyses. If they continue to elongate as the calyptra develops, then there may be some advantage that would favor that prolonged use of energy for their development.

**Cuticle**

It is likely that many bryophyte sporophytes have a cuticle. For example, the large, waxy-looking capsule of *Buxbaumia viridis* (Figure 141), and most likely the other members of the genus, has a layered cuticle (Koch *et al.* 2009). And in *B. viridis* this cuticle is waxy with massive wax layers having small embedded and superimposed platelets and granules on top of this complex. Although until recently the only documented sporophyte cuticles had been those of the *Polytrichales*, this complex of cuticle components is common in various groups of tracheophytes.

Pressel and Duckett (2011), suspecting that capsule waxes were more common than those of these two groups, examined a wider array of taxa, particularly those with shiny surfaces. They demonstrated that *Bartramia* (Figure 142), *Plagiopus* (Figure 143-Figure 144), and *Mnium* (Figure 145-Figure 147) invested as much in surface waxes of the capsule as did *Polytrichum*. They interpreted these waxes as having a role in preventing accumulated water from depressing gas exchange in the capsules, that is, prevention of water logging.
Figure 141. *Buxbaumia viridis* capsule showing shiny, waxy cuticle. Photo by Bernd Haynold, through Wikimedia Commons.

Figure 142. *Bartramia pomiformis* capsule showing waxy surface. Photo by Walter Obermayer, with permission.

Figure 143. *Plagiopus oederiana* with capsules showing waxy surface. Photo by Michael Lüth, with permission.

Figure 144. *Plagiopus oederiana* waxy capsule with calyptra at near maturity. In this case, the calyptra does little to protect the nearly mature capsule, most likely making the cuticle more important. Photo by Janice Glime.

Figure 145. *Mnium* sp. with water on young capsules, illustrating the potential for water logging. Photo by Alan S. Heilman, through Creative Commons.

In *Orthotrichum* many species have immersed stomata (Figure 146). These openings are surrounded by protruding cells that maintain an air space between the capsules and the calyptra (which remains attached and covers most of the capsule until the spores are ripe; Figure 140) (Pressel & Duckett 2011). The waxes repel the water on the capsule and prevent it from being drawn under the calyptra by capillary action. That is, a primary role for these surface waxes may be to prevent water logging in this and other species.

Figure 146. *Orthotrichum pusillum* immersed stoma on calyptra. Photo by Bob Klips, with permission.
But this cuticle story apparently does not begin with the capsule. The young sporophyte is covered by a calyptra. And in *Funaria hygrometrica* (Figure 148-Figure 151) this calyptra is covered by a waxy cuticle four layers thick at all stages, hence providing protection long before the developing sporophyte develops its own cuticle that ultimately arises on the sporangium (Budke et al. 2012). When the calyptra cuticle is removed during periods of low moisture, the sporophyte suffers significant damage, including decreased survival, increased tissue damage, incomplete sporophyte development, more peristome malformations, and decreased reproductive output (Budke et al. 2013). This is in contrast to the conclusion of Pressel and Duckett (2011) that the cuticles function primarily to prevent water logging. I have for my entire career as an ecologist failed to understand why ecologists get into so many arguments over two or more different explanations for the same thing, in this case the presence of stomata. There seems to me to be no evolutionary argument against multiple functions for the same thing, at the same or at different times. Just consider the many functions of our brains, or the many uses for fingernails.

Budke et al. (2012) examined the development of the cuticle on both the calyptra and the capsule, using *Funaria hygrometrica* (Figure 148-Figure 151) as a model organism. These researchers found that the sporophyte cuticle does not mature until the formation of the capsule.
Peristome: Peristome teeth likewise have cuticles, with differences related to habitat conditions at the time of spore discharge (Pressel & Duckett 2011). In Polytrichales, the spores are dispersed when raindrops pounce on the diaphragm (epiphragm; Figure 154) that connects the teeth (Watson 1971). For this mechanism to work, the teeth must not only remain dry, but must repel water so that it does not block the small openings between the teeth where spores must exit (Pressel & Duckett 2011).

On the other hand, most mosses disperse their spores when it is dry. Water is taken up and lost rapidly from between the ornamentation on these peristomes (Pressel & Duckett 2011). These include all Bryopsida they tested: Amblystegium (Figure 155), Bryum (Figure 102), Coscinodon (Figure 156), Dicranella (Figure 157), Didymodon (Figure 158), Fissidens (Figure 159), Funaria (Figure 160), Grimmia (Figure 160), Hypnum (Figure 161), Mnium (Figure 145-Figure 147), Rhyynchostegium (Figure 162), Schistidium (Figure 75), Syntrichia (Figure 18), Tortula (Figure 1, Figure 136). These water gains and losses permit rapid closure in wet conditions and accelerate opening under dry conditions.
Figure 156. *Coscinodon cribrosus* peristome. Photo by Michael Lüth, with permission.

Figure 157. *Dicranella varia* capsules showing peristome. Photo by Kristian Peters, through Wikimedia Commons.

Figure 158. *Didymodon rigidulus* with capsules. Photo by Michael Lüth, with permission.

Figure 159. *Fissidens adianthoides* peristome. Photo by Kristian Peters, with permission.

Figure 160. Peristome teeth of *Funaria hygrometrica*, a species in which teeth move in response to drying conditions and spores escape from the spaces between the teeth. Photo by George Shepherd, through Creative Commons.

Figure 161. *Hypnum cupressiforme* peristome. Photo by Walter Obermayer, with permission.

Figure 162. *Rhynchostegium confertum* with capsules. Photo by Michael Lüth, with permission.
But what happens in capsules with no teeth or only rudimentary peristomes? As an example, in *Weissia* (Figure 163) water is prevented from entering the capsule by a highly water-repellent capsule rim (Figure 163). If water entered the capsule, it could cause premature germination or interfere with ultimate dispersal.

Figure 163. *Weissia fallax* capsule showing rudimentary peristome. Note the waxy appearance of the reddish annulus around the teeth. Photo by Michael Lüth, with permission.

**Guard Cells and Stomata**

Capsules of many (most?) mosses have guard cells and stomata. The guard cells usually resemble those of tracheophytes, having a doughnut shape, and surrounding the stoma (opening). These are mostly located at the base of the capsule. In addition to the cuticle, we might expect the guard cells to play a role in water relations of the capsule. After all, the stomata and guard cells have existed through 400 million years of land plant evolution (Chater et al. 2011).

Like the cuticle, the role of the pores and stomata has been overlooked in bryophytes. Although we have known about the stomata in moss capsules for a long time, and used them as taxonomic characters in genera such as *Orthotrichum*, we have largely ignored their function, failing even to ask what it might be.

When thinking about adaptations to drought, we usually think of the survival of the gametophyte. What danger could there be to a dry capsule full of spores, right? But before that capsule is full of spores, it is a photosynthetic body in need of water. Perhaps the young seta with no capsule has little problem, but once the capsule starts to differentiate, water needs most likely increase dramatically. And once meiosis begins, water needs are critical. An interruption during meiosis could lead to a variety of anomalies, many of which could cause spore death.

Paton and Pearce (1957) reviewed the early literature on stomata in bryophytes, pointing out that in *Sphagnum* they do not mature. In fact, the capsule pores of *Sphagnum* are considered pseudostomata. Their function seems to be limited, facilitating capsule dehydration, shape change, and dehiscence (Duckett et al. 2009; Merced 2015). This is not surprising, because in *Sphagnum*, the seta is only a few cells high. Instead, the capsule is elevated on a pseudopodium that is developed from the gametophyte. This pseudopodium does not extend until the capsule is mature. Hence, the role of the pseudostomata to create a transpiration stream for nutrient transport would seem futile. Rather, Duckett and coworkers (2009) provide evidence that the pseudostomata remain open when the capsule is mature, causing the capsule to dry and shrink, forcing the spores out.

Stomata also are absent in the liverworts (thallus pores excepted), present in at least some hornworts, and absent in the moss order Andreaeales (Figure 164-Figure 165) (Paton & Pearce 1957). As in the tracheophytes, the number of guard cells associated with a stoma is usually two. Known exceptions (single circular guard cells) occur in *Funariaceae* (Figure 148-Figure 151) and *Buxbaumia aphylla* (Figure 166). Larger numbers of guard cells (3-4) occur but do not seem to be consistent in any single taxon and are thus considered an anomaly.

The walls of the guard cells are strongly cuticularized (Paton & Pearce 1957). The number of stomata in capsules that have been examined varies from 4 to over 200. Species with a long seta generally have more stomata than species with a short seta or immersed capsules. This supports the hypothesis that they are needed to provide an adequate transpiration stream to transport nutrients from the leafy gametophyte to the sporophyte capsule (Haig 2013).

Figure 164. *Andreaea rothii* with capsules that have no stomata. Photo by Jan-Peter Frahm, with permission.

Figure 165. *Andreaea* capsule SEM, a capsule that lacks stomata. Photo by George Shepherd, through Creative Commons.
Guard cells are usually located at the base of the capsule. In reality, they tend to be located below the area covered by the calyptra, where gas exchange and water loss are possible. This is consistent with a role to permit water loss, but could they also serve in gas exchange? It appeared that the capsule guard cells did not respond to changes in humidity (Copeland (1902). Rather, they are only able to close when the sporophyte is dehydrated or reopen when it is remoistened. This is consistent with their potential role in bringing nutrients upward.

In the moss *Physcomitrella patens* (Figure 167-Figure 168), the stomata of the sporophytes do indeed respond to environmental signals with the hormone abscisic acid (ABA) serving as a signalling component. In fact, the genes controlling ABA in *P. patens* can be moved to mutant *Arabidopsis thaliana* (flowering plant) that has lost its ABA-regulatory gene and cause stomata in that plant to behave normally. When *P. patens* mutants lack the ABA regulatory gene, the response to ABA is greatly reduced.

Figure 166. *Buxbaumia aphylla* capsules, a species with single circular guard cells. Photo by Štěpán Koval, with permission.

Figure 167. *Physcomitrella patens* capsule stomata SEM. Photo courtesy of Jeff Duckett and Silvia Pressel.

But wait! While ABA may affect guard cell closure in *Physcomitrella patens* (Figure 168), it appears that the guard cells in mosses have a somewhat different function. First of all, there seems to be no potassium-regulating mechanism (Duckett *et al.* 2010a). Instead, their primary role seems to be to permit water to escape when the capsule is mature (Boudier 1988; Beerling & Franks 2009; Duckett *et al.* 2009, 2010b). This loss of water causes the capsule to become distorted enough to force the rather stiff circular cap (operculum) to pop off.

It appears that the stomata endow the capsule with multiple advantages. Loss of water during development could be important to create a transpiration stream that moves nutrients upward from the gametophyte to the capsule of the sporophyte (Haig 2013). If this interpretation is correct, the water loss is essential to maintain continuous movement of water and associated nutrients upward. In fact, Haig interprets the elevation of the capsule on an elongated seta to be an adaptation that increases the movement of water by placing the capsule into the zone of turbulent air above the quiet boundary layer. The placement of the stomata at the base of the capsule gives them exposure while the calyptra reduces water loss from the part of the capsule where spores are developing.

Ziegler (1987) pointed out that in some mosses the sporophyte guard cells have thick walls and do not open and close. This type of guard cell occurs in species that have reduced photosynthetic tissue in the capsule and have been considered evolutionarily reduced. Bryophyte guard cells also differ from those of tracheophytes in that they are larger than the surrounding cells, whereas in tracheophytes they are smaller.

Paton and Pearce (1957) found that the stomata become functionless at a relatively early stage in capsule development, suggesting that this loss in function protects the developing spores against desiccation. They were able to demonstrate this early loss of function in the hornwort *Anthoceros* (Figure 169-Figure 170) and in mosses in the Bryales. Based on their studies on the hornwort *Phaeoceros*, Duckett and Ligrone (2003) say no to the function of capsule guard cells in gas exchange, at least in hornworts; they could find no response to moisture changes or to ABA in the hornwort *Phaeoceros* stomata (Figure 171).
On the other hand, in greenhouse-grown *Funaria hygrometrica* (Figure 172) the stomata (Figure 173) open on the fourth day of capsule expansion (Garner & Paolillo 1973). By the fifth day, continuing through the tenth day, they close in darkness and reopen in light. They also can be closed by the application of abscisic acid (ABA) (Garner & Paolillo 1973; Chater et al. 2011). Thus far we have no evidence to demonstrate the usefulness of this opening and closing. It could enhance gas exchange; it could control water loss during the critical stages of meiosis; and it could serve as a transpiration stream to bring nutrients from the gametophyte. And the function could change or disappear at maturity.

Indeed it appears that the function changes as the *Funaria hygrometrica* (Figure 172-Figure 173) capsule ripens. As maturity approaches, the stomatal responsiveness declines and about half the stomata remain open day and night (Garner & Paolillo 1973)! Furthermore, more stomata become exposed when the calyptra is shed (Duckett et al. 2009, 2010a). The stomata no longer provide a mechanism to conserve water.

Further complicating our interpretation of stomatal function during capsule development is the apparent lack of relationship between the presence of stomata and habitat. In the liverworts, stomata are totally absent. But liverworts produce mature capsules before elongation of the stalk occurs, negating the necessity for long distance

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**Figure 169.** *Anthoceros agrestis* with capsules. Photo by Michael Lüth, with permission.

**Figure 170.** *Anthoceros punctatus* SEM image of sporophyte showing stomata. Photo courtesy of Jeff Duckett and Silvia Pressel.

**Figure 171.** *Phaeoceros laevis*, open stoma flanked by desiccated and shrunken epidermal cells well above dehiscence point. Photo courtesy of Jeff Duckett, Ken PNG, Karen Renzaglia, and Silvia Pressel.

**Figure 172.** *Funaria hygrometrica* capsules. Photo by Li Zhang, with permission.

**Figure 173.** *Funaria hygrometrica* stomata. Photo courtesy of Jeff Duckett and Silvia Pressel.
translocation of nutrients and being consistent with the observations of Paton and Pearce (1957) that fewer stomata occurred on moss capsules with short or absent setae than on those with emergent, longer setae. The widespread absence of stomata in at least some species among so many moss taxa [e.g. *Atrichum* (Figure 174), *Pogonatum* (Figure 175), *Acaulon* (Figure 176), *Campylopus* (Figure 177), *Leucobryum* (Figure 178), *Cinclidotus* (Figure 179), *Discelium* (Figure 180), *Nanomitrium, Fontinalis* (Figure 183), *Tetraphis* (Figure 182), *Catoscopium, Leucodon, Cyclodictyon*] (Paton & Pearce 1957) suggests they are not essential for gas exchange. Furthermore, since most of these genera have long setae, one could argue against their function in creating a transpiration stream for nutrient transport. One might also argue that the well developed vascular tissue in both gametophytes and sporophyte setae of the *Polytrichaceae* makes the presence of stomata to create a transpiration stream unnecessary for nutrient transport, yet some members of the family have stomata and guard cells. And the stomata in tracheophytes are certainly necessary to maintain function of the xylem tissue in these larger plants.

Figure 174. *Atrichum crispsulum* capsules – in a genus in which at least some species lack stomata. Photo by Robert Klips, with permission.

Figure 175. *Pogonatum urnigerum* capsules, member of a genus in which some species lack stomata. Photo by Kristian Peters, with permission.

Figure 176. *Acaulon muticum* with capsules, a genus in which species lack stomata. Photo by Michael Lüth, with permission.

Figure 177. *Campylopus nivalis* capsules, a species that lacks stomata. Photo by Michael Lüth, with permission.

Figure 178. *Leucobryum glaucum* with capsules, member of a genus in which capsules often lack stomata. Photo by Janice Glime.
Figure 179. *Cinclidotus fontinaloides*, a species that lacks stomata. Photo by Michael Lüth, with permission.

Figure 180. *Discelium nudum* capsule, a genus in which at least some members lack stomata. Photo by Des Callaghan, with permission.

Figure 181. *Catoscopium nigritum*, a genus with capsules that lack stomata. Photo by Michael Lüth, with permission.

Figure 182. *Tetraphis pellucida* capsule, a genus that lacks stomata. Photo by Walter Obermayer, with permission.

Figure 183. *Fontinalis squamosa* var *curnowii* with capsules, a species that lacks stomata. Photo by David Holyoak, with permission.

Merced and Renzaglia (2013) demonstrated the remarkable similarity between stomata in the highly developed *Oedipodium* (Figure 184) and the very reduced *Ephemerum* (Figure 185) capsules. The capsule structure differs, with *Oedipodium* having extensive spongy tissue along the capsule apophysis where stomata are concentrated and *Ephemerum* lacks such tissue but has minimal substomatal cavities. Although *Oedipodium* (Figure 184) has numerous long-pored stomata and *Ephemerum* has few round-pored stomata, the stomatal ultrastructure and wall thickenings of these two taxa are quite similar. Both have sporophytes with a cuticle that is thicker on the guard cells and extends on the walls surrounding the stomata. When the capsules are older, epicuticular waxes and pectin clog the pores, closing them much like the stomata of fir trees in winter. Merced and
Renzaglia argue that the cuticle, water-conducting cells, and spongy tissues of *Oedipodium* all support the role of stomata in facilitating gas exchange and water transport as the sporophyte develops. They also contend that the existence of stomata exclusively on capsules may indicate a function in drying and dispersal of spores.

Hence, we have four potential functions for the stomata of capsules. These include a role in creating a transpiration stream to aid in nutrient transport, prevention of water logging that inhibits gas exchange, regulation of gas exchange, and drying that contracts the capsule and aids in spore expulsion.

**Summary**

Bryophytes gain water in their cells both through external (*ectohydric*) capillary movement and internal (*endohydric*) transport. When fully hydrated, their water content is typically high, up to more than 1200% of their dry mass. When dry, they can survive months to many years. Structural adaptations of stems and whole plants such as growth form, branch and leaf arrangements, rhizoidal tomentum, mucilage, central strand, hydroids, paraphyllia, ventral scales, cuticles, and stomata aid in moving water, facilitating entry, or reducing loss.

Thallose liverworts benefit from ventral transport by rhizoids and scales. The dorsal surface is covered by a cuticle but gas exchange may occur through pores overlying photosynthetic chambers. The pores are ringed by cells with cuticular ridges that prevent water drops from entering but that allow water vapor to escape. Midribs may help to gather and direct water both externally and internally. For many taxa, dormancy is a "last resort" to avoid the effects of desiccation. Fungal partners occur in both thallose and leafy liverworts, but their role is not known. Smooth rhizoids facilitate fungal entry; pegged rhizoids transport water and the pegs prevent collapse upon drying.

In mosses and leafy liverworts, growth form can help in both movement and conservation of water. Clumps reduce transpiration and provide additional capillary spaces. Mixed species can help each other, especially if one is good at moving water and one is good at retaining it. Mosses may have a central strand where water moves, but this is apparently absent in all liverworts except the *Haplomitriopsida*. Leaf cuticles occur in both mosses and liverworts and may repel water to avoid water logging or reduce loss by transpiration. *Rhizoids* and *tomentum* help in the movement of water upward. *Mucilage* in some liverworts, especially *Haplomitriopsida*, can be of great value in holding water about the plants.

The protonema can usually withstand slow drying. Like the guard cells in some stomata, it is responsive to ABA. ABA may be linked to *inducible desiccation tolerance* in the gametophores. *Constitutive desiccation tolerance* is the most common form of desiccation tolerance in bryophytes, but as the plants age they may switch to inducible desiccation tolerance. *Hardening* can occur following slow drying and may last more than a few days.

The sporophyte and calyptra both have cuticles, and at least in *Funaria hygrometrica*, the cuticle in the calyptra matures first, helping the calyptra to protect the young embryo. Calyptra hairs, thallus hairs,
paraphyllia, and paraphyses all function to help in movement of water and reduce rate of drying. Capillary spaces provided by these can further facilitate absorbing and holding water, bathing the tissues in water and reducing water loss.

The sporophytes of most (?) mosses and hornworts have guard cells and stomata that cease to function at sporophyte maturity. Their function(s) are ambiguous, but they may contribute to creating a transpiration stream to move nutrients upward, regulating capsule hydration during development, and drying the capsule prior to dehiscence and dispersal.

Acknowledgments

An earlier version of this chapter benefitted from the help of Beth Seafone and Medora Burke-Scoll, who helped me explain things without leaving too much to one's imagination, but at the same time not repeating myself. In an earlier version Linda Luster checked the literature citations, proofread, and made glossary suggestions from a layperson's perspective.

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# CHAPTER 7-4a

## WATER RELATIONS: LEAF STRATEGIES – STRUCTURAL

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CHAPTER 7-4a
WATER RELATIONS: LEAF STRATEGIES – STRUCTURAL

Figure 1. *Campylopus introflexus* demonstrating the ability of water to cling and collect on the thin, wiry leaves. Photo by Michael Lüth, with permission.

Much of what we know about water uptake by bryophytes has been through observation. While the observations are probably valid, broad generalizations have emerged and these have been applied to all mosses, especially by non-bryologists, and can lead to inappropriate experiments and conclusions.

Larson (1981) experimented with three species of bryophytes (and 8 lichens) using a "raining" wind tunnel environment to determine the effects of various structures on water uptake and storage. Larson found that the time required to reach saturation did not differ between lichens and mosses, varying from three minutes in the moss *Polytrichum juniperinum* (Figure 2) to over 300 minutes in the lichen *Stereocaulon saxatile*. The rate of absorption increases with the ratio of surface area to weight, making it extremely rapid in finely divided plants. Hence, comparison of leaf structure and plant form become important in considering the role of bryophytes in the water cycling of an ecosystem (Proctor *et al*. 1998; Wu *et al*. 2007).

Schofield (1981) considered leaf shape, arrangement, orientation, surface ornamentation, and detailed anatomy to be important in influencing water movement. These adaptations are complemented by branch arrangement, stem cortical cells, rhizoid structure, and presence of paraphyllia.

Figure 2. *Polytrichum juniperinum* hydrated (left) and dry (right) showing change in leaf position to wrap around stem. Photo by Michael Lüth, with permission.
Bryophytes hold their water in three ways (Proctor et al. 1998): **apoplastic** water in cell-wall capillary spaces and held by matric forces; **symplastic** (internal osmotic) water; external capillary water. For many bryophytes, the external capillary water is a highly important, albeit variable, component. This external water complicates any measurements of relative water content (RWC) because it makes measurement of the bryophyte at full turgor a difficult endeavor. Proctor et al. found that full-turgor water ranged from 110% dry weight (dw) in *Syntrichia ruralis* (Figure 3) and *Andreaea alpina* (Figure 4) to 1400% dw or more in *Dumortiera hirsuta* (Figure 5) and *Conocephalum conicum* (Figure 6–Figure 7). Most species had an osmotic potential ($\Psi_\pi$) at full turgor of -1.0 to -2.0 MPa, but thallose liverworts had values that were much less negative (-0.35 to -0.64 MPa).

Figure 3. *Syntrichia ruralis* with raindrops, a moss with low water content. Photo by Peggy Edwards, with permission.

Figure 4. *Andreaea alpina*, a moss with low water content. Photo by Andrew Hodgson, with permission.

Figure 5. *Dumortiera hirsuta*, a thallose liverwort that holds a high water content. Photo by Li Zhang, with permission.

Figure 6. *Conocephalum conicum*, a thallose liverwort that holds a high water content. Photo by Robert Klips, with permission.

Figure 7. *Conocephalum conicum* thallus section with pore From website of the Botany Department, University of British Columbia, with permission.

Pressel et al. (2009) pointed out that despite the ancient history of liverworts, we know little about the physiology of their desiccation tolerance. Desiccation causes a number of cytological changes in liverworts, including fragmentation of the vacuole, rounding of the chloroplasts and mitochondria with thylakoids, and cristae becoming rearranged but remaining undamaged, all responses that are similar to those of mosses and **tracheophytes** (non-bryophyte plants; plants with lignified vascular tissue). Furthermore, chlorophyll fluorescence shows half–recovery within minutes to 2 hours, but requires 24–48 hours to reach normal, unstressed values. And like desiccation tolerance in mosses, the de- and repolymerization of the cortical microtubule cytoskeleton
are associated with de- and rehydration. But liverworts have oil bodies, and these play a role unknown in mosses, as will be seen below.

Guerra et al. (1992) described the adaptations of xeric mosses in the gypsiferous zones of the southeast Iberian Peninsula, listing 15 modifications for conserving water. I have included these and some of my own observations here.

**Overlapping Leaves**

Most bryophytes have their leaves inserted at angles on the stem. In some cases, especially leafy liverworts (Figure 8), these are incubous in arrangement [leaves overlapping from base to tip like shingles on a roof, with the part of the leaf closer to the stem base being nearer the substrate (ventral) and the more apical side emerging on the upper (dorsal) side of the stem], whereas others are succubous [basal edge dorsal, apical edge ventral – the leaf succumbs to the leaf above it].

Figure 8. Succubous leaf arrangement of liverworts such as *Jungermannia* (left) and incubous arrangement of those such as *Calypogeja* (right). Note the decurrent leaf bases in the liverwort on the left. Redrawn by Margaret Minahan from Iwatsuki.

Clee (1937) found that in the succubous *Plagiochila asplenioides* var. *major* (Figure 9), water could move up to 3.7 cm in one minute. However, with the incubous arrangement, water moved less than 1 cm per minute. On the other hand, Basile and Basile (1987) questioned the role of the incubous vs. succubous leaf orientation in water conduction. They found that conduction proceeds equally in both orientations and that there is no correlation between the direction of leaf overlap and the angle of the substrate slope where they commonly grow. This seems reasonable since water coming from the top in rainfall would be presented with the opposite direction from water coming from beneath the branch. Hence, we could consider the branches in Figure 8 to be the above and below presentations of the same plant. Certainly if water is available from both above and below, it should make little difference if the plant is succubous or incubous. We need experiments to compare the effect on liverworts that form protruding shelves, those that are growing upright from a substrate, those that are adnate to a vertical surface, and those that grow horizontally adnate to a substrate. Then we need to compare the direction of the water source – base or tip of plant, dorsal or ventral surface.

Among mosses, Bowen (1933) considered the erect habit of leaves to hold and conduct more water than spreading leaves. This effect is enhanced if the leaves have decurrent bases (extensions of the leaf base down the stem; Figure 8).

Bayfield (1973) found that as water content declined in *Polytrichum commune* (Figure 10), the leaf arrangement changed (see also changes in *Polytrichum juniperinum* Figure 2). As the moisture decreased, the leaves wrapped closer around the stem, seemingly increasing moisture retention, a phenomenon that makes *Hedwigia ciliata* (Figure 11) almost unrecognizable when wet if one is only familiar with the dry state. Bayfield also found that external conduction is possible in the capillary spaces between the stem and the overlapping leaf bases. In the endohydric *Polytrichum* species, the loss of water is controlled by a complex series of changes in the leaf arrangement, whereas in the ectohydric *Racomitrium lanuginosum* (Figure 12-Figure 13), little or no mechanical control is exercised over water loss. It is likely that all *Polytrichum* (Figure 2, Figure 10) species benefit from this movement of the leaves upon drying.

Figure 9. *Plagiochila asplenioides* with overlapping, succubous leaves. Photo by Michael Lüth, with permission.

Figure 10. *Polytrichum commune* showing the dry lower leaves that are beginning to wrap around the stem compared to the wide-spreading upper leaves that are well hydrated. Photo by Michael Lüth, with permission.
Chapter 7-a: Water Relations: Leaf Strategies – Structural

Leaves Curving or Twisting upon Drying

Many species have leaves that curve or twist when they dry, particularly those in xeric habitats. These leaves curve toward the stem and thus reduce the exposed surface area. Among these are *Campylostelium pitardii* (Figure 14), *Phascum cuynetii*, and *Pterygoneurum sampaianum*.

Thickened Leaf

Many leaves partially protect themselves from water loss by having all or part of the leaf more than one cell thick. This is a common character for the borders and costa, where it most likely serves for support and possibly water movement, but in the leaf lamina, this reduces the exposed surface area (Figure 17).

Some leaves are bistratose in the upper part of the leaf, i.e. the part most exposed when the plant is dry. Among these are the xerophytic species *Syntrichia caninervis* (Figure 115) subsp. *spuria*, *Dicranella varia* (Figure 15), and *Didymodon australasiae* (Figure 16) (Guerra *et al*. 1992).

Figure 11. *Hedwigia ciliata* showing wet leaves (upper left) and dry leaves (diagonally across lower right) as a result of drying from the edge of the mat inward. The plants were growing on exposed boulders at the base of a cliff. Photo by Janice Glime.

Figure 12. *Racomitrium lanuginosum* dry showing twisted leaves and prominence of awns at the leaf tips, but little mechanical control over water loss. Photo by Michael Lüth, with permission.

Figure 13. *Racomitrium lanuginosum* wet showing transparent awns that are much less conspicuous than in dry plants. Photo by Des Callaghan, with permission.

Figure 14. *Campylostelium pitardii* with capsules, a species whose leaves curve or twist when dry. Photo by Proyecto Musgos, through Creative Commons.

Figure 15. *Dicranella varia*. Note the twisted leaves on the dry mosses in the foreground. Photo by J. C. Schou, with permission.
Some species protect the photosynthetic cells with hyaline cells, as in *Leucobryum* (Figure 18) and *Octoblepharum* (Figure 19). *Fissidens grandifrons* (Figure 20) differs from most other members of the genus *Fissidens* by having leaves that are multiple cell layers thick, most likely an adaptation to its habitat in fast-flowing water of streams and waterfalls. *Fissidens* accomplishes a degree of protection and provides capillary water-holding spaces by creating a pocket (Figure 21-Figure 24), giving this region a thickness of two layers of cells; the next leaf toward the apex often fits into this pocket. But this flattened moss nevertheless moves water slowly through its external surface (Table 1).
Concave Leaves

Proctor (1979a) found that many taxa of ectohydric mosses have concave leaves (e.g. Figure 25-Figure 26). When examined in moist weather, the concavities on the upper sides of the leaves will generally be full of water. This helps to solve the problem of gas exchange by exposing one surface to the atmosphere while keeping the other surface bathed in water. And most of the CO₂ needed for photosynthesis comes from respiration in the soil and litter. Gas diffusion in air is about 10⁴ times faster than in water (Proctor 1982). Other mosses, like Campylopus (Figure 1) and Polytrichum (Figure 2, Figure 10), are able to roll their leaves, like some grasses, when they are dry. In this mode, mosses like Syntrichia ruralis (Figure 28) can look much darker and expose less surface area to the atmosphere, whereas the wet cells change the optical properties, making the cell walls more translucent (Glime & Church, unpubl.).
Leaf spreading upon re-moistening is rapid in most bryophytes. Yenhung Li (unpublished data) found that in *Sphagnum* sp., *Ptilium crista-castrensis* (Figure 29), *Pleurozium schreberi* (Figure 30), and *Dicranum polysetum* (Figure 32), the first leaves spread within 1.5 to 2 seconds of receiving water (Table 1). To wet all the leaves in pieces 0.7 cm long required less than 2 minutes for most taxa, but required 24 minutes in *Rhodobryum ontariense* (Figure 31). The highest rate of conduction among the 15 taxa was in *Pleurozium schreberi* (140 mm min⁻¹).

Table 1. Mean time required for leaf spreading and conduction rate after rewetting along 0.7 cm branches in 15 species of bryophytes (n = 30 & 10 respectively). Based on Yenhung Li, unpublished data.

<table>
<thead>
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<th>Species</th>
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<th>conduction mm/min</th>
</tr>
</thead>
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<tr>
<td><em>Ptilium crista-castrensis</em></td>
<td>2</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Dicranum polysetum</em></td>
<td>2</td>
<td>70.00</td>
</tr>
<tr>
<td><em>Pleurozium schreberi</em></td>
<td>5</td>
<td>140.00</td>
</tr>
<tr>
<td><em>Hedwigia ciliata</em></td>
<td>5</td>
<td>11.48</td>
</tr>
<tr>
<td><em>Climacium dendroides</em></td>
<td>8</td>
<td>21.00</td>
</tr>
<tr>
<td><em>Fontinalis duriae</em></td>
<td>9</td>
<td>2.60</td>
</tr>
<tr>
<td><em>Dicranella heteromalla</em></td>
<td>10</td>
<td>11.48</td>
</tr>
<tr>
<td><em>Lophozia barbata</em></td>
<td>10</td>
<td>24.10</td>
</tr>
<tr>
<td><em>Anomodon attenuatus</em></td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Fontinalis antipyretica var. gigantea</em></td>
<td>26</td>
<td>27.5</td>
</tr>
<tr>
<td><em>Porella platyphylla</em></td>
<td>34</td>
<td>0.75</td>
</tr>
<tr>
<td><em>Sphagnum</em> sp.*</td>
<td>90</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Bryum pseudotriquetrum</em></td>
<td>149</td>
<td>0.82</td>
</tr>
<tr>
<td><em>Fissidens adianthoides</em></td>
<td>284</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Rhodobryum ontariense</em></td>
<td>1421</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Li found some indication that small leaves can spread more quickly than large ones, at least in *Fontinalis*. *Fontinalis duriae* (Figure 33) has smaller and thinner leaves than does *F. antipyretica var. gigantea* (Figure 34-Figure 35), and *F. duriae* can spread its leaves in 1/3 the time required for *F. antipyretica var. gigantea*. However, the difference may be due to the stiffness of the keel (leaf fold; Figure 35) in *F. antipyretica var. gigantea*, whereas *F. duriae* has flat leaves.
Figure 33. *Fontinalis duriae*, a species with flat, relatively narrow leaves that spread more quickly than larger leaves with a keel in *Fontinalis antipyretica* var. *gigantea*. Photo by Janice Gline.

Among the slowest species to re-wet in Li’s study were *Fissidens adianthoides* (Figure 36) and *Rhodobryum ontariense* (Figure 31), both for rate of conduction and leaf wetting. *Fissidens adianthoides* has leaves that are large and partly two-layered. There is little overlap between the leaves in this genus except at the two-layered pocket (Figure 37), and Church and Nelson (unpubl data) noted that when the leaves of *F. adianthoides* are dry there is little or no overlap even at the pocket. Therefore, lack of capillary space may account for its slow response. The slowness of *Rhodobryum ontariense*, which has all its leaves crowded at the top of the stem like a palm tree (Figure 31), may likewise be explained by lack of capillary spaces (Figure 38). Below the crowded rosette of leaves at the apex are very reduced scale-like leaves along the stem, providing little capillary space and rendering it the slowest among the 15 species observed by Li. It required 123 minutes for the water to travel 0.7 cm up the stem! Although Li’s data indicate a slight trend for rapid conduction to be coupled with rapid leaf spreading, there are enough exceptions to indicate that the relationship is not so simple.

Figure 34. *Fontinalis antipyretica* showing keeled leaves that spread slowly but that conduct water externally relatively rapidly. Photo by Jan-Peter Frahm, with permission.

Figure 35. *Fontinalis antipyretica* leaf showing keel (lower side of image). Photo by Malcolm Storey, through Creative Commons.

Figure 36. *Fissidens adianthoides*, a moss providing little capillary space, hence slow external conduction. Photo by Niels Klazenga, with permission.

Figure 37. *Fissidens arnoldii* showing the overlap created by leaf pockets where the leaf blade has two, but separated, layers. Photo by Michael Lüth, with permission.

Figure 38. *Rhodobryum ontariense* dry, with its leaves twisted upward. Note the bare stem that seemingly provides no capillary spaces for external conduction. Photo by Michael Lüth, with permission.
Cuculate Leaves

Cuculate is hooded or boat-shaped, referring to the apex of leaves in this case. The cavity created by this leaf form is able to hold water, in part due to surface tension. An example of this is the moss *Phascum cuynetii*; some *Sphagnum* (Figure 39) species also have cuculate leaves.

Figure 39. *Sphagnum* sp. from the Neotropics showing cuculate leaves. Photo by Michael Lüth, with permission.

Plications

Plications, or Japanese fanfolds, in the leaf may reduce evaporation by reducing the exposed area and creating nearly dead space between the folds. On the other hand, it might simply be a means of neatly folding the leaf as it dries and loses the turgidity that kept it concave. These plications are present in *Brachythecium* (Figure 40), *Coscinodon* (Figure 41-Figure 43), and *Hamatocaulis vernicosus* (=*Drepanocladus vernicosus*; Figure 44), among others. Some taxa exhibit these only as they are drying or dry, so the system is responsive to water loss. When it is rehydrated, the plications permit the leaf to expand.

Figure 40. *Brachythecium* leaves showing plications. Photo by Bob Klips, with permission.

Figure 41. *Coscinodon cribrlosus*. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 42. *Coscinodon cribrlosus* leaf with plications. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 43. *Coscinodon cribrlosus* leaf cross section showing plications. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.
Revolute and Involute Margins

Just as elongate cells of the border permit leaves to become contorted as they dry, the involute (Figure 45-Figure 48) and revolute (Figure 49-Figure 50) margins add structural support to the margin that causes contortions when the leaf dries (Figure 50). This contorted condition is known as crispate.
Both *Pottiaceae* and *Grimmiaceae* exhibit crisp, contorted leaves where the lamina is able to shrink and the leaf can roll with marginal rolling increasing as the plants dry (Kürschner 2004). The leaves wind spirally around the stem as they dry, reducing water loss and protecting the chlorophyll and DNA from excessive sunlight. The untwisting of the leaves provides another service – removal of trapped sand particles and other particles held by the leaves. When the lamina folds inward, it reduces desiccation. Kürschner suggests that the shiny costa may increase reflection of sunlight, further reducing desiccation. In these two families that occupy dry, open habitats, parallel evolution has adapted them to their similarly dry niches.

**Borders**

Borders are usually elongate cells that may be light in color or heavily pigmented. But in some species, the leaf margin may be heavily pigmented with chlorophyll in multiple cell layers. Such is the case in species of *Pseudocrossidium* (Figure 51-Figure 54) (Kürschner 2004). These species have marginal cells that form a well developed chlorophyllous region (Figure 52). They are protected by the *revolute* (rolled under; Figure 52-Figure 53) leaf margin that helps to maintain their hydration (Herzog 1926; Kürschner 2004).

So if the costa conducting cells all have protoplasm (*leptoids*), this leaves us with the question of water transport within the leaf. **Leaf borders** with elongate cells such as those in *Atrichum* (Figure 55) and the *Mniaceae* (Figure 56) provide benefits similar to those of the costa and seem to speed the movement of water from the base of the leaf to more distal parts, or in some cases from the tip toward the middle, but unfortunately, I have been unable to find any published study to verify this memory. Other roles are discussed in Chapter 7-4.

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**Figure 50.** *Bryoerythrophyllum recurvirostrum* leaf showing strong costa and *revolute* leaf margin that cause its *crispate* appearance when dry. Photo by Michael Lüth, with permission.

**Figure 51.** *Pseudocrossidium crinitum* hydrated. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

**Figure 52.** *Pseudocrossidium crinitum* underside of leaf showing thickened, revolute, chlorophyllose margin. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

**Figure 53.** *Pseudocrossidium crinitum* leaf cross section showing revolute margin. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

**Figure 54.** *Pseudocrossidium revolutum* showing curled leaves and revolute margins in dry condition. Photo from Proyecto Musgo, through Creative Commons.
Figure 55. *Atrichum sebewnii* leaf showing border with elongated cells and double border teeth. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 56. *Plagiomnium affine* leaf border showing elongate cells compared to wider but shorter leaf lamina cells. Photo by Ralf Wagner <www.dr-raff-wagner.de>, with permission.

It appears that long border cells (Figure 56) are able to move water and facilitate uptake. But they may provide an additional role in the wet to dry state transition of the leaf in at least some taxa (Lowell 1998). When the leaf of *Atrichum undulatum* (Figure 57) is wet, the elongate cells of the border are turgid and extend the leaf lamina out into a nearly straight surface. But as the leaf dries, the opposing forces of the drying leaf cells and the border result in the contorted leaf shape that is exhibited by the dry *Atrichum undulatum* leaf (Figure 57). The margins roll toward each other and the tip rolls toward the base, creating a "boat" shape. The border acts much like a wire sewn into the edges of a cloth ribbon, but somewhat more flexible.

In *Atrichum* (Figure 57) the leaf is prestressed; that is, it has a natural dry state that is highly convoluted, but when wet the turgor forces it to become straight (Lowell 1998). Thus, when the leaf dries, the leaf itself contorts into a form that is able to trap and hold water next to the leaf and stem surface. As Lowell describes it, the border is like the party toy that you blow into and it extends straight out, but when it is relaxed, it forms a coil. Species of *Mniaceae* (Figure 58) with borders seem to have similar responses, with the borders causing the leaf margins to curl toward each other, the leaf to become somewhat concave, and the leaf to become contorted.

Figure 57. *Atrichum alcecrisstatum* drying (lower plants) and moist (upper plants). Photo courtesy of Eric Schneider.

Figure 58. *Plagiomnium* branch with contorted leaves due to drying. Although this moss has been rewet, it is slow to hydrate and regain its shape. Photo source unknown.

A similar adaptation appears in *Lejeuneaceae* and *Porella*, where a hyaline row of marginal leaf cells function in water storage (Daniels 1998). Perhaps the same function occurs in some of the mosses such as some *Fissidens* (Figure 59-Figure 60) or *Plagiomnium* (Figure 56) with well-developed borders. Because of their elongate structure, water can be expected to move more quickly along the border because of fewer end walls to traverse. Yet there seems to be little experimentation to demonstrate that these cells are of any advantage in gaining or moving water to vital parts, or holding water.

Figure 59. *Fissidens bryoides* leaf cells and border, showing elongate border cells. Photo by Dick Haaksma, with permission.
Leaf Teeth

Lots of ideas have been presented to suggest the evolutionary significance of teeth in tracheophytes, from deterrents to insects (making the leaf look like something has eaten it, stimulating production of antiherbivore compounds or being spiny) to dripping points for water to help reduce growth of fungi and epiphytes. But what might their value be to bryophytes (Figure 61-Figure 62)?

One interesting observation is that teeth and lobed leaves of deciduous trees are more common in deciduous forests, but they are rare in tropical forests (Baker-Brosh & Peet 1997). Baker-Brosh and Peet hypothesized that they might provide sites for early season photosynthesis. They found that eight species with prominent teeth or lobes did indeed have early season photosynthesis on the margins of the leaves, but not in seven others and none in the four entire-leafed species in the experiments.

Obeso (1997) found that spines on the European holly (Ilex aquifolium) deterred browsing by ungulates, and that the spines were inducible, decreasing significantly when browsing was prevented for one year.

Another possibility for the adaptive value of teeth is their bearing on water relations. Royer et al. (2009) found that among the 227 sites they studied in the Australian subtropical rainforest, both the percentage of species and abundance of toothed species declined from riparian (wetlands adjacent to rivers or streams) habitats to ridge-top habitats. Hence, we can rule out any protective value that teeth might have against desiccation. On the contrary, this correlation suggests that teeth could have a role in reducing water in saturated leaves.

Do these tracheophyte models help us to suggest roles for teeth in bryophytes, or are they simply not a detriment to the mosses and liverworts that have them? Do leaf teeth suggest that something has eaten the leaves? We don't know if antiherbivore compounds are inducible in bryophytes, so there may be no disadvantage to having teeth as a warning unless most of the leaves with teeth do have antiherbivore compounds, inducible or not. It seems unlikely that the teeth have any painful effect to deter browsers. And we don't even understand how deciduous tree leaves benefit from teeth in more moist climates.
It is possible that the bryophyte teeth do have a photosynthetic role in spring when new leaves are forming. The apex, especially of acrocarpous mosses, has the most exposure to light, and the marginal parts of the leaves will have the most exposure, so it is possible that they have such a role. But experiments to demonstrate such a benefit are lacking.

Teniolae

The teniola is a border-like row of differentiated cells (Figure 63), differing from a true border by being intramarginal (i.e. not at the margin). They are more than one cell thick and this condition may extend also throughout the blade portion. These are found in *Calypneres* (Figure 64) and function for support, but may also provide water transport (Reese 1993).

Costa

The costa is the supporting structure for many moss leaves, often also providing an avenue of water transport (Frahm 1985) (Figure 65-Figure 66). It resembles a midrib both in appearance and function (Figure 67). Habitat seems to play some role in its development, although its predisposition to presence or absence is usually genetically determined.

Figure 63. Portion of leaf showing the intramarginal border, the teniola. Drawing by Janice Glime.

Figure 64. *Calypneres motleyi*, member of a genus that has teniolae. Photo by Jan-Peter Frahm, with permission.

Figure 65. *Mnium hornum* showing distinct costa and teeth. Photo by Bob Klips, with permission.

Figure 66. *Mnium hornum* leaf showing elongate cells of costa and border. Photo by Bob Klips, with permission.

Figure 67. Cross section of *Trichodon cylindricus* showing costa. Photo by Janice Glime.

The costa of some species may be shorter, thinner, and even disappear when it develops in water (Zastrow 1934). For example, the submerged forms of *Warnstorfia exannulata* (=*Drepanocladium exannulatum*) (Figure 68-Figure 69) have a costa that only reaches midleaf, whereas the terrestrial forms have a strong costa; similarly, *Cinclidium stygium* (Figure 70) normally has a strong costa above water, but when grown submerged it becomes thin and small (Zastrow 1934). When cultured in artificial streams where the leaves were exposed to air, *Fontinalis novae-angliae* developed short double costae, although
these are normally absent when it grows submersed (Glime, unpubl.). The broad costa in \textit{Campylopus} (Figure 71-Figure 72) not only serves as the photosynthetic organ, but as a water reservoir as well, adding to the possible advantages of growing a costa above water.

\textbf{Stereids}

In the stem, stereids are thick-walled cells that contain living protoplasm and have been compared to xylem parenchyma cells (Hébant 1970). In leaves, they form ribs on one or both sides of the costa (Figure 73) and may function as protection against desiccation (Frahm 1985). They occur in a variety of families, including \textbf{Dicranaceae} (Figure 74) and \textbf{Pottiaceae} (Figure 75-Figure 76).
It appears that the structure of the costa can have adaptive value relating to moisture conditions. Those *Campylopus* taxa surviving habitats with changeable conditions have well-developed costal stereids (Frahm 1985). Frahm found that dorsal costal lamellae (Figure 95) aid in water uptake, whereas the ventral costal stereids (Figure 77) common among *Campylopus* species help to reduce desiccation. *Campylopus savannarum* survives its savannah habitat with the aid of such stereids, whereas *Campylopus* taxa occurring on wet cliffs, dripping rocks, and swamps lack stereids (Figure 78).

**Figure 74.** *Dicranum scoparium* (*Dicranaceae*) leaf cross section. This leaf has few sclereids but has relatively large conducting cells, in this case smaller than the leaf lamina cells. Photo from Botany website, University of British Columbia, Canada.

**Figure 75.** *Syntrichia inermis* (*Pottiaceae*) leaf cross section. Note the enlarged costa with stereid cells on the bottom and conducting cells near the top. In this case, the lamina cells are covered with papillae that may help in water intake, a function thus far demonstrated for only one species. More likely they channel the water. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

**Figure 76.** *Syntrichia princeps* (*Pottiaceae*) leaf cross section showing costa with stereids (pinkish color on lower portion) and large leptoids. Photo by Paul S. Wilson.

**Figure 77.** *Campylopus flexuosus* leaf cross section showing ventral (lower) stereids. Photo by Amelia Merced, Duke Herbarium.

**Figure 78.** *Campylopus tallulensis* leaf cross section showing thin-walled ventral costal cells typical of the more humid mountainous regions. Photo by Amelia Merced, with permission.

**Lamellae**

The term *lamella* shares the same root word as laminate and refers to layers, in this case vertical stacks of cells that form rows, often reaching the length of the leaf (Figure 82, Figure 83). They may cover the costa, the blade, or a liverwort thallus. These rows are arranged in such a way that they somewhat resemble a book that has just been opened and laid to rest, with its pages still parting and standing upward from the middle. Some of the most xerophytic (referring to plants of dry habitats) mosses, such as *Aloina* (Figure 79), have branched filaments over the costa, giving it a succulent (fleshy) appearance; *Crossidium* (Figure 80-Figure 81) achieves a similar effect with dense filamentous outgrowths from the costa in the upper half of the leaf.
Figure 79. *Aloina brevirostris*, illustrating the succulent appearance caused by the numerous filaments on the costa. Photo by Michael Lüth, with permission.

Figure 80. *Crossidium crassinerve* with filaments on leaf costae. Photo by Michael Lüth, with permission.

Members of the *Polytrichaceae*, such as *Polytrichum* and *Dawsonia*, which are all *endohydric* (having internal water transport), have vertical lamellae (Figure 82), on their leaves that provide capillary spaces and create dead air spaces that can reduce water loss across the broad surface of these atypically large moss leaves (Figure 82-Figure 85). In addition, some species [*Polytrichum hyperboreum* (Figure 86-Figure 87), *P. piliferum* (Figure 88-Figure 89), *P. juniperinum* (Figure 90-Figure 91)] have the edge of the leaf lamina (flattened part of leaf not including costa or border) rolled over the lamellae, creating an internal structure somewhat like the *palisade mesophyll* (columnar cells of inner leaf tissue) of a flowering plant, with the lamina behaving in some ways like an epidermis. The leaves have the additional ability to flex like a hinge when water fills the thin-walled leaf base cells (van Zanten 1975), causing the leaves to be spread lengthwise away from the stem under moist conditions but be straight or curved around the stem when dry (Figure 2). Such behavior retards water loss and protects the chlorophyll during dry periods, while permitting maximum use of light during wet periods.

Figure 82. Cross sections of lamellae of *Polytrichaceae*. Top: stained section of *Polytrichum*. Bottom: *Polytrichastrum alpinum* with papillose terminal cells on the lamellae. Photos by Janice Glime.

Figure 83. Lamellae on leaf of *Polytrichum ohioense*, viewed down onto leaf surface at 100X. Photo courtesy of John Hribljan.
Figure 84. SEM of *Dendroligotrichum squamosum* (Polytrichaceae) showing tops of lamellae. Photo courtesy of Jeff Duckett and Silvia Pressel.

Figure 85. SEM of *Dendroligotrichum squamosum* leaf showing terminal cells of lamellae. Photo courtesy of Jeff Duckett and Silvia Pressel.

Figure 86. *Polytrichum hyperboreum* showing leaf lamina rolled over the lamellae. Photo by Michael Lüth, with permission.

Figure 87. *Polytrichum hyperboreum* leaf cross section showing lamina folded over lamellae. Photo by Michael Lüth, with permission.

Figure 88. *Polytrichum piliferum* showing leaf lamina rolled over the lamellae. Photo from Botany Department website, University of British Columbia, Canada, with permission.

Figure 89. *Polytrichum piliferum* leaf cross section showing leaf lamina rolled over the lamellae. Photo from Botany website, University of British Columbia, Canada, with permission.
Polytrichum juniperinum showing leaf lamina rolled over leaf lamellae. Overlap can be seen easily near leaf bases where the overlap is incomplete, permitting water to enter the basal cells. Photo by Janice Glime.

Polytrichum juniperinum leaf cross section showing leaf lamina rolled over leaf lamellae. Photo by John Hribljan, with permission.

In Pilopogon laevis (Figure 92) the costa is ribbed on the back of the leaf; in P. peruvianus (Figure 93-Figure 94) it has 3-4-cell-high lamellae on the back of the leaf, adapting this species to its dry coastal desert habitat. Likewise, Campylopus pilifer (Figure 95) has similar lamellae and prefers such dry habitats as rocks, soil-covered boulders, and gravel. On the other hand, C. introflexus (Figure 96) has only 1-2-cell-high lamellae and lives on humus, wet sand, and peat.

Pilopogon laevis, a species with a ribbed costa. Photo by Jan-Peter Frahm, with permission.

Pilopogon peruvianus in its desert habitat. Photo by Michael Lüth, with permission.

Pilopogon peruvianus leaf cross section showing 3-4 cell high lamellae. Photo by Michael Lüth, with permission.

Campylopus pilifer, a plant of rocks and gravel, leaf cross section showing deep lamellae. Photo by Amelia Merced, Duke Herbarium, with permission.
Although Frey and Kürschner (1991) found a correlation between costal lamellae and increasing aridity, the lamellae of *Polytrichum* seem not to be so much an adaptation to prevent water loss as to provide for additional surface area [2.4-fold in *Polytrichum commune* (Figure 97-Figure 98)] and gas exchange during photosynthesis (Thomas *et al.* 1996). Proctor (1979a, b) and Thomas *et al.* (1996) described wax on the terminal cells of the lamellae of *Polytrichum* and attributed to this wax the repulsion of water, preventing it from entering between the lamellae. Perhaps lamellae are adapted to increasing gas exchange and are more important in water retention or repulsion than in absorption, at least in some species.

The genus *Atrichum* (*Polytrichaceae*) also has shallow to deep lamellae, and these have been used to justify separation into different species. The lamellae shown in Figure 99-Figure 101 fall within *Atrichum undulatum* var. *undulatum*, but any lamellae more than 4 cells high would indicate a different variety (Crum 1983), or species (The Plant List 2010).
Daniels (1998) has compared leafy liverworts growing in a variety of habitats. Xerophytic (dry habitat adapted) taxa such as Frullania (Figure 104) have helmet-shaped leaf lobules and Radula (Figure 105) has a saccate lobule, both functioning for water storage. Porella (Figure 102), capable of both an epiphytic (living on plants) and a saxicolous (living on rock) habit, has leaf folds underneath (lobules) and large underleaves. Liverwort plants in the humid rainforests such as those in the Lejeuneaceae (Figure 106-Figure 108) have smaller lobules than those growing in drier, more exposed habitats (Cornelissen & ter Steege 1989; Gradstein 1995). Such structures help to hold water in capillary spaces in the absence of multiple rows of leaves. Some aquatic invertebrates, especially rotifers, live in these watery lobules (see Volume 2, Chapter 4-5 on Rotifers). It is likely that the pockets of Fissidens (Figure 21-Figure 24, Figure 37) may have similar water-holding functions.

Lobules and Storage Organs

Liverworts have an evolutionary history that separates some of the major groups by their water relations (Heinrichs et al. 2005). In the Jungermanniidae, two clades split. The Porellales are predominantly epiphytes that have specialized lobules (Figure 102) or water sacs and endosporous protonemata. The Jungermanniales (Figure 103) are frequently terrestrial, lack water sacs, and normally develop exosporous protonemata.

Figure 101. Atrichum undulatum leaf (costa) cross section showing small, thick-walled stereids above and below the large, transparent conducting cells. Lamellae are on top of the costa and are only 2-3 cells high. Photo by Walter Obermayer, with permission.

Figure 102. Ventral side of Porella platyphylla showing underleaves along stem and lobules on each side of them. Photo by Paul Davison, with permission.

Figure 103. Lophozia wenzelii, a member of the Jungermanniales, showing the absence of lobules. Photo by Des Callaghan, with permission.

Figure 104. Frullania tamarisci showing lobules. Photo by Michael Lüth, with permission.

Figure 105. Radula from the tropics with saccate lobules (arrows). Photo by Michael Lüth, with permission.
Hair Points

Hair points are common on leaves of xerophytic mosses, including species of *Campylopus* (Figure 109-Figure 110), *Grimmia* (Figure 111), *Schistidium* (Figure 112-Figure 113), *Hedwigia* (Figure 134), and *Syntrichia* (Figure 114). As discussed earlier, Loeske, in 1930, demonstrated that in *Schistidium apocarpum* (Figure 112-Figure 113) hair points are actually lost when the mosses are kept in damp air or deep shade. Proctor (1979a) and Kürschner (2004) consider these hairs to be organs that reflect some of the solar radiation, thus reducing energy absorption, temperature, and evaporation. But they reduce water loss more directly as well; hair points on *Syntrichia intermedia* (Figure 114) and *Grimmia pulvinata* (Figure 111) reduce the boundary layer conductance by about 20-35% in experiments (Proctor 1980). Not only does this thicker boundary layer trap stagnant air, thus reducing evaporation loss, but it increases the distance from the leaf surface to the surrounding air, thus decreasing the diffusion gradient (Proctor 1982).
Hair points may also help in trapping and absorption of water vapor from fog and dew (Figure 109-Figure 110). Dry tips can reflect sunlight (Figure 109), reducing water loss (Kürschner 2004).

Zhang et al. (2009) considered the effect of dew as an important moisture source in the Gurbantunggut Desert, Northwestern China. They measured dew quantities with micro-lysimeters and demonstrated the increase in dew deposition as the crust grew larger. Mosses had the highest deposition compared to that of lichen crusts, cyanobacterial crusts, and bare sand (p < 0.05). Interestingly, the retention time for the moisture gained from dew did not follow this pattern. Instead, it was held longest by sand, followed by the cyanobacterial crust, moss crust, and lichen crust, in that order.

Tao and Zhang (2012) further examined the function of hair points in the desert moss Syntrichia caninervis (Figure 115). The hair points in this case comprised only about 4.8% of the shoot weight, but they were able to increase the absolute water content by 24.9%. And, during dehydration, those moss samples with hair points always
had a higher water content than did those without. Furthermore, the shoots with hair points took 20 minutes longer to become completely dehydrated. And of course there was greater dew accumulation on the shoots with leaf hair points, increasing the dew on the crusts by 10.3%. Following short simulated rainfall events, the evaporation of water from the crusts was always slower when the leaves had hair points in contrast to the rapid loss of water trapped from dew (Zhang et al. 2009).

Yuan Ming Zhang’s research team filmed the events following application of a drop of water on the hair points of Syntrichia caninervis (Figure 115). The water moved quickly down the hair point and was absorbed by the leaves within seconds. Like a fine wire, the hair tips serve as a conduit for the water. This mechanism permits these mosses to extract water from dew or fog, and to benefit from rapid absorption of the first few drops of rain, maximizing its period of hydration. Zhang et al. (2011) supported the significance of this rapid rewetting. In lab experiments they showed that within the first minute the photosynthetic yield (Fv/Fm) recovered to 90% of its rate after 30 minutes. Cytological changes occurred rapidly, indicating no damage to membranes or organelles. This rapid recovery makes it possible for it to use the water collected by the hair points from fog, dew, rain, and melting snow for immediate recovery, making it possible to attain positive photosynthetic gain in its desert ecosystem.

Duration of the rainfall or dew fall event is important. Proctor (2004) found that in Grimmia pulvinata (Figure 111), dew fall did not enter the moss sufficiently to rehydrate it. Could these hair points prevent wetting and drying cycles that are too frequent for adequate repair of dehydration damage in mosses regularly subjected to hot, dry days? Is this a mechanism to prevent the leaf from becoming hydrated at a time when it will dehydrate again within hours? This is reminiscent of the dormancy mechanism in desert seeds wherein a chemical must be washed off before the seed will germinate. This keeps the seed from germinating unless there is enough rainfall to sustain the young seedling until it reaches a size where it can survive. In these mosses, it requires a rainfall that will hydrate the moss long enough for it to repair the damage of desiccation and make a positive photosynthetic gain before becoming dehydrated again.

**Nucleation**

It appears that bryophytes are good nucleators. This is a phenomenon in which a small object, known best from bacteria and proteins, causes the formation of ice around itself. Moffett et al. (2009) suggest that this phenomenon is widespread among bryophytes. Nucleation occurs when the difference in vapor pressure over ice and water is at or close to the maximum. At these temperatures, typically -8 to -18°C, ice grows at the expense of supercooled water. Moffett et al. suggest that the nucleation ability permits the bryophytes to collect water from fog, dew, and cloud water. It is interesting to note that airborne bryophytes may use this nucleation to initiate precipitation.

**Papillae**

Papillae in bryophytes are small projections from cells, especially common in the Pottiaceae (Figure 117-Figure 118). Kou et al. (2014) attempted to limit the confusion of many terms in their descriptions by providing four terms to describe them: simple, forked, branched, and pedicellate.

Papillae can both facilitate rapid water uptake (Proctor 1979a; Longton 1988; Kürschner 2004) and accelerate water loss (Pressel et al. 2010). Species that benefit from these papillae must, as a consequence, shut down under drying conditions. This is consistent with the role of surface waxes (discussed in Chapter 7-4b of this volume). The thick surface waxes of tracheophytes are usually associated with conditions of drying. In bryophytes, however, they are often characteristic of species from constantly flowing aerated water or other places where water logging depresses gas exchange (Pressel et al. 2010). In other words, often they are important for their hydrophobic (water-repelling) nature.

The role of papillae, those little bumps and extensions on cell walls (Figure 118), has been controversial for a long time, but their common appearance on bryophytes of dry habitats cannot be ignored. Nevertheless, Loeske (1926) points out that papillae are also found in a number of wetland and aquatic taxa, including Dichodontium pellucidum (Figure 119-Figure 120), Philonotis (actually prorate cells – end walls overlap and protrude; Figure 121-Figure 122), Aulacomnium palustre (Figure 123-Figure 124), Helodium blandowii (Figure 125-Figure 126), and Paludella (Figure 127). Loeske observed that the papillae
are maintained in a number of species through a wide range of wet to dry habitats. On the other hand, these taxa are common in wet meadows, lake shores, and other wet habitats where they may periodically be dry while being exposed to high sunlight, suggesting that the papillae may be of value under those exposed conditions.

Figure 117. *Barbula convoluta* leaf cells showing papillae (especially visible as tiny projections along the margins). Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 118. *Chrysothamnus chilensis* leaf cross section showing papillae. This leaf is well endowed with stereids in the costa. Photo by Juan Larrain, with permission.

Figure 119. *Dichodontium pellucidum* showing dull, waxy look that results from surface papillae. Photo by Jan-Peter Frahm, with permission.

Figure 120. *Dichodontium pellucidum* leaf cells in cross section showing papillae. Photo by Amelia Merced through Duke University Plant Biology website, with permission.

Figure 121. *Philonotis fontana* exhibiting dull appearance resulting from prorate cells. Photo by Malcolm Storey, through Creative Commons.

Figure 122. *Philonotis fontana* leaf lamina showing prorate cells that have an appearance similar to papillae. Photo by Kristian Peters, through Creative Commons.

Figure 123. *Aulacomnium palustre*, wetland moss with papillae. Photo by David T. Holyoak, with permission.
Some papillae are quite decorative, adorning species that typically live on limestone rocks or other highly desiccating habitats. *Encalypta ciliata* (Figure 128-Figure 130) has branched papillae and lives on limestone rocks and other dry locations.

Figure 128. *Encalypta ciliata* in a hydrated state, showing the nearly translucent appearance of the leaves. Photo by Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 129. *Encalypta ciliata* in a dry state, showing the dull surface of the contorted leaves. Photo by Li Zhang, with permission.
Hedwigia ciliata (Figure 133-Figure 134) is a moss that has white tips on its leaves, presumably protecting the underlying leaves from sun damage. But we need to examine the role of these tips in water uptake as well. The leaf and awn cells are heavily endowed with papillae that give the leaves a waxy appearance despite the absence of waxes.

At least some leaf papillae (Andreaeobryum macrosorum, Figure 135) are constructed in such a way that they provide a channel for the uptake of water (Crandall-Stotler & Bozzola 1990, 1991). This channel is within each papilla and is different from the channels formed between the papillae (cf. Proctor 1984). SEM observations indicate the channel within the papilla facilitates the rapid uptake of water during rehydration (Crandall-Stotler & Bozzola 1990, 1991). So far, this channel has not been demonstrated in any other species.

So how can papillae function both for water absorption and water loss, and why would evolution tolerate such a seeming contradiction? Pressel et al. (2010) may have answered this question. They found that in Rhacocarpus purpurascens (Figure 136), the trilamellate (having 3 layers) walls have a porous outer layer that permits rapid uptake of water, whereas its cuticle-like layer is highly hydrophobic and prevents water-logging. Could it be that the papillae of bryophytes create that space needed to prevent water-logging? But Pressel and coworkers contend that papillae in R. purpurascens accelerate water loss.
resulting in a metabolic shutdown when the plants are water-stressed. With the wide variety of shapes, sizes, and density of papillae among the bryophytes, it is still possible that some have the ability to prevent water-logging during the critical periods when the plants are wet in normally dry habitats. If this ability exists, it may be of considerable importance in at least some cases.

Figure 135. *Andreaeobryum macroporum*, a moss for which papillae are known to aid in uptake of water through a channel in the papilla. Photo from Botany website, University of British Columbia, Canada, with permission.

Figure 136. *Rhacocarpus purpurascens* showing shiny leaves. Photo by Michael Lüth, with permission.

One such species is the desert moss, *Syntrichia caninervis* (Figure 115, Figure 137-Figure 139). When Wu et al. (2014) compared absorption of rhizoids to that of leaves, the leaves were clearly the greater absorptive organs. They tested absorption by dropping water onto the upper and lower leaf surfaces, both of which have C-shaped papillae (Figure 137) (Zheng et al. 2010). Wu and coworkers found that the adsorption by the papillae is so rapid that they could not determine the leaf angles. They concluded that in this case the papillae are superhydrophilic (having a highly efficient water absorption mechanism). The spaces between the papillae form microcapillary spaces that serve as an efficient conducting system (see also Koch et al. 2008).

Figure 137. *Syntrichia caninervis* leaf papillae. Photo by Michael Lüth, with permission.

Figure 138. *Syntrichia caninervis* side view of leaf papillae that appear C-shaped from above. Photo by Terry McIntosh, with permission.

Figure 139. *Syntrichia caninervis* var. *caninervis* showing long papillae on costa and smaller ones on cells. Photo by M. T. Gallego.

The only thing that seems clear about papillae is that our understanding of them is not clear. It is likely that papillae cannot be lumped into one function, but that shapes, structure, and arrangement may create different capabilities, and these must coordinate in various ways with surface waxes, cell wall components, and other leaf surface features to optimize their role in the climates where the bryophytes live.
Leaf Bases and Alar Cells

Many mosses have the advantage of enlarged, thin-walled cells at the base of the leaf (alar cells) (Figure 141-Figure 142). These serve as entry points for water into the leaf and stem, but in many species their enlargement when fully hydrated also forces the leaf away from the stem, exposing greater surface area for photosynthesis, and perhaps even for water capture.

Wu et al. (2014) demonstrated the importance of adjusting the leaf angle in the desert moss Syntrichia caninervis (Figure 115, Figure 144). Although this moss is extremely desiccation tolerant, it must balance the need for water conservation with the need for light for photosynthesis when it is hydrated. This is accomplished by the movement of the leaves in response to moisture changes. As leaves become hydrated, they can move from a steep angle of 69-84° with the horizontal axis (Figure 144) to one of only 30° (Figure 115) within 7 seconds of becoming hydrated, with the first leaves moving within 1 second. They are able to obtain maximum net photosynthetic gain at a shoot relative water content of only 60%. The hyaline cells at the leaf base facilitate the rapid absorption of water, but they also swell and force the leaf away from the stem mechanically. It is interesting that the loss of leaf hair retards the leaf angle adjustment. When water was added to the soil instead of being added as an aerial source of water, the absorption rate was reduced, indicating that most water absorption is through the leaves.
Leaf Cell Shape

Bill Buck once asked me what I thought about the elongate cells in mosses such as 
*Fontinalis* and what the significance of such elongate cells might be, predominant in pleurocarpous mosses but rare in acrocarpous ones. I don't know that either of us has a better answer than we did then, but long, narrow cells should have an advantage in water movement. Elongate cells mean that fewer end walls must be crossed for water and other substances to traverse the interior of the leaf from tip to base or vice versa. The split between acrocarpous and pleurocarpous mosses suggests to me that the innovation of elongate cells, perhaps unnecessary in aquatic ancestors, occurred early in the evolution of pleurocarpous mosses and was rarely achieved among the acrocarpous species.

In the acrocarpous moss *Bryum pseudotriquetrum*, this elongation is partially achieved (Figure 145). This is a moss of wet habitats that dry out. The leaves are usually out of the water, and having somewhat elongated cells should improve transport.

Porose Cells

Porose cells provide more cause for speculation. These cells, uncommon among bryophytes, would seem to provide linkages to adjoining cells while permitting the cells to have otherwise thick walls. Such porosity is easily seen in *Dicranum polysetum* (Figure 146). I am unaware of any experiments to demonstrate that this is actually true or to compare the rate of transport in leaves with such cells to those in leaves with non-porose cell walls.

Hyalocysts

Colorless or hyaline cells (Figure 147) are typical of leaves of *Sphagnum* (Figure 148) and *Hedwigia* (Figure 134), and the awns of numerous xerophytes. Frahm (1985) examined the correlation between hyalocysts and habitat in *Campylopus* (Figure 149). *Campylopus shawii* occurs in wet swamps where it can obtain and store water easily; it has large ventral hyalocysts. *Campylopus setifolius*, on the other hand, grows on wet, dripping rocks that dry out occasionally; it has smaller hyalocysts, presumably to reduce the water loss to evaporation from these cells. The presence of ventral hyalocysts in *C. flagelliferus* (Figure 149) seem to adapt it to its life restricted to the bark of living trees where it needs a means of rapid water uptake.
Figure 148. *Sphagnum papillosum* leaf cells showing large hyaline cells with fibrils and green photosynthetic cells. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 149. *Campylopus flagelliferus*, an epiphyte with ventral hyalocysts. Photo by Jan-Peter Frahm, with permission.

Species of the cushion moss, *Leucobryum* (Figure 18, Figure 150), appear very succulent because of the hyalocysts among the photosynthetic cells. In this case, the leaf is several cells thick and the hyalocysts give them a whitish appearance. *Leucophanes* (Figure 151-Figure 152) has two different types of hyalocysts. The base of the leaf has a V-shaped arrangement of hyaline cells and the leaf lamina has an upper and lower layer of hyaline cells surrounding the photosynthetic cells.

Figure 150. *Leucobryum juniperoides*, showing the thick, whitish leaves. Photo by Michael Lüth, with permission.

*Sphagnum* species are considered xerophytic hydrophytes with many adaptations to deal with periodic drought (Andrus 1986). Living in a watery mire for most of the year, this genus has no internal conducting system and must face a severe threat of drying in the full sun of the summer when the water table is low. The ectohydric *Sphagnum* is a poor drought tolerator, but a relatively good drought avoider (Li *et al.* 1992). It has two types of leaf cells, small photosynthetic cells and large hyaline cells (Figure 153).

Figure 151. *Leucophanes molleri* leaf showing V-shaped hyaline base. Photo courtesy of Noris Salazar Allen.

Figure 152. Cross section of *Leucophanes molleri* leaf showing hyaline cells surrounding the photosynthetic cells. Photo courtesy of Noris Salazar Allen.

*Sphagnum* species are considered xerophytic hydrophytes with many adaptations to deal with periodic drought (Andrus 1986). Living in a watery mire for most of the year, this genus has no internal conducting system and must face a severe threat of drying in the full sun of the summer when the water table is low. The ectohydric *Sphagnum* is a poor drought tolerator, but a relatively good drought avoider (Li *et al.* 1992). It has two types of leaf cells, small photosynthetic cells and large hyaline cells (Figure 153).

Figure 153. *Sphagnum* leaf cell types and pores. Left: *Sphagnum* leaf cells stained with crystal violet. Photo by Janice Glime. Right: *Sphagnum palustre* photosynthetic and hyaline cells as seen in cross section (upper) and flat (lower). Drawings by Margaret Minahan.

Hyaline cells bathe the photosynthetic cells in water by providing a reservoir. Since the hyaline cell is a dead cell, its sole purpose seems to be to supply water to the photosynthetic portion of the leaf. These cells give some species of *Sphagnum* (Figure 153-Figure 154) the ability to hold up to 25 times their own mass in water (Andrus 1986).
Transplant studies indicate that species of *Sphagnum* differ in abilities to inhabit different heights above the water level, and these differences seem to correlate with the positions they occupy in the field (See Li et al. 1992, Rydin 1993, and discussion in competition chapter). Studies by Hintikka (1972) hint that the mechanism for some of these adaptive differences may not relate to water, but to other factors associated with submersion. When grown in sterile culture, *S. fallax* (Figure 154) produced no hyaline cells in the presence of high ammonium, high organic nitrogen, or low carbohydrates. In nature, ammonia from decomposing plant matter would be greater under water than around emergent plants, quickly diffusing away in the atmosphere. Likewise, amino acids from organic decomposition would be present only in submersion water, not in rainfall. Response to low carbohydrates may be a limit in carbon available for making additional cell wall tissue, a need for an energy source, or it could relate to CO$_2$ from decomposing plant material in interstitial bog or fen waters.

*Sphagnum* seems to require a tremendous water content to achieve its maximal net photosynthesis, probably supplied by the large reservoir of water in its non-photosynthetic hyaline cells. In *S. fuscum* (Figure 155), a hummock top species, 600-1000% saturation was optimal, whereas in *S. angustifolium* (Figure 156), which tends to occur somewhat closer to the water surface, 900-1300% was optimal (Silvola & Aaltonen 1984), indicating the greater need for water in species that live closer to the water level. The photosynthetic decrease with water reduction was steeper for *S. fuscum*, and plants in the field generally occurred where their water content was within this 600-1000% range. In *S. angustifolium*, however, plants often occurred where their water content was outside their optimum range, thus defining narrow and broad relative niches.

*Sphagnum* is well known for its morphological plasticity in response to water availability (Miller 1991). For example, *Sphagnum magellanicum* (Figure 157) and *S. papillosum* (Figure 158) in dry conditions produce leaves that are longer (Figure 159) with more pores per cell (Figure 160). Li and coworkers (1992) suggest that these modifications may promote water-holding and absorbing properties.
Yet, these two species also differ in their water relations (Li et al. 1992). *Sphagnum magellanicum* (Figure 157) seems to be a better competitor for water than is *S. papillosum* (Figure 158) under dry conditions. This is exhibited by its better water transport ability and greater water content under the same atmospheric moisture conditions (Figure 161). This greater ability may be facilitated by its greater stem diameter due to larger hyaline cells, greater pore number, and smaller leaf size. On the other hand, *S. papillosum* (Figure 158) seems to be a better drought tolerator, having a higher survivorship following severe drought conditions.

Superiority in water transport permits *S. magellanicum* (Figure 157) to occupy a higher position in the hummock than does *S. papillosum* (Figure 158). Li and coworkers (1992) found that when the two species grow intermixed in the higher hummock positions, both species grow better than if either is alone, provided at least half the plants are *S. magellanicum*. They suggest that lateral transport among stems may occur to facilitate this, with *S. magellanicum* providing water for both species. If *S. papillosum* is dominant, even at somewhat lower positions in the hummock, both dry out more quickly.

The cancellinae (sing. cancellina) occur in few bryophytes, but especially in the Calymperaceae, Pottiaceae, Encalypta (Figure 163-Figure 164), and some species of Leptodontium (Figure 162). They are large, empty basal leaf cells, usually hyaline, that form a lattice. In the Calymperaceae, these are porate (having pores), and may serve as water storage cells.

Superiority in water transport permits *S. magellanicum* (Figure 157) to occupy a higher position in the hummock than does *S. papillosum* (Figure 158). Li and coworkers (1992) found that when the two species grow intermixed in the higher hummock positions, both species grow better than if either is alone, provided at least half the plants are *S. magellanicum*. They suggest that lateral transport among stems may occur to facilitate this, with *S. magellanicum* providing water for both species. If
Cell Structure

Cells structures can differ in a variety of ways that can affect water uptake, movement, and conservation. These differences include cell wall thickness, cell wall components, pores in the walls, internal papillae, presence of oil bodies, and vacuole size. These differences have the potential to alter the water relations of the leaves.

Cell Walls

Guerra et al. (1992) included incrassate cell walls among the adaptations of xerophytic mosses. Examples of these include Aloina aloides (Figure 165-Figure 166) and Didymodon fallax (Figure 167-Figure 168).

Proctor (1979a) contends that coarse leaf cell walls (Figure 169) seem to aid water movement, possibly creating more internal capillary spaces among the fibrils of the cell wall (Proctor 1982). Proctor (1984) noted that mosses of dry habitats tend to have thick cell walls that can occupy more than half the cross section of the leaf. Fajuke (2010) further found that six mosses from Nigeria had thick cell walls that helped them survive desiccation.
On the other hand, Frey and Kürschner (1991) could find no correlation between thickened cell walls and increasing aridity. Proctor (1982) also pointed out that such xerophytic mosses as Syntrichia (Figure 170-Figure 171), Encalypta (Figure 172-Figure 173), and Anomodon viticulosus (Figure 174-Figure 175) have quite thin walls and external conduction, suggesting that the thick walls are associated with species having internal conduction.

Figure 170. Syntrichia ruralis, a moss of xeric habitats. Photo by Michael Lüth, with permission.

Figure 171. Syntrichia ruralis leaf lamina cells showing thin walls. Photo by Kristian Peters, with permission.

Figure 172. Encalypta rhabdocarpa showing xeric habitat in Europe. Photo by Michael Lüth, with permission.

Figure 173. Encalypta vulgaris leaf cells with branched papillae and thin cell walls. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 174. Anomodon viticulosus leaf cells and papillae. Photo by Janice Glime.

Figure 175. Anomodon viticulosus leaf cells and papillae. Proctor (1982) considered this species to have thin cell walls, but that does not appear to be the case in this example. Photo by Walter Obermayer, with permission.

The moss Rhacocarpus purpurascens (Figure 136) appears to have a unique means of facilitating rapid absorption of fog, dew, and rain (Barthlott & Schultze-Motel 1981; Edelmann et al. 1998). It has four layers of cell wall with a "peculiar architecture," forming cavities within the wall.
Flexibility of the cell wall is undoubtedly an aid to cell survival. This permits the cells to shrink upon dehydration, up to 50-70% in *Syntrichia ruralis* (Figure 176), without allowing for air to enter the drying cells (Moore *et al.* 1982).

Figure 176. *Syntrichia ruralis* leaf cells with c-shaped papillae and thin walls. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Popper and Fry (2003) suggest that the addition of xyloglucans to the cell wall components may have been an important contribution to the ability of bryophytes to invade land. The presence of high concentrations of uronic acids would have permitted these plants to hold nutrient ions until such time as water was available for transport.

Cell walls seem like the first line of defense against desiccation. Autofluorescing compounds that can strengthen these walls are present in sporangial epidermis, spiral thickenings of elaters, and rhizoids, and leaf cells in the special case of *Sphagnum* (Figure 177) Kroken *et al.* (1996). In charophytes, these resistant compounds have multiple functions that include desiccation resistance and microbial resistance in lower charophytes, a role in embryogenesis in *Coleochaete* (Figure 178) and embryophytes, and decay resistance in structures that characterize bryophytes, such as rhizoids, sporangial epidermis, and elaters.

Figure 177. *Sphagnum palustre* cells showing the spiral thickenings on the hyaline cells. Photo by Malcolm Storey, through Creative Commons.

It appears that the resistance of cell walls to desiccation is an ancient trait, already present in the green alga *Coleochaete* (Figure 178) (Kroken *et al.* 1996). In fact, it seems to be unique to *Coleochaete* among the charophytes and the resistance is produced in response to desiccation stress.

But bryophytes also have this ability – sexual reproduction induces autofluorescence in the cell walls of well-hydrated tissues at the placental junction, suggesting that these cell walls are endowed with compounds (phenols?) that endow them with desiccation resistance (Kroken *et al.* 1996). A similar phenomenon occurs in the gametophyte tissue at the apical end of the *Sphagnum* pseudopodium (gametophyte stalk that suspends the *Sphagnum* capsule away from the plant; Figure 179), suggesting a similar role to that of other bryophytes and even *Coleochaete* (Figure 178).

Figure 178. *Coleochaete*, an alga with slime papillae and other characters that are more common among bryophytes. Photo by Yuji Tsukii <http://protist.i.hosei.ac.jp/>, with permission.

Figure 179. *Sphagnum pseudopodia* supporting capsules. The swollen upper end is desiccation tolerant and houses the foot of the sporophyte. Photo by Joan Edwards, with permission.
**Lignin:** The presence of lignin in bryophytes has been a controversial topic for ages. Lignins are present in xylem and sclerenchyma cells of tracheophytes. To demonstrate whether these substances might be present in bryophyte and charophyte cell walls, Ligrone et al. (2008) examined the charophyte *Nitella* and a number of bryophytes. Using polyclonal antibodies that labelled lignified walls in tracheophytes, they found that these also bound to the cell walls of bryophytes. But rather than the specific locations found in tracheophytes, the locations in mosses and liverworts were not tissue-specific. Hornworts (*Megaceros flagellaris* and *M. fuegiensis*; Figure 180) differed somewhat in that labelling was stronger in pseudoelaters and spores than in other cell types. Cell walls were likewise labelled in the charophyte *Nitella*, but a lack of binding suggested that lignins or lignin-like substances were absent in *Coleochaete*.

![Figure 180. *Megaceros* spores and elaters, a genus in which lignin labelling is stronger in spores and elaters than in other cell types. Photo by Christine Cargill, with permission.](image1)

**Oil Bodies**

Oil bodies are common in the leaf cells of leafy liverworts (Pfeffer 1874; Garjeanne 1903; Müller 1905, 1939; Schuster & Hattori 1954; Pihakaski 1972a, b; Stewart 1978; Schuster 1992; Asakawa 2004), but similar structures are generally absent in mosses. Kronestedt (1983) found that they had seasonal variability in the nature of the matrix and the amount of lipophilic material in the floating liverwort *Ricciocarpos natans* (Figure 181). The globules can coalesce to form larger units. Their role has remained a mystery (He et al. 2013), but recently several researchers have provided evidence that they may have a crucial role in desiccation tolerance.

Oil bodies seem to have different developmental pathways in different species. Pihakaski (1966, 1968, 1972a) compared their development in two leafy liverworts — *Bazzania trilobata* (Figure 182-Figure 183) and *Lophozia ventricosa* (see Figure 184). The component parts are the same in both species: an outer membrane that envelopes the whole oil body, a granular stroma layer that varies in size and thickness, specific globules enveloped by the stroma layer, and a thin inner membrane that surrounds the specific globules. But the oil bodies in these two species develop in different ways. In *B. trilobata*, they develop from vacuole-like formations in the shoot apex or in leaf primordia where certain substances segregate. In this species, granular dense bodies are visible in the cells of the shoot apex, but these shrink in size as oil bodies develop and are absent in the mature leaf cells. In *L. ventricosa* they originate by aggregation and fusion of lipid bodies.

![Figure 181. *Ricciocarpos natans*. Photo by Štěpán Koval, with permission.](image2)

![Figure 182. Oil bodies (transparent) in leaf cells of *Bazzania trilobata*. Photo by Walter Obermayer, with permission.](image3)

![Figure 183. Oil bodies (transparent) in leaf cells of *Bazzania trilobata*. Photo by Walter Obermayer, with permission.](image4)
Duckett and Ligrone (1995) followed the development of oil bodies in gemmae of *Odontoschisma denudatum* (Figure 185). They appear suddenly early in development, forming flat structures associated with the endoplasmic reticulum. Suire (2000) provided evidence that liverwort oil bodies are secretory cell compartments that originate from the endoplasmic reticulum. The oil bodies remain closely associated with the cytoplasmic lipid bodies throughout development but do not fuse with them. Finally they take on their ultimate shape and become suspended by fine cytoplasmic bridges within the vacuoles.

Oil bodies are notorious for disappearing in herbarium specimens. Pressel *et al.* (2009) described this behavior for desiccation-tolerant liverworts. They found that while they are dry, they remain substantially unchanged, but when they are rewet, they initially change drastically, becoming flattened. It requires up to 48 hours for them to regain their normal shapes. However, if the liverworts are dried faster than would typically happen in nature, they, and other organelles, disintegrate when the liverwort is rewet. Pressel *et al.* suggested that loss of shape upon normal rewetting could be evidence of a shift in soluble carbohydrates or other substances into the cytosol, indicating that the oil bodies may be critical to the desiccation tolerance of liverworts.

Galatis *et al.* (1978) found phenolic and "polysaccharidic" compounds but no protein in the oil bodies of *Marchantia palacea* (Figure 186). On the other hand, He *et al.* (2013) reported that in *Marchantia polymorpha* (Figure 187) the oil bodies (Figure 188) contain a protein complex that is immunologically related to plastid and cytoplasm enzymes of the isoprenoid synthesis (isoprenoids belong to a class of organic compounds composed of two or more units of hydrocarbons, with each unit consisting of five carbon atoms in a specific pattern; they have a wide range of roles in physiological processes of plants and animals). Suire *et al.* (2000) similarly found isoprenoid biosynthetic enzymes similar to those found in plastids and the cytosol of *Marchantia polymorpha*. The suggested paucity of protein in the oil droplets of liverworts (Galatis *et al.* 1978) is likewise in sharp contrast with that found in the green alga *Chlamydomonas reinhardtii* (Moellering & Benning 2010). In this alga, 259 proteins were associated with lipid droplets.

Figure 186. *Marchantia paleacea* thallus with archegoniophores, a species with phenolic and "polysaccharidic" compounds but no protein in the oil bodies. Photo from Briofitas de Mexico, through Creative Commons.

Figure 187. *Marchantia polymorpha* with ice crystals. This species has oil bodies that contain a protein complex. Photo by David Taylor, with permission.
Oil bodies of liverworts produce mono-, sesqui-, and diterpenoids, aromatic compounds such as bibenzyl, bis-bibenzyls, and aceto-genins (Asakawa 2008; Asakawa et al. 2013). These often aromatic compounds have such activities as causing allergenic contact dermatitis, antimicrobial action, antifungal and antiviral action, cytotoxicity, insecticidal action, insect antifeedant, superoxide anion radical release, 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase, DNA polymerase β, and α-glucosidase and NO production inhibition, antioxidant, piscicidal, neurotrophic, and muscle relaxation. But these are mostly uses of interest to humans and do little to tell us how the liverwort benefits from them.

It appears that mosses do have their own version of oil bodies. Huang et al. (2009) reported abundant oil bodies in the photosynthetic gametophyte and the spores of Physcomitrella patens (Figure 189-Figure 190). These researchers found that neutral lipids in these oil bodies in the gametophyte were largely steryl esters and triacylglycerols, and unlike some reports on the liverwort oil bodies, they had proteins. These proteins were programmed by three oleosin genes. The expression of these oleosin genes were tissue specific. Structural proteins cover the surfaces of the lipid droplets and prevent them from coalescing during desiccation (Huang et al. 2009; He et al. 2013).

The number of oil bodies in apical gametophyte tissue decreases during the production of sex organs in Physcomitrella patens (Figure 189-Figure 190) (Huang et al. 2009). In spores, the oil bodies serve as food reserves for gluconeogenesis (formation of glucose from smaller molecules) and are equivalent to those of seed oil bodies. It appears that these oil bodies have an energy function for reproduction, but could they be important in providing the energy needed during rehydration as well?

Vacuoles

Bryophytes, for some reason, were long thought to lack vacuoles. However, this is not the case, as demonstrated in the liverwort Lunularia cruciata (Figure 191) (Carginale et al. 2004), the mosses Physcomitrella patens (Figure 189) (Nagao et al. 2005), Ephemerum cohaerens (Figure 192) (Kwok & Rushing 1999), and Fontinalis antipyretica (Figure 193) (Bruns 1998). In fact, the vacuoles can be quite large, as witnessed by the chloroplasts crowded around the periphery of the cell in many species. But there has been no systematic study to indicate which bryophytes have vacuoles and which do not. We might ask if there is some correlation between the ability to withstand drought or to take up water, or even to hold on to cellular water as the environment dries and the presence of one or more vacuoles.

Vacuoles are known in plants to contain solutes that control the water uptake by the vacuole (Taiz & Zeiger 1991). In bryophytes, Nagao et al. (2005) have demonstrated that ABA affected the appearance of vacuoles during treatment with freezing. Since ABA is also involved in drought tolerance and has resulted in the increased osmotic concentration of protonemal cells, this mechanism of vacuolar preparation should be explored for possible relationships to drought tolerance in various
bryophytes. Could presence of a vacuole help the cell take in water more quickly by storing solutes that create an osmotic gradient, yet are safely out of the way of cellular metabolism? Could it also have a role in the ability of the cells to shrink as they dry and expand when wet?

Figure 191. *Lunularia cruciata* thallus section through gemmae cup. This is a species of thallose liverwort with demonstrated vacuoles. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 192. *Ephemerum cohaerens* leaf, a species with demonstrated cell vacuoles in the leaves. Photo by Dick Haaksma, with permission.

Figure 193. *Fontinalis antipyretica* leaf cells, a species with demonstrated cell vacuoles in the leaves. Photo by Janice Glime.

Slime Papillae

In leafy liverworts, slime papillae on marginal leaf cells can help to absorb and hold water, as in the leaf margins of *Porella* (Figure 194) and *Heteroscyphus* (Figure 195) (Daniels 1998). The presence of slime papillae in *Takakia* (Figure 196) was among the reasons why several bryologists originally considered that genus to be a liverwort, but capsule structure confirmed its similarity to mosses.

Figure 194. *Porella pinnata*, a species with slime papillae on the leaf margins. Note white margins at arrows on right. Photo by Des Callaghan, with permission.

Figure 195. *Heteroscyphus coalitus*, a leafy liverwort with slime papillae. Photo by Tom Thekathyil, with permission.

Figure 196. *Takakia lepidozioides* slime papillae. Photo from the Herbarium of Hiroshima University, with permission.
Summary

Bryophytes gain water in their cells both through external (ectohydric) capillary movement and internal (endohydric) transport. Structural adaptations such as overlapping leaves, concave leaves, crispate leaves, plications, revolute or involute margins, lamellae, multi-layered leaves, lobules, hair points, papillae, costae, stolons, borders, leaf teeth, teniolae, alar cells, hyaline cells, cancellinae, resistant cell walls, oil bodies, and vacuoles, aid in moving water, facilitating entry, or reducing loss. In areas with high fog occurrence and little or no rainfall, fog can be a major contributor to the bryophyte water budget.

Overlapping leaves, concave leaves, revolute margins, and involute margins help to hold water in capillary spaces. Alar cells provide a point of entry through thin walls that balloon up and mechanically spread the leaves. The costa and border cells may move water more quickly because the cells are long and have fewer end walls to be crossed. Plications permit leaf expansion in hydrated leaves and conserve moisture in drying conditions, as do twisting and contorted leaves. Hair points collect water from fog and dew and slow down drying by reducing exposed surface area of the leaf above. In leafy liverworts, lobules retain water for species of dry habitats. Lamellae may repel water and prevent water logging in some species, but hold water in capillary spaces in others.

Cell walls may contain phenols and other fluorescing materials similar to lignin to resist water loss. Oil bodies may provide rehydration energy, but their role in water relations is still poorly understood. Vacuoles hold water within the cell and permit expansion and contraction of the cell. Slime Papillae may contribute to absorption and holding of water.

Acknowledgments

An earlier version of this chapter benefitted from the help of Beth Seafone and Medora Burke-Scoll, who helped me tow the line in explaining things without leaving too much to one's imagination, but at the same time not repeating myself. Ron Gratz translated part of the work by Loeske. Linda Luster checked the literature citations, proofread, and made glossy suggestions from a layperson's perspective. I was fortunate to have Ken Kellman ask on Bryonet for examples of bryophytes with a demonstrated cuticle; he was kind enough to share his collected references with me. Thank you to Noris Salazar Allen for sending me the leaf images of Leucophanes.

Literature Cited


## CHAPTER 7-4b

**WATER RELATIONS: LEAF STRATEGIES – CUTICLES AND WAXES**

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CHAPTER 7-4b

WATER RELATIONS: LEAF STRATEGIES – CUTICLES AND WAXES

Figure 1. *Pohlia wahlenbergii* var. *glacialis* in Norway. The drops of water on the surface are being repelled by hydrophobic surface waxes, preventing water logging. Photo by Michael Lüth, with permission.

Bryophytes Do Have Cuticles

Anyone who has observed the speed with which many mosses and leafy liverworts absorb water would assume that they lack waxes. But as we examine these bryophytes with chemical and SEM methods, we find that this assumption is not reliable (Buda *et al.* 2013). For example, the simple moss *Physcomitrella patens* (Figure 2) has a cuticle with a chemical composition and structure similar to that of flowering plants. It is likely that the cuticle was a necessary factor in the evolution to land, regulating water status and providing protection from biotic and abiotic stresses. Using knockout genes to create mutant plants that were "severely deficient in cuticular wax accumulation" Buda *et al.* found that these plants also had reduced desiccation tolerance. The gene responsible for the cuticle wax formation in *Physcomitrella patens* is the same one as that in *Arabidopsis thaliana*, indicating its evolution early in the invasion of land.

Figure 2. *Physcomitrella patens*, a moss from which the cuticular wax gene has been isolated. Photo by Janice Glime.
Although thickened waxy cuticles seem to be rare in moss gametophytes, various mosses have some sort of cuticular covering. In some mosses, this is expressed as granules, platelets, or ribbons that are soluble in chloroform (Proctor 1982), satisfying the test for cuticular wax in tracheophytes. This type of cuticle endows *Pohlia cruda* (Figure 3), *P. wahlenbergii* (=*P. albicans*; Figure 4), *Saelania glaucescens* (Figure 55), *Schistostega pennata* (Figure 5), *Pogonatum urnigerum* (Figure 6), and many *Bartramiaceae* with their *glaucous* (whitish) appearance (Proctor 1982). We shouldn't be surprised that *Pogonatum urnigerum* has surface waxes similar to those of tracheophytes, but even primitive mosses such as *Andreaea rupestris* (Figure 7) have surface waxes that are similar to the epicuticular waxes of tracheophytes (Haas 1982).

In addition to most of the species already named, Stránsky et al. (1967) found n-alkanes (19-33 carbon atom chains) in *Leucobryum glaucum* (Figure 8), *Rhytidiadelphus triquetrus* (Figure 9), *Conocephalum conicum* (Figure 10), *Porella platyphylla* (Figure 11), *Pellia fabbroniana* (Figure 12), and *Pellia epiphylla* (Figure 13). Even *Sphagnum* is known to have waxes (lignoceryl alcohol) in *S. capillaceum* (*S. nemoreum*; Figure 59), *S. fuscum* (Figure 60), and *S. magellanicum* (Figure 61) (Ives & Neill 1958).
The documentation of cuticle in bryophyte leaves is somewhat scant. Nevertheless, Proctor (1979b) examined 43 species of mosses and determined that 12 of these have a well developed surface wax on the leaves that is comparable to that of flowering plants. Eight more have traces of wax. Not surprisingly, all of these species are endohydric (have internal conduction). If a moss has a waxy or glaucous look, it most likely has surface wax. A good example of this is the leaves of Polytrichum (Figure 14-Figure 18). On the other hand, Atrichum undulatum (Figure 19-Figure 21), in the same family (Polytrichaceae), has no discernable wax and lacks the waxy appearance. Instead of having leaves that curl inward and wrap around the stem upon drying, members of Atrichum have wavy leaf surfaces and become contorted when they dry (Figure 22-Figure 23).
Figure 14. *Polytrichum commune* leaves showing waxy surface. Photo by James K. Lindsey, with permission.

Figure 15. *Polytrichum commune* leaf lamellae where waxes are present on the terminal cells. Photo from Botany Department, University of British Columbia, with permission.

Figure 16. SEM of *Polytrichum commune* leaf cuticle. Photo by Michael Proctor, with permission.

Figure 17. *Polytrichum juniperinum* showing leaves with lamina rolled over lamellae and waxy appearance. Photo by Michael Lüth, with permission.

Figure 18. *Polytrichum juniperinum* side view of lamella with thick waxy layer on top. Photo by Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 19. *Atrichum undulatum* showing lack of glaucous coloring and presence of wavy leaves that curl and twist when drying. Photo by Janice Glime.
Figure 20. *Atrichum undulatum* leaf showing tips of lamellae. Photo by Walter Obermayer, with permission.

Figure 21. *Atrichum undulatum* leaf cross section showing lamellae. This moss has little or no wax on its leaves. Photo by Walter Obermayer, with permission.

Figure 22. *Atrichum altecrisatum* drying (lower part of image). Photo by Eric Schneider, with permission.

The array of species with demonstrated waxes is a mixed group of xerophytes and hydrophytes (Proctor 1979b, 1982). *Pohlia wahlenbergii* (Figure 24-Figure 26), a glaucous moss of wet habitats, has a high content of wax, whereas *Pohlia nutans* (Figure 27-Figure 28), a ubiquitous moss often found in dry habitats, has little wax. *Pohlia cruda* (Figure 29-Figure 31), a species of moist places, has a high content like that of *P. wahlenbergii*. It appears that the wax in these species is important to prevent water logging, allowing for gas exchange for photosynthesis.

Figure 23. *Atrichum undulatum* leaf lamellae and out of focus areas that indicate undulations. Photo by Walter Obermayer, with permission.

Figure 24. *Pohlia wahlenbergii* showing a wet habitat that is typical for it. Photo by Michael Lüth, with permission.

Figure 25. *Pohlia wahlenbergii* showing water droplets that are repelled by the waxy surface. Photo by Michael Lüth, with permission.
Figure 26. SEM of *Pohlia wahlenbergii* cuticle. Photo by Michael Proctor, with permission.

Figure 27. *Pohlia nutans* showing a typical dry, exposed habitat for the species. Photo by Michael Lüth, with permission.

Figure 28. *Pohlia nutans* showing the lack of a glaucous or waxy appearance. Photo by Michael Lüth, with permission.

Figure 29. *Pohlia cruda* in a crevice where moisture can be maintained, showing a waxy appearance. Photo by Michael Lüth, with permission.

Figure 30. *Pohlia cruda*, a glaucous moss with a high leaf wax content. Photo by Michael Lüth, with permission.

Figure 31. SEM of *Pohlia cruda* cuticle. Photo by Michael Proctor, with permission.

Bartramia pomiformis* (Figure 32-Figure 34) has a whitish appearance and has a high degree of wax covering (Figure 34) (Proctor 1979b). In the same family, Conostomum tetragonum* (Figure 35) has an intriguing 3-d mesh of wax (Figure 36).
Xu et al. (2009) found surface wax on the leaves of the desert moss *Syntrichia caninervis* (Figure 37). These waxes were comprised of fatty acids, alcohols and alkanes. In this species, the wax crystals shift as the leaf ages, increasing the percentage and weight (13.6%; 1150 μg g\(^{-1}\) DW) of very long-chain components in young leaves to 37.2% and 2640 μg g\(^{-1}\) in older leaves. Furthermore, when juvenile leaves experienced dehydration followed by rehydration the wax content of juvenile leaves increased by 35.17%. In lab-cultivated leaves subjected to three wet/dry cycles, the wax content increased by 1900%.
Some bryophytes are puzzling at first glance. For example, Hedwigia (Figure 38-Figure 40) species appear to be waxy, yet absorb water rapidly. But members of the Hedwigiales lack waxes (Pressel & Duckett 2011). This puzzle unravels when we understand the role of the papillae (which typically make leaves look whitish) on the leaves of Andreaeales, Grimmiales (Figure 41-Figure 42), Pottiales (Figure 43-Figure 46), Hedwigiales, and Orthotrichales. Based on experiments by Proctor (1979a) and confirmed by Pressel and Duckett (2011), the water enters these leaves by flowing within channels in the striated (having linear marks, slight ridges, or grooves on surface, often one of number of similar parallel features) cell walls and between the papillae, causing rapid uptake of water through the leaf surface between the papillae. These taxa lack waxes.
In genera such as *Aloina* (Figure 47), *Crossidium* (Figure 48-Figure 49), and *Pterygoneurum*, (Figure 50-Figure 52) the water enters between the leaf lamellae (Figure 51-Figure 52) (Proctor 1979a; Pressel & Duckett 2011). All these taxa grow in habitats where intermittent dehydration/rehydration, often in rapid sequence, is common. However, in the *Polytrichaceae* (Figure 14-Figure 18), water logging between the lamellae is an issue, depressing gas exchange needed for photosynthesis (Proctor 1979a, 1982, 1984). These leaves are protected by abundant waxes that prevent water from entering the spaces between the leaf lamellae. Instead, air bubbles are trapped in these spaces.
One of the problems that bryophytes must face is having ice or ice crystals on their surfaces (Figure 53-Figure 54). These crystals are very hygroscopic, potentially causing the kind of dehydration that can occur to your meat in the freezer. A waxy cuticle could serve like a plastic freezer bag, in this case preventing the water from being drawn from the cells. This role for the wax, if present, remains to be tested.
The presence of a white flocculent material on the moss *Saelania glaucescens* (Figure 55-Figure 57) is widely known. *Saelania glaucescens* has been a puzzle to bryologists and biochemists. Its whitish covering is predominantly on the backs of the leaves and takes the form of a hoary appearance, not a smooth or shiny surface. Although this material has been identified as kauranol plus several minor waxes (Nilsson & Märtensson 1971), the reason for the peculiar arrangement that looks like a thin layer of minute angel hair remains a mystery. Bryologists have suggested that it might be caused by parasitic fungi or bacteria, but there is no evidence to support these ideas (Märtensson & Nilsson 1974). Likewise, it does not seem to be the result of any normal metabolic product. Proctor's (1979b) analysis demonstrates that this is a heavy coating of waxes with webleike ridges covered by a fine, cobwebby matrix of wax.
**Sphagnum**

*Sphagnum fimbriatum* (Figure 58-Figure 62), a peatmoss of wet habitats, has an osmiophilic layer (one that stains with osmium tetroxide, indicating wax) that resembles the early developmental stage of tracheophyte cuticle (Cook & Graham 1998). One might think that a wet habitat moss would not need such protection, but in summer these peatmosses can become quite dry, so such a layer may help to reduce desiccation. On the other hand, this layer may prevent water logging at times when this moss is submersed (*cf.* Pressel & Duckett 2011). The cuticle in *S. fimbriatum* is sheetlike with regular ridges that run parallel to the edges of the "thalli." Our next question is how can a leaf with a cuticle use it for protection from desiccation and yet be able to absorb water. In *Sphagnum*, this may be facilitated by the pores, but might the structure of the cuticle play a role?

Figure 58. *Sphagnum fimbriatum*, a moss with a known osmiophilic layer resembling an early developmental cuticle of tracheophytes. Photo by Michael Lüth, with permission.

Figure 59. *Sphagnum capillaceum* (= *S. nemoreum*), a *Sphagnum* species known to have waxes (lignoceryl alcohol). Photo by Michael Lüth, with permission.

Figure 60. *Sphagnum fuscum*, a *Sphagnum* species known to have waxes (lignoceryl alcohol). Photo by Michael Lüth, with permission.

Figure 61. *Sphagnum magellanicum*, a species known to have waxes. Photo by Michael Lüth, with permission.

Figure 62. *Sphagnum fimbriatum* leaf cross section, a moss that has an osmiophilic (waxy) layer on the outside of the leaf cells. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

**Leafy Liverworts**

Even the leafy liverworts can have waxes. Heinrichs *et al.* (2000) support the contention of Cook and Graham (1998) that this innovation occurred prior to the evolution of bryophytes from their algal ancestor. Although only six (5 of *Plagiochila* plus *Plagiochilion mayebarae*) of the 81 species of *Plagiochilaceae* in the study had surface waxes on their leaves (Heinrichs *et al.* 2000), this is a family with
many members in damp or wet, even submersed, habitats. They found that *P. tabinensis* contains 1.4% of its dry weight as surface waxes. These are comprised of steryl esters, triacylglycerols, and free fatty acids.

By using the electron microscope, Heinrichs and Reiner-Drehwald (2012) found surface wax in the leafy liverworts *Lejeunea flava* (Figure 63), *Mytilopsis albifrons*, *Dinckleria pleurata*, and *D. fruticella*, representing the families *Lejeuneaceae*, *Lepidoziaceae*, and *Plagiochilaceae*, respectively.

They found that many members in damp or wet, even submersed, habitats. These are comprised of steryl esters, triacylglycerols, and free fatty acids.

Figure 63. *Lejeunea flava*, a leafy liverwort known to have a cuticle with surface wax. Photo by Jonathan Sleath, with permission.

**Admissibility of Water**

If leaves of bryophytes are covered with waxes, how does water enter these largely ectohydric plants? One suggestion is that the bases of the leaves may lack a cuticle, but that would seem to slow down water entry and be maladaptive for gathering dew or taking advantage of short desert rainfall events. But the structure of the wax itself may solve this problem. Shepherd and Wynne Griffiths (2006) describe the layered sheets of wax as probably discontinuous and surrounded by further solid amorphous regions. The crystalline regions are considered impermeable, but water and solutes are able to diffuse through the cuticular wax by way of the amorphous zones. This requires a greater travel distance than a straight path through the surface, but it seems a better route than travelling to the leaf base, then travelling internally through cell contents and cell walls to reach the tip of the leaf.

**Multiple Forms – Multiple Roles**

The cuticle, like many plant features, can serve multiple advantages for plants. In tracheophytes it is able to reduce water loss, prevent water logging, protect against high light intensity, reduce the temperature, reduce osmotic stress, prevent physical damage, protect against altitudinal stresses (light, extreme temperatures, wind), and protect against pollution (Shepherd & Wynne Griffiths 2006).

In tracheophytes, waxes exist in several forms, including rods, ribbons, filaments, tubes, and plates (Shepherd & Wynne Griffiths 2006). Among the bryophytes, for five *Plagiochila* (leafy liverwort) species studied, two exhibited wax platelets and three exhibited wax rodlets (Heinrichs *et al.* 2000). Proctor (1979b) demonstrated several forms among mosses (Figure 31, Figure 34, Figure 36, Figure 57).

Among the tracheophyte forms, wax tubes are associated with mid-chain oxy-substituents, such as β-diketones, hydroxy-β-diketones, diols, and secondary alcohols (Shepherd & Wynne Griffiths 2006). Platelets are associated with primary alcohols with a terminal oxy-substituent. Are these same factors influential in bryophyte wax morphology? If so, is there any adaptive significance for these differences?

**Temperature**

We know that in tracheophytes temperature, light intensity, and humidity influence the wax morphology, but since these three factors typically act together, it is often difficult to tease out cause and effect (Shepherd & Wynne Griffiths 2006). For example, in *Citrus aurantium*, a higher daytime temperature during leaf development reduces the quantities of alkanes, primary alcohols, fatty acids, and alkyl esters per unit area (Riederer & Schneider 1990). But except for the esters, the amounts of these same compounds increase with higher night-time temperatures.

At higher temperatures, the waxes are more likely to form plates and flakes, whereas at lower temperatures they are more likely to form vertical structures such as rods and tubes (Shepherd & Wynne Griffiths 2006). But waxes at higher temperatures also often form complex dendritic shapes. Tubular forms of waxes are thermodynamically unstable due to their high surface area to volume ratio, so an input of energy, typically heat, can transform them into compact planar forms that are thermodynamically more stable. And tubes can turn into dendrites when the temperature is raised. On the other hand, rapid cooling can also favor dendrite formation. Furthermore, more waxes are produced at lower temperatures.

**Light**

Shorter, less elaborate wax structures are often associated with greater illumination (Shepherd & Wynne Griffiths 2006). Thick waxes such as those in *Eucalyptus* leaves increase reflectance and reduce photosynthesis, but in "non-waxy" leaves (i.e. not appearing waxy or glaucous), there is no effect. In wheat, reflectance is proportional to the amount of wax present, with higher reflectance reducing light transmission to underlying mesophyll cells (Johnson *et al.* 1983). Higher radiation levels can cause an increase in wax thickness in many plants, suggesting an inducible mechanism to protect the cells (Baker 1974; Giese 1975; Reed & Tukey 1982; Shepherd *et al.* 1995).

But it seems unlikely that the thin cuticle of bryophytes has much of an effect on reflectance or photosynthesis. Nevertheless, as will be seen in the chapter on light relations, many bryophyte leaves transmit more light when wet than when dry, suggesting that papillae or other surface features may screen light, thus protecting the DNA and chlorophyll, but that when water fills in the spaces, light is transmitted rather than scattered. Are these waxes protective agents against UV radiation when the...
bryophytes are dry? But UV reflectance is an uncommon adaptation among tracheophytes. It can range from <10% in most plant species, to 70% in only a few others (Caldwell et al. 1983; Barnes et al. 1996).

Waxes may play yet another role for the light-limited bryophytes. Droplets held on wettable tracheophyte leaves can focus solar radiation up to 20 times (Brewer et al. 1991). We might imagine that bryophyte leaves or multiple plant tips might trap water droplets that likewise focus the light in some low-light habitats. This focussing would occur at the actively growing tips in most acrocarpous mosses.

Salt Stress

Waxes also protect leaf cells from salt stress (Shepherd & Wynne Griffiths 2006). Examples from tracheophytes suggest that an increase in wax production may be an inducible response to increased salt exposure. Fujiwara et al. (2002) found that pre-treating cabbage seedlings with NaCl induced hardening, improving drought resistance. It would be interesting to compare wax content among bryophytes that grow within areas affected by salt spray to the same species grown away from its influence.

Contact Angles and Entry

Contact angles are important for water entry. Brewer et al. (1991) found that changes in the contact angle and wettability are also associated with changes in wax composition and morphology in tracheophytes. These observations present interesting questions for bryophytes. If leaves have waxes over the leaf lamina, but lack waxes at the base, water will roll to the leaf base where uptake is easy. This movement to the leaf base would further facilitate the solution of deposited nutrients and carry them to the base for absorption (Cape 1996 for tracheophytes). Because of the mode of water uptake in bryophytes, this feature is likely to be more important than it is in tracheophytes. Hence, we should expect the angle of the leaf to be important in this nutrient and water gathering. At the same time, it presents dangers for collecting deposited pollutants.

Combined Role and Water Relations

Let's continue under the assumption that at least in some cases the waxes on bryophytes may play a role in reflectance and scattering of light to a degree that can lower the temperature of the bryophyte cells they cover. This will, in turn, reduce the vapor pressure difference between the leaf tissue and the air, reducing the loss of water through transpiration. But all these assumptions remain to be tested in bryophytes.

Altitude Protection

High altitude imposes stresses that include weathering, dehydration, low temperatures, and greater UV light intensity. In conifers, a thicker wax coverage is characteristic of high altitude growth (Günthardt 1984; Riolo 1999). We need to look for a similar relationship in bryophytes.

Pollution Protection

Ozone

In tracheophytes, ozone causes a severe reduction in the formation of new wax, but it does not seem to affect existing wax (Carlsson et al. 1994; Hellgren et al. 1995). Its effect on bryophyte cuticles remains unknown, but failure to replace cuticle could have severe consequences in prevention of water logging and protection from UV radiation.

CO₂

The effects of CO₂ on tracheophytes are varied, in some cases causing an increase in waxes and in others a decrease (Shepherd & Wynne Griffiths 2006). The effect on bryophyte cuticles remains to be demonstrated.

Repelling Water

Despite all of these reports on waxes on the cuticle of bryophytes, we know little of their role. Mårtensson and Nilsson (1974) comment that not all of the aforementioned waxy species are shiny, including Rhytidiadelphus triquetrus (Figure 9), although I would consider it to be shiny. Others, such as Sphagnum subnitens (Figure 64-Figure 65) and S. subfulvum (Figure 66) are shiny when dry, but lose their shine when moist, suggesting that the relationship is complex and is not a matter of simple reflection. It would be interesting to determine their role in repelling water to avoid water logging vs retaining water in times of drought.
Figure 66. *Sphagnum subfulvum*, a species that is shiny when dry due to waxes. Photo by Michael Lüth, with permission.

The role of repelling water may be more important than that of retaining water. I have mentioned a potential role of repelling water, as seen in Figure 1. Gas exchange works poorly through a wet surface. Waxy or oily surfaces help to repel the water, yet allow a higher rate of gas exchange than does water. Hence it is not uncommon to find such surfaces among aquatic bryophyte taxa (Proctor 1984).

Proctor (1984) contends that the ability to shed surface water is important to bryophytes in their low-light habitats of crevices and caves or in waterside habitats of waterfalls. He cites the waxy surfaces of the tops of photosynthetic lamellae of the *Polytrichaceae* (Figure 6, Figure 14-Figure 18) as support for this contention. This might also be supported by the waxy surface of *Pohlia cruda* (Figure 29-Figure 31), a common species in crevices.

As discussed in Chapter 7-3 of this volume, the pores of *Marchantia* (Figure 67), as in the stomata of tracheophytes, have strongly water-repellent ledges (Schönherr & Ziegler 1975; Figure 68), like the waxy ridges of tracheophytes, preventing water from entering and interfering with the photosynthetic interior.

Mosses like *Plagiomnium* (Figure 69) have both upright and horizontal stems. But this genus has a different problem from most mosses in obtaining water. Its leaves repel water, as known by anyone who has tried to wet them to make a slide. It has perhaps solved this problem by its well-developed hydroids and leptoids, and even false leaf traces (Figure 70).

Figure 67. *Marchantia polymorpha* with gemmae cups and tiny white dots that indicate pores. Photo by Jan-Peter Frahm, with permission.

![Marchantia polymorpha](image1)

Figure 68. Section through pore of *Marchantia polymorpha*, with waxy ledge indicated by arrows. Photo by George Shepherd, through Creative Commons.

Figure 69. *Plagiomnium ellipticum* with drops of water on its water-repellent (waxy) surface. Photo by Des Callaghan, with permission.

Figure 70. *Plagiomnium ellipticum* stem cross section. This is a moss with both upright and horizontal (plagiotropic) stems. It most likely benefits from having both hydroids and leptoids to transport substances because its leaves are very resistant to getting wet and typically repel water. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.
In some cases, there are special adaptations for bringing water into the leaves while at the same time being able to prevent water loss. In *Rhacocarpus purpurascens* (Figure 71), a moss of exposed habitats that experience frequent alternation of drought and heavy precipitation, the cell walls are trilamellate (Pressel et al. 2010). The outer layer is porous and ensures rapid uptake of water and retention. At the same time its very hydrophobic cuticle-like layer prevents waterlogging. The middle lamellar stratum permits extension of protoplast hydration, allowing the metabolism to remain active under drying conditions. *Sphagnum*, on the other hand, can become waterlogged and experience depressed metabolism as a result.

![Figure 71. *Rhacocarpus purpurascens*, a moss that repels water. Photo by Michael Lüth, with permission.](image)

**Summary**

Bryophytes gain water in their cells both through external (ectohydric) capillary movement and internal (endohydric) transport. Structural adaptations such as overlapping leaves, concave leaves, crispate leaves, plications, revolute or involute margins, lamellae, multi-layered leaves, lobules, cuticles, hair points, papillae, costae, stereids, borders, cancellinae, teniolarae, alar cells, hyaline cells, pores, oil bodies, vacuoles aid in moving water, facilitating entry, or reducing loss. In areas with high fog occurrence and little or no rainfall, fog can be a major contributor to the bryophyte water budget.

Waxes are known from all the major groups of bryophytes, including such aquatic taxa as *Sphagnum*, and occur in a wide range of habitat moisture, but many species seem to lack them. Glaucous species may be so because of waxes or because of dense papillae. The water repellent nature of waxes keeps water from entering pores of a thallus and prevents water from remaining on plants in a way that blocks gas exchange. The presence of waxes may relate to endohydry or to living in places where water lingers on the plants. The role may be more to keep water away than to hold water in.

Waxes need to be arranged on a leaf to admit water, at least at the base, but they may have an amorphous layer that transmits water. Multiple forms of waxes seem to be correlated with various environmental parameters such as UV light, temperature, salt stress, contact angle, and altitude, but these correlations have not been explored in bryophytes. Cuticles can offer protection from such pollutants as ozone and CO\(_2\) and may play a role in preventing absorption of airborne pollutants that land on the surfaces of the leaves.

**Acknowledgments**

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Figure 1. *Riccia cavernosa*, a thallose liverwort that dries out during drought and recovers in the fall when rain returns. Photo by Jan-Peter Frahm, with permission.

Water Relations on Land

Proctor (2014) points out that one of the basic needs of bryophytes is that of coping with the intermittent availability of water. To this end, poikilohydry is efficient at the small scale of a bryophyte, whereas endohydry is more beneficial for the large tracheophytes.

Physiological adaptations relate on one end to the morphology and on the other to the biochemistry. Although we have recognized morphological characters for a very long time, few have actually been tested experimentally on a large scale for their adaptive value in altering physiology. The biochemical adaptations, on the other hand, constitute a new and emerging field of bryology, one that coincides closely with physiology of tracheophytes. By using the more easily studied bryophytes, we have gained the possibility of better understanding of the physiology of tracheophytes. This unusual interest in bryophytes is largely because of the relative ease with which genes can be moved into them or knocked out of them and their expressions be observed. And both bryophyte and fern gametophytes exhibit desiccation tolerance, whereas this ability is rare among sporophytic seed plants (Watkins *et al.* 2007). Long live the gametophytes! Even the lichens seem to have less desiccation tolerance than the bryophytes (Green *et al.* 2011).

Oliver *et al.* (2000) hypothesized that for photosynthetic plants to move onto land, desiccation tolerance was crucial. Using species of "resurrection plants" from both bryophytes and tracheophytes, Fisher (2008) concluded that desiccation tolerance arose among propagules as a means of survival. In bryophytes, nearly every part is a potential propagule in most species. For example, Maheu (1902) found that the moss *Tortula muralis* (Figure 2) would regenerate protonemata after
being stored dry for 14 years. Physiological adaptations may permit the bryophyte to retain water or to recover from loss of water, and to change its strategies with the seasons or the climate.

Alpert (2000) presented two main puzzles from the observed habitat patterns of desiccation-tolerant plants. "What are the mechanisms by which plants tolerate desiccation?" and "Why are desiccation-tolerant plants not more ecologically widespread?" There appear to be multiple mechanisms of tolerance, including protection from oxidants and loss of normal configuration of macromolecules during dehydration. Alpert suggests that their inability to occupy a wide ecological range is due to their inability to maintain a cumulative positive carbon balance during their repeated wet/dry cycles and the tradeoffs between desiccation tolerance and growth rate.

**Drought Tolerance vs Avoidance**

As clear as the two words *tolerance* and *avoidance* may seem, they can lead to confusion because of differences in perspective. During (1979) tells us that *drought tolerance* is the ability to survive and maintain activity despite a lack of water in the environment. Proctor (2000) gives a more physiological definition that considers drought-tolerant plants to be those that are able to maintain a more or less normal metabolism at lowered cell volume and water potential, while tolerating elevated ionic concentrations in the cytoplasm and external environment. This physiological type of maintenance may be in evidence for the drought-tolerant *Hedwigia ciliata* (living on exposed boulders; Figure 82) and *Grimmia pulvinata* (often living on concrete; Figure 3). During a 5-day sequence of natural field drying, they showed no sign of plants drying and both maintained their photochemical efficiency, exhibiting normal day-night patterns (Schroeter et al., 1999).

Plants that show *tolerance* have vegetative parts that endure the stress period as best as possible (During 1979). But where is that lack of water, in the environment, or in the plant? I prefer to clarify this and say that *drought tolerance* is the ability of the plant to survive in a *habitat* that becomes dry. *Desiccation tolerance* is the ability of the *plant* to survive periods during which the *cells* are water-stressed and the plant itself has become dry; it suffers dehydration of all its metabolic systems. Such vegetative desiccation tolerance is rare among tracheophytes, with few species withstanding vegetative desiccation: 60-70 species of fern and fern allies and 60 species of angiosperms (Oliver et al. 2000). Instead, most tracheophytes survive through reproductive structures. Bryophytes (and lichens), on the other hand, exhibit vegetative desiccation tolerance as well as through reproductive structures (Kappen & Valladares 1999; Proctor et al. 2007).

For sake of clarity, let us consider *drought* to be a condition of the environment and *desiccation* to be a condition of the plant, in this case the bryophyte. For tracheophytes, drought in the environment nearly always causes desiccation in the plant, but for bryophytes, this may not so often be the case.

Using that terminology, *drought tolerance* can be accomplished in two ways: *desiccation tolerance* and *desiccation avoidance*. Desiccation avoidance is the ability to prevent desiccation from occurring within the plant or the ability to go into a *dormant stage* during periods of low water availability; it is often characterized by plants that die and leave stress-tolerant *diaspores* (any structures that become detached from parent plant and gives rise to new individuals) that will grow the next season. Note the use of the word *stage* here, not *state*. For bryophytes, spores and gemmae provide dormant *stages*, although the entire mature sporophyte might be considered a stage that does not require water. On the other hand, a desiccation-tolerant vegetative plant can go into a dormant *state*, where metabolic activity slows to an imperceptible level, but where this same plant stage will regain its ability to gain carbon and grow.

Using these concepts, Smith (1986) considers that true desiccation tolerance among plants is rare or non-existent. The tracheophytes may in fact never be desiccation tolerators (Larcher 1983), generally relying on avoidance by storing water or by going into a dormant life cycle stage until the return of sufficient water (Smith 1986). Bryophytes, on the other hand, can be true desiccation tolerators, and suffer relatively little damage at relative humidity levels far below those tolerated by tracheophytes (Table 1). They do this in a vegetative stage through mechanisms that avoid desiccation damage.

**Desiccation resistance**, the ability to maintain an adequate water supply under drought conditions, is actually *drought avoidance*. Drought avoidance also includes the
ability to revert to a dormant stage that requires no water, such as spores and tubers. Water is essential to all life, and the ability to obtain it under limiting conditions or to store it until more is available permits some organisms to live in conditions that are intolerable for others. Most perennial bryophytes do not have the option of disappearing into the soil for the winter, and in fact the period of greatest drought for many of them is in the summer. However, bryophyte growth generally ceases during this hot and often dry time and metabolic activity is slowed considerably, if not completely.

Table 1. Comparison of desiccating percent relative humidity levels tolerated by various groups of plants. Table modified from Larcher (1983).

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>%RH Tolerated without injury</th>
<th>%RH Moderate without injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine algae</td>
<td>99-97</td>
<td>14-41</td>
</tr>
<tr>
<td>Deep water algae</td>
<td>95-86</td>
<td>69-204</td>
</tr>
<tr>
<td>Algae of the ebb line</td>
<td>86-83</td>
<td>204-252</td>
</tr>
<tr>
<td>Intertidal algae</td>
<td>95-86</td>
<td>69-204</td>
</tr>
<tr>
<td>Liverworts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygrophytes</td>
<td>usually 95-90</td>
<td>92-90</td>
</tr>
<tr>
<td>Mesophytes</td>
<td>usually 92-50</td>
<td>90-36</td>
</tr>
<tr>
<td>Xerophytes</td>
<td>usually 36-0</td>
<td>0</td>
</tr>
<tr>
<td>Mosses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water mosses and hygrophytes</td>
<td>95-90</td>
<td>69-141</td>
</tr>
<tr>
<td>Mesophytes</td>
<td>usually 90-50</td>
<td>96-92</td>
</tr>
<tr>
<td>Xerophytes</td>
<td>usually 5</td>
<td>95-90</td>
</tr>
<tr>
<td>Fern gametophytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest ferns</td>
<td>&gt;90</td>
<td>50-90</td>
</tr>
<tr>
<td>Rock ferns</td>
<td>40-60</td>
<td>20-30</td>
</tr>
<tr>
<td>Tracheophytes (tissue sections)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf epidermis</td>
<td>96-92</td>
<td></td>
</tr>
<tr>
<td>Mesophyll</td>
<td>95-90</td>
<td></td>
</tr>
<tr>
<td>Root cortex</td>
<td>97-95</td>
<td></td>
</tr>
</tbody>
</table>

Both desiccation avoidance and desiccation tolerance strategies are available to bryophytes (Figure 4-Figure 5). You will soon see that whereas desiccation tolerance may be unavailable to tracheophytes, it is of considerable importance for bryophytes.

**Desiccation Tolerance**

In 1702 Anthony von Leeuwenhoek examined dry sediment from a gutter after hydrating it for an hour, and found tiny animals swimming about (Alpert 1982, 2000). These animals, rotifers, seemingly had arisen from the dead. Leeuwenhoek followed with experiments that showed these animals could remain in this dry state for months. But he did not imagine that they had lost all moisture because they retained their normal oval shape. During the next century, experiments demonstrated that rotifers, nematodes, and tardigrades all could undergo a dry, dormant state. In fact, some organisms can survive for over ten years without water, reaching immeasurably low water potentials (Alpert 2000). In this desiccated state they can endure temperature extremes from 0ºC to 100ºC.

But what was this dormant state? Words such as anabiosis, abiosis, revivification, and resuscitation, arose to describe the dry state and ability to return from it (Alpert 1982). The term cryptobiosis, however, seems most appropriate, avoiding the question of whether or not the organism is still alive. Instead, it refers to the state of an organism when it shows no visible sign of life, when its metabolic activity is immeasurable (hidden life).

Figure 4. Distribution of a number of genera of mosses relative to mesic and xeric conditions and their strategies of avoidance vs tolerance. Modified from Vitt et al. 2014.

Figure 5. Distribution of a number of genera of mosses relative to mesic and xeric conditions and their strategies of avoidance vs tolerance. Modified from Vitt et al. 2014.

Low temperature physics helped to clarify the issue. Recognizing absolute zero as -273ºC, the temperature at which everything freezes and all molecular movement stops, Becquerel (1950a, b, c, 1951) subjected tardigrades, rotifers, algae, seeds, bacterial and fungal spores, fragments of the lichen Xanthoria parietina, and leaves of the mosses Grimma (Figure 79-Figure 80) and Barbula (Figure 6) to two-hour treatments at temperatures very close to 0.0ºK (0.05-0.008ºK). These organisms returned to their active state and bacteria even reproduced. Syntrichia ruralis (Figure 77-Figure 93) survived after 24 hours at -198ºC.
(Bewley 1973). Based on typical reduction in metabolism of $\frac{1}{2}$ for every 10°C drop in temperature, Becquerel calculated that at absolute zero metabolism would be 7.13 trillion times as slow as the normal rate at 15°C (see Alpert 2000).

This did not support the hypothesis that life ceased and then was reactivated. In fact, three arguments can be made against that hypothesis, some of which have been demonstrated for desiccated mosses. First, Dilks and Proctor (1976b) have demonstrated that mosses recover more slowly as duration of desiccation increases, ultimately reaching a duration from which they are unable to recover. Second, for most organisms in this cryptobiotic state, there is still a minute uptake of oxygen (Pigón & Weglarska 1955a,b), indicating retention of metabolism. Third, there is a point at which all these organisms die.

Such desiccation tolerance, a common phenomenon among bryophytes, seems to have been lost in the evolution of tracheophytes. Rather, tracheophytes have experienced increased growth rates, more structural and morphological complexity, and mechanisms for conserving water rather than recovering from its loss (Oliver et al. 2000). Only in their reproductive structures, particularly seeds and underground storage organs, have tracheophytes retained and diversified the strategy of desiccation tolerance.

Norris (1990) contends that four dimensions of water relations must be understood to understand the problems of bryophytes compared to tracheophytes. To this I have added the fifth as a result of more recent experiments:

1. hydration/dehydration frequency
2. hydration duration
3. dehydration duration
4. degree of water loss
5. rate of water loss.

With the need for repair whenever moss cells become dry, it is not surprising that the frequency of the wet-dry cycle and the duration of the hydration period are important in determining survival. Even in such xerophytic bryophytes as Grimmia pulvinata (Figure 3), living on rock walls in Britain, the median length of wet and dry periods is generally between 5 and 15 hours (Proctor 2004). The longest dry periods in early summer are typically 15-17 days, with the longest continuously wet period lasting nearly 28 days. The moss cushions typically remain wet about 1.7 times the duration of rain. It appears that dew fall is insufficient to cause hydration in this species, perhaps because water drops are trapped by the long hairs instead of reaching the leaf lamina. Such a mechanism could protect the species against frequent (daily) wet-dry cycles in which the nightly wet period is insufficient for damage repair before the moss becomes dry again. Growth occurred primarily in autumn when the moss was wet for long periods, despite relatively low levels of irradiation.

Like others, Stark et al. (2013) argued that desiccation tolerance is the most important evolutionary innovation permitting plants to colonize land. They used the desert moss Pterygoneurum lamellatum (Figure 7) and chlorophyll fluorescence to test recovery from drying of 30 minutes to 53 hours. As in other studies, rate of drying is a major factor in recovery, with only the shoot apex escaping the severe damage of very rapid drying. Rapidly desiccated shoots have slower growth rates, fewer regenerative shoots, and a compromised photosynthetic system. The responses to differences in rate of drying indicate that this xerophytic moss has inducible desiccation tolerance, in contrast to the assumption that xerophytic bryophytes have only constitutive desiccation tolerance.

Although Sphagnum (Figure 63) may not be a good model for other kinds of bryophytes, it gives us an idea of the evaporative relationships of these non-tracheophytes. Sphagnum in a foggy coastal blanket bog in Newfoundland demonstrated that the bog surface loses little water during foggy periods, due, in part, to absence of a vapor pressure deficit. On the other hand, during dry, clear periods the surface of the bog dries, increasing the surface resistance to evaporation (Price 1991); at the same time, higher available energy from the sun causes the rate of evaporation to be higher than on foggy days. This results in a daily evaporation rate of 1.5 mm per day on clear days, contrasting to 0.7-1.1 mm per day for foggy or rainy days.

If we put the two strategies, avoidance and tolerance, into a different perspective, we find that some species tend to avoid drought by holding water more effectively while some survive better at a lower water content. Table 2 lists the survival time of a number of bryophytes. Mechanisms to accomplish survival vary. As we have seen already (in Chapter 7-4a & b of this volume; Li et al. 1992)
**Sphagnum magellanicum** (Figure 8) is superior to *S. papillosum* (Figure 9) at retaining water and transporting it from lower parts of its environment, but *S. papillosum* has a greater rate of survival (95%) after laboratory drying (80% for *S. magellanicum*). Thus, *S. magellanicum* is more of a drought avoider whereas *S. papillosum* is more of a short-term drought tolerator. On the other hand, *S. papillosum* death (65%) surpasses that of *S. magellanicum* (50%) when both are dried for 30 days.

**Figure 8.** *Sphagnum magellanicum*, a moss with good water retention and transport but inferior desiccation survival. Photo by Michael Lüth, with permission.

Lloyd Stark (pers. comm. 18 July 2015) found conflicting results among the publications on the duration of the dry period of *Sphagnum*. Desiccation tolerance alone did not explain the conflicting results. Sagot and Rochefort (1996) dried fragments three species of *Sphagnum* (*S. fallax* (Figure 14-Figure 16), *S. fuscum* (Figure 10), *S. magellanicum* (Figure 8)) and dried them at 60% relative humidity. These species were able to tolerate up to 14 days of desiccation under these conditions. On the other hand, when Schipperges and Rydin (1998) completely dried *S. fuscum* and *S. magellanicum*, and three other species, none of the five species survived. On the other hand, if the water content was maintained above 100% (normal hydration of *Sphagnum* is much greater than that), all the species survived 3-12 days in this "dry" condition. But with the standard water content considered to be near 10% dry weight (=equilibration with 50% relative humidity), this hardly qualifies as dry.

Hájek and Beckett (2008) likewise found that hummock species *Sphagnum magellanicum* (Figure 8) and *S. fuscum* (Figure 10) under desiccation conditions lose more water before turgor starts dropping than do other *Sphagnum* from less exposed habitats (73% vs 56% on average). Nevertheless, the osmotic potentials at full turgor are similar in all species (-1.1 MPa). Unlike the desiccation-tolerant *Racomitrium lanuginosum* (Figure 78) and *Syntrichia ruralis* var. arenicola (Figure 77), the hummock *Sphagnum* species have more rigid cell walls than those of wet habitats. Thus, the leaves of hummock species lose turgor at higher relative water contents (0.61) than species lower in the hummock-hollow complex (0.46). Hummock species also begin a photosynthetic decline sooner during drying. On the other hand, the hummock species recover more completely after rehydration.

Hajek and Vicherova (2014) were able to harden 13 species of *Sphagnum* (Figure 8-Figure 9) to desiccation. Hardening agents included drought, slow drying, ABA application, and chilling or frost. They measured tolerance by recovery of chlorophyll fluorescence parameters after severe desiccation. The hardening was accomplished by subjecting the shoot apices to a very high relative humidity (98-99%) for seven days prior to exposing them to desiccating conditions. With that preparation, the bryophytes were able to tolerate 56% relative humidity. This indicates that in *Sphagnum* desiccation tolerance is inducible.

Despite its ability to induce desiccation tolerance, one important role of *Sphagnum* (Figure 8-Figure 9) as an ecosystem engineer is its ability to retain water (Hajek & Vicherova 2014). Its ability to survive desiccation is seasonal. Following initial dehardening in the lab, untreated shoots of *Sphagnum* lack desiccation tolerance. Nevertheless, desiccation tolerance was induced by all hardening treatments except chilling, and especially by slow drying, even in the aquatic section *Cuspidata*. Under field conditions, *Sphagnum* species in hollows and lawns developed desiccation tolerance several times during the growing season as the precipitation and lowered water table created changing conditions. On the other hand, hummock and aquatic species responded only to frost in late autumn, becoming desiccation tolerant. The protonemata did not develop desiccation tolerance, suggesting that this may be a limiting stage in the life cycle. The desiccation avoiders do

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**Table 2. Known durations of desiccation survival in bryophyte gametophyte plants.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphagnum fuscum</em></td>
<td>2-4 d</td>
<td>Schipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td><em>Sphagnum papillosum</em></td>
<td>2-4 d</td>
<td>Schipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td><em>Sphagnum balticum</em></td>
<td>2-4 d</td>
<td>Schipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td><em>Sphagnum cuspidatum</em></td>
<td>2-4 d</td>
<td>Schipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td><em>Sphagnum magellanicum</em></td>
<td>14 d</td>
<td>Sagot &amp; Rochefort 1996</td>
</tr>
<tr>
<td><em>Sphagnum fallax</em></td>
<td>14 d</td>
<td>Sagot &amp; Rochefort 1996</td>
</tr>
<tr>
<td><em>Fontinalis flaccida</em></td>
<td>3 mos</td>
<td>Glime unpubl</td>
</tr>
<tr>
<td><em>Barbula torquata</em></td>
<td>18 mos</td>
<td>Moore et al. 1982</td>
</tr>
<tr>
<td><em>Oxytiria</em></td>
<td>4 yrs</td>
<td>Volk 1984</td>
</tr>
<tr>
<td><em>Riccia canescens</em></td>
<td>7 yrs</td>
<td>Volk 1984</td>
</tr>
<tr>
<td><em>Grimmia laevidaga</em></td>
<td>10 yrs</td>
<td>Breuil-Sée 1993</td>
</tr>
<tr>
<td><em>Syntrichia ruralis</em></td>
<td>14 yrs</td>
<td>Breuil-Sée1993</td>
</tr>
<tr>
<td><em>Tortula muralis</em></td>
<td>14 yrs</td>
<td>Maheu 1902</td>
</tr>
<tr>
<td><em>Anoectangium compactum</em></td>
<td>19 yrs</td>
<td>Malta 1921</td>
</tr>
<tr>
<td><em>Riccia macrocarpa</em></td>
<td>23 yrs</td>
<td>Breuil-Sée1993</td>
</tr>
</tbody>
</table>

**Figure 9.** *Sphagnum papillosum*, a moss with poor transport and water holding ability, but good desiccation survival. Photo by Michael Lüth, with permission.
not develop desiccation tolerance and must live in compact hummocks or submerged. Thus, there seems to be a tradeoff between desiccation tolerance in species lower on the hummocks and submerged vs resources spent on water retention and desiccation avoidance at higher positions.

Hájek and Beckett (2008) suggest that the higher water-holding capacity of hummock *Sphagna* (Figure 8-Figure 10) would allow them to continue their metabolism longer during desiccation, i.e., they have greater desiccation avoidance. On the other hand, their faster recovery makes them desiccation tolerators. Species in lower positions suffer fewer wet-dry cycles but have more elastic cell walls, permitting them to maintain turgor through a wider range of conditions and thus continue metabolism.

Hájek and Beckett (2008) found that *Atrichum androgynum* (Figure 11), a moss of the New Zealand forest floor, behaved in a manner similar to hummock *Sphagnum* (Figure 8-Figure 10) species. Proctor (2000) suggests that it is the ability to use external water conduction that permits bryophyte leaf cells to maintain full turgor most of the time. Their carbohydrate content is similar to that of embryos in desiccation-tolerant seeds. They are furthermore able to recover rapidly without protein synthesis. As larger plants evolved, vegetative desiccation tolerance was lost; growth rates increased, structural and morphological complexity evolved (Oliver et al. 2000), and water conservation mechanisms were selected over rapid intake and recovery.

Oliver et al. (1993) noted that carbon balance, damage limitation, and cellular repair are necessary components of desiccation tolerance. Using desiccation tolerance of three desiccation-tolerant species of *Syntrichia* (Figure 12-Figure 13, Figure 77), they learned that electrolyte leakage is not an important measure of tolerance, but that differences in protein synthesis could be used to assess damage limitation. Using this assessment, they found the order of tolerance in descending order to be *Syntrichia caninervis* (Figure 12), *S. ruralis* (Figure 77), and *S. norvegica* (Figure 13). This basis of classification and ranking correlates well with the water stress considered to be present in their natural habitat.

Wagner and Titus (1984) compared two *Sphagnum* species – *S. fallax* (Figure 14-Figure 16), a hollow species that lives close to the water table, and *S. capillifolium* (Figure 17-Figure 18) (= *Sphagnum nemoreum*), a hummock species. Here, the relationship is somewhat surprising. The hollow species *S. fallax* is more desiccation tolerant than the hummock dweller *S. capillifolium*. *Sphagnum fallax* not only recovers a greater proportion of its predesiccation photosynthetic rate, but it also has a higher survival rate after 5-10 days of desiccation. This relationship can be explained by events in its habitat. *Sphagnum fallax* dries more frequently and for longer periods of time than does the hummock-dwelling *S. capillifolium*. *Sphagnum capillifolium* is able to retain moisture longer in the field. Growth habit may explain this ability, with *S. fallax* being larger and having a widely spreading head, whereas *S. capillifolium* has a compact capitulum (head) (Figure 18) and lives in tightly packed clumps (Figure 17).
Figure 13. *Syntrichia norvegica*, the least desiccation-tolerant of three *Syntrichia* species on the basis of protein synthesis. Photo by Michael Lüth, with permission.

Figure 14. Habitat of *Sphagnum fallax* on hummocks in the pool where they undergo water level fluctuations. Photo by Michael Lüth, with permission.

Figure 15. *Sphagnum fallax* showing spreading branches in capitula and large spaces between plants. Photo by Michael Lüth, with permission.

Figure 16. Close-up view of a hummock of *Sphagnum fallax*. Photo by Michael Lüth, with permission.

Figure 17. *Sphagnum capillifolium* hummock showing the tight relationship between plants. Photo by Barry Stewart, with permission.

Figure 18. *Sphagnum capillifolium* capitulum showing the tightness of the branches. Photo by Bernd Haynold through Creative Commons, with permission.

Bu et al. (2013) consider that peatlands have hummocks with drought-tolerant species and hollows with drought-intolerant species. They found that drought reduces the biomass production, height increment, and side shoot production of both hummock species *Sphagnum palustre* (Figure 19) and *S. capillifolium* (Figure 17-Figure
Bu and coworkers found that the leaf hyaline cell percentage increases in the hummock species but not in the hollow species. Furthermore, the nitrogen and carbon contents of the hummock species respond more to drought than they do in the hollow species. Instead, it is the presence of neighboring species of *Sphagnum* that causes the decrease in carbon in all three species. Despite this effect, there is no change in the competition under wet or dry treatment for any of the six species combinations. Contrary to expectations, *Sphagnum fallax* exhibits a change from facilitation in wet conditions to competition under dry conditions. This suggests that hummock species can facilitate the hollow species in wet environments but can outcompete them for water under drying conditions. The inability of hollow species to grow on hummocks could be the combination of superior competitors and the greater drought.

![Figure 19. *Sphagnum palustre*, a drought-tolerant hummock species. Photo by Michael Lüth, with permission.](image)

Wood (2007) summarized vegetative desiccation tolerance of bryophytes. Defining it as the “unique ability to revive from the air-dried state,” he considered desiccation-tolerant species to be those that can survive equilibration with either modestly dry air (*i.e.*, 70-80% RH) or extremely dry air (*i.e.*, 0-30% RH). He considered these desiccation-tolerant species to comprise seven bryological classes: *Andreaeopsida*, *Bryopsida*, *Polytrichopsida*, and *Tetraphidopsida* (mosses), *Jungermanniopsida* and *Marchantiopsida* (liverworts), and the *Anthocerotopsida*. This omits the *Andreaeobryopsida* and the *Sphagnopsida*. The *Andreaeobryopsida* may be omitted simply due to lack of data. The *Sphagnopsida*, on the other hand, do indeed have desiccation tolerance in at least some species. In defense of the omissions, only 210 out of ~21,000 bryophyte species (*ca.* 1.0%) have been experimentally determined to possess vegetative desiccation tolerance – 158 species of mosses, 51 species of liverworts, and 1 species of hornwort.

**Desiccation Avoidance**

Many options of desiccation avoidance are available to tracheophytes that are not available to bryophytes. Bryophytes cannot make use of deep roots or increase the length of their roots (or in bryophytes - rhizoids), as do many tracheophytes, because this would have little effect at the scale of a bryophyte. Nor do they have large underground storage organs to permit dormancy. But many do have underground **tubers** (see Chapter 4-10 of this volume) that store significant quantities of lipids or starches (Duckett & Pressel 2003) and that seem to be an adaptation to drought avoidance (El-Saadawi & Zanaty 1990).

Bryophytes cannot conserve water by using an alternate photosynthetic pathway to store CO₂ (Rundel et al. 1979, James 1981) because it would provide no water conservation advantage due to their lack of leaf stomata. Their developmental structure does not permit the loss of leaves because no buds occur at the base of each leaf, and one must wonder if such a small stem could store sufficient energy to support the growth of new leaves prior to any new input from photosynthesis.

The plants protect each other from desiccation and may hide buds of younger shoots within the clump. In *Bazzania trilobata* (Figure 20), field plants are able to tolerate drying, whereas lab drying is lethal (Sollows et al. 2001). Field conditions do not provide the desiccation level one might suppose by measuring air moisture. But it is also likely that the drying rate is different, and the integrity of the clump may have been altered in the lab.

![Figure 20. *Bazzania trilobata* illustrating overlapping leaves and layering of branches. Photo by Janice Glime.](image)

Many bryophytes can roll their leaves, as do some vascular plants, and they have several other related options to reduce the exposed surface area. These include curling and contorting the leaves (see Chapter 7-4 in this volume), a mechanism that creates small air spaces and presumably decreases air movement across the leaf surface. Others appress their leaves closely to the stem, protecting the upper surface from exposure and overlapping leaves sufficiently to protect even portions of the back surface of the leaf from exposure. And, despite their lack of specialized energy-storing organs (with some exceptions), they do have life cycle options. Perhaps the most important of these adaptations is the ability to withdraw water from the cell and form extracellular ice, with desiccation tolerance being an important adaptation (Dilks & Proctor 1975). (See Chapter 7-9 and 7-10 for further information on effects of freezing.)

**Life Cycle and Life Strategy Adaptations**

Hedderson and Longton (1996) evaluated the relationship between life history traits and taxonomic group, relating these to water relationships. They found...
that 40-50% of the life history variation was related to water relations. The capacity for water uptake and retention arranges species from short-lived monoicous (having both sexes on same plant) taxa that produce few, large spores to those dioicous (having separate sexes) taxa with the opposite traits. The endo-ectohydric (internal vs external water control) gradient similarly relates to the investment in spores as a function of life expectancy.

One way to survive dry periods is to avoid them by leaving your spores behind to carry on the species. In the Murray River Valley, Australia, where flooding occurs every spring, long dry periods ensue and many taxa such as the ephemeral mosses persist there as spores (Peintinger 1988). In such genera as Riccia (Figure 1), which typically inhabit seasonally dry areas, dispersal of spores by animals, aided by the ornamentation of the spore, is important (Volk 1984; see Chapter 4-8 in this volume). Survival is facilitated by the ability to endure temperatures as high as 80ºC when dry, whereas temperatures higher than 50ºC when wet will injure them.

Alternatively, ephemeral bryophytes such as Riccia cavernosa (Figure 1), Physcomitrella patens (Figure 21), and Physcomitrium eurystomum (Figure 22) are able to grow on the muddy floodplain soil (Peintinger 1988), then become dormant in the fall until water returns again.

For some bryophytes, altering their phenology according to available water is an adaptive strategy to take advantage of water when it is available. Octoblepharum albidum (Figure 23) in Nigeria produces antheridia and archegonia two months earlier when watered regularly (Egunyomi 1979). In nature, they produce archegonia during the rainy season, then produce capsules and take advantage of the dry season for dispersal of spores. This moss furthermore has leaves that can regenerate after as much as 29 weeks of dry storage, permitting an alternate means of propagation in those years when weather is not favorable for sexual fertilization.

In the very hot and dry summers of Kuwait, El-Saadawi and Zanaty (1990) found that a different avoidance strategy can be used. Bryum bicolor (Figure 24) forms subterranean rhizoidal tubers (see Figure 25) (Risse 1993) and stem tubers that permit it to be dormant as an avoidance mechanism, but it also exhibits tolerance in its protonemata, main stems, and stem apices (El-Saadawi & Zanaty 1990). Funaria hygrometrica (Figure 26) survives only by avoidance in the same conditions, using subterranean corm-like or bulbiform bases and bulbils to span the drought period.
Chapter 7-5: Water Relations: Physiological Adaptations

Figure 25. *Bryum sauteri* rhizoidal tubers, a means of surviving drought. Photo by David T. Holyoak, with permission.

Figure 26. *Funaria hygrometrica* with young sporophytes, growing abundantly on charcoal, where it will continue growth for several years until competition moves in. Note the bulbiform basal leaves that can protect the plant and young sporophyte during drought. Photo by Janice Glime.

Even in less xeric conditions, drought-resistant tubers (Figure 25) are present in such taxa as *Atrichum tenellum* (Figure 27), *A. crispum* (Figure 28) (Arts 1987), and *Fissidens cristatus* (Figure 29) (Arts 1986). In *Haploodontium notarisi* (Figure 31), tubers are viable for up to 10 years (Arts 1988).

Figure 27. *Atrichum tenellum*, a moss that can survive drought as tubers. Photo by Michael Lüth, with permission.

Figure 28. *Atrichum crispum*, a moss that can survive drought as tubers. Photo by Jan-Peter Frahm, with permission.

Figure 29. *Fissidens cristatus*, a moss that is able to survive drought and freezing as tubers. Photo by Jan-Peter Frahm, with permission.

Some bryophytes actually require a dry season. In *Orthotrichum anomalum* (Figure 30), this dry period is necessary for the operculum (capsule lid) to dehisce (Johnsen 1969). The leafy gametophyte grows only when it is cool and moist, but watering during the dry period is detrimental.

Figure 30. *Orthotrichum anomalum* with dehisced capsules. Photo by Michael Lüth, with permission.

Seasonal Changes

As we have just seen, the physiological state of the bryophyte, and hence desiccation tolerance, varies with the season. Many bryophytes [*e.g.*, *Plagiochila spinulosa*...
(Figure 32), *Hylocomium splendens* (Figure 61-Figure 62), *Scorpiurium circinatum* (Figure 33), *Syntrichia ruralis* (Figure 93), *Racomitrium aquaticum* (Figure 34)] seem to be most sensitive during autumn and early winter, the times when most bryophytes resume growth after a hot summer (Dilks & Proctor 1976a). Desiccation tolerance increases from spring to a maximum in early summer, the season when many species become dormant. Some degree of acclimation may be occurring, resulting in increased tolerance as summer approaches (Richardson 1981).

Bryophytes apparently adjust their desiccation tolerance and resistance according to their experiences with the environment (Dilks & Proctor 1976a), as will be discussed in greater detail later with regard to rehydration. That is at least part of the reason for different studies showing different results, even from the same researchers. It is interesting that not all bryophytes adjust in the same way, with one group of bryophytes having their least desiccation tolerance time in autumn and winter and others in late summer in Britain (Figure 35), in this case coinciding with differences among their habitats. *Andreaea rothii* (Figure 36) seems to have no response to season.

Ochi (1952) examined the effects of season on drought tolerance and concluded that mosses with active buds at the beginning of the growing season are generally more drought resistant than in other seasons. Seemingly in contrast to this statement, Ochi showed that in Japan *Dicranum japonicum* (Figure 37) survives drought longer (28 weeks) if the plant has active buds in early January rather than in early September or April (~4 weeks),
whereas *Polytrichastrum formosum* (=*Polytrichum attenuatum*; Figure 38), when dried on the same dates, survives longest when buds become active in September (>56 weeks compared to 28 in January and 11 in April). He concluded that these seasonal strategies represent three types of seasonal fluctuations in osmotic value: higher values in summer (dry season), lower in winter (wet season); higher in winter, lower in summer; no seasonal fluctuations (those from wet habitats).

Figure 35. Relationship between season and maintenance of photosynthesis during desiccation of British bryophytes. *P*<sub>50</sub> is the number of days (in this case) of desiccation at which photosynthesis upon rehydration is reduced to 50% its initial value. Redrawn from Dilks and Proctor (1976a).

Figure 36. *Andreaea rothii*, a season-neutral moss with respect to its desiccation tolerance. Photo by Michael Lüth, with permission.

Davey (1997) found that in Antarctic bryophytes, the photosynthetic rate following a desiccation/rehydration cycle decreased from spring to summer to autumn. The pattern was clearest in the hydric taxa, with less effect in the xeric species.

Figure 37. *Dicranum japonicum*, a moss where early January buds result in ability to survive drought longer. Photo by Li Zhang, with permission.

Figure 38. *Polytrichastrum formosum*, a moss that survived longest when buds became active in September. Photo by David T. Holyoak, with permission.

Akande (1984, 1985) likewise attributed seasonal differences in desiccation tolerance of four epiphytic (growing on other plants, especially trees) bryophytes to changes in osmotic values, with osmotic values increasing from wet to dry season. He found that the mosses *Entodontopsis nitens* (=*Stereophyllum nitens*) and *Calymperes palisotii* (Figure 39) had a greater osmotic potential and greater desiccation tolerance than the leafy liverworts *Mastigolejeunea florea* and *Frullania spongiosa*.
Physiological Adaptations

All of us have observed that bryophyte assemblages differ with habitats (e.g. Šinžar-Sekulić et al. 2005). Oliver et al. (2000) note that most of the desiccation-tolerant plants are bryophytes, in addition to algae and lichens. They agree that desiccation tolerance was an important step in the evolution of land plants. They suggested that such tolerance requires constitutive cellular protection coupled with active cellular repair. But as evolution progressed, plants gained structural and morphological complexity. Plants developed mechanisms that conserve water within the plant, and vegetative desiccation like that seen in bryophytes was no longer necessary.

But Alpert and Oechel (1985) contend that desiccation-tolerant plants are rarely present in the most xeric microhabitats, suggesting that in these locations they are unable to maintain a positive cumulative carbon balance. They demonstrated this in Grimmia laevigata (Figure 83), the dominant green plant on exposed granitic boulders in the California, USA, chaparral by measuring the response of net CO2 flux to light, temperature, plant balance. They demonstrated this in

Among desiccation-tolerant bryophytes, rehydration is rapid, with leaves returning to normal form in as little as 2 minutes and chloroplasts returning to normal conformation in 2-5 minutes in such desiccation-tolerant mosses as Syntrichia ruralis (Figure 77) (Tucker et al. 1975; Oliver & Bewley 1984). On the other hand, following rapid drying such intolerant species as Cratoneuron filicinum (Figure 40) still have misshapen organelles after 24 hours, and about half the cells of slow-dried plants still contain misshapen organelles (Oliver & Bewley 1984). In slow-dried plants respiration recovers, but it does not in rapid drying of desiccation-intolerant plants.

Charron and Quatrano (2009) considered two general mechanisms for survival in the xeric aerial environment. The descendants of the early land plants evolved specialized transport tissues while the bryophytes retained and perfected their co-equilibrium of their water content with that of their surroundings, relying on cellular processes to recover from damages due to water stress.

Bates (1997) examined the effects of wet/dry cycles on the nutrient economy of two pleurocarpous mosses of different habitats – Brachythecium rutabulum (Figure 56- Figure 57; wet ground, among grasses, logs; shade or open) and Pseudoscleropodium purum (Figure 41; grasslands and heaths). When provided with weekly drying periods of 24 hours every week, these plants had noticeably less biomass production than those plants that were continuously hydrated. Brachythecium rutabulum experienced bleaching of green tissues, unlike Pseudoscleropodium purum. When NPK (mix of nitrogen, phosphorus, and potassium salts) was added to the growing solutions once a week, Pseudoscleropodium purum exhibited growth stimulation even among the weekly desiccated plants. Uptake of N was similar in both hydrated and desiccated plants of both species. P and K+ were considerable in B. rutabulum, but in desiccated plants they was greatly reduced. As with phosphorus, uptake of P and K+ differed little between hydrated and intermittently desiccated Pseudoscleropodium purum. In both species, P and K+ were leaked from cells during desiccation, were retained on the cells by cation exchange, and taken up again during rehydration. But even this maintenance has a cost. K+ and Mg2+ intracellular levels in new growth are maintained at the expense of exchangeable cations. Uptake is greatest during the early stages of recovery, most likely due to damaged membranes, and that is when the NPK application has the greatest effect on growth. These experiments suggest that P. purum has a lower nutrient requirement than B. rutabulum and they explain why B. rutabulum requires a more continuous hydration to maintain its greater production. An interesting revelation is the ability of these species to initiate new growth without additional nutrient absorption.

Bohnhart (2000) asked what makes desiccation tolerable. He considered that bryophytes tolerated rapid desiccation, using protective mechanisms. Most research has focussed on repair mechanisms. The photosynthetic apparatus and cell integrity are maintained during desiccation, but rehydration leads to cellular damage. Despite this damage, recovery is rapid. mRNA (messenger RNA, the molecule that carries information from DNA to the ribosome) exists in RNPs (nucleoproteins that contain RNA) before the stress conditions arise. During recovery,
non-reducing sugars, dehydrins (group of proteins produced in response to cold and drought stress), and rehydrins (transcripts used during rehydration) appear. Hoekstra (2005) reported on the importance of fatty acid saturation in membranes in imparting survival of desiccation.

Figure 41. *Pseudoscleropodium purum*, a species in which intermittent desiccation seems to have little effect on K and P uptake. Photo from Proyecto Musgo, through Creative Commons.

Yang et al. (2012) sought the genetic determinant(s) for stress tolerance. Using *Syntrichia caninervis* (Figure 12) they identified ScALDH21, a gene that responds to ABA (abscisic acid, a stress hormone; see Chapter 7-7 Water Relations – Biochemistry) and desiccation and that plays an important role in response to desiccation and salinity stresses.

When the desiccation-tolerant *Syntrichia ruralis* (Figure 77) is desiccated, it retains all its pigments, chlorophyll included, and is able to recover physiological function rapidly upon rehydration (Hamerlynck et al. 2002). But all is not equal among these plants of both sun and shade habitats. *Syntrichia ruralis* has lower plant mass, as well as lower tissue N, C, total photosynthetic pigment concentrations, and carbon isotope discrimination (A) values compared to shade plants. The ratio of carotenoid to chlorophyll in sun plants is typical of high light plants, but the ratio of chlorophyll $a$ to chlorophyll $b$ in these plants is lower than expected, resembling those of plants adapted to shade. As a consequence, the levels of optimal quantum efficiency of PS II ($F_v/F_m$) (= variable fluorescence / maximum fluorescence; PS II is photosystem II of photosynthesis, where oxygen is liberated from water) are lower in the sun plants. Reciprocal transplants reveal that *Syntrichia ruralis* is able to adjust to altered light levels. This is evidenced by increases in $F_v/F_m$, NPQ (non-photochemical quenching), light-adapted PSII yield ($\phi_{PSII}$) in transplanted sun plants, and concurrent decreases in sun-transplanted shade plants. Nevertheless, the transplanted sun plants did not adjust sufficiently to reach performance levels exhibited by the undisturbed shade plants. These plants demonstrate at least some ability to adjust to the loss of shade canopy or other disturbance in the light regime.

*Sphagnum* (Figure 8, Figure 9, Figure 14–Figure 18) has a unique cell structure (Figure 42) providing a water reservoir. We might expect that this reservoir increases the drying time, sparing the moss from the detrimental effects of rapid drying. But differences do exist among *Sphagnum* species.

Figure 42. *Sphagnum hyaline leaf cells* and pores. Photo from Botany Department website, University of British Columbia, Canada, with permission.

In the hummock-forming *Sphagnum fuscum* (Figure 10) and *S. magellanicum* (Figure 8), desiccation results in a greater water loss before turgor sets in, compared to other non-hummock species (mean of 73% water loss vs 56%, respectively) (Hájek & Beckett 2008). The hummock species have more rigid cell walls than those of wet habitat species (epsilon = 3.55 vs 1.93 MPa, respectively). This rigidity results in loss of turgor in chlorophyllous cells at a higher relative water content in hummock species compared with species of wet habitats (0.61 vs 0.46) and at less negative osmotic potentials (-2.28 vs -3.00 MPa, respectively). Compared with other species, hummock *Sphagnum* (Figure 8, Figure 9) species that have been desiccated to -20 or -40 MPa recover more completely after rehydration. The mesophytic (intermediate habitat based on moisture) *Atrichum androgynum* (Figure 43) responds similarly to the hummock *Sphagnum* species.

Figure 43. *Atrichum androgynum*, a moss that behaves similarly to hummock *Sphagnum* species when it loses water. Photo by Jan-Peter Frahm, with permission.
Under a given rate of desiccation, the hummock species of *Sphagnum* (Figure 8-Figure 10), with their higher water content, continue their metabolism longer than species with lower water-holding capacities (Hájek & Beckett 2008). And these species recover faster, indicating a higher drought tolerance. These behaviors permit them to survive in the drought-exposed hummocks. The species growing in wet habitats have smaller water-holding capacities but are able to maintain turgor and have more elastic cell walls that permit them to metabolize longer during drying.

Most *Sphagnum* (Figure 8-Figure 10) species live where intermittent desiccation is inevitable. Hence, this genus appears to have inducible desiccation tolerance (Hájek & Vicherová 2013). In experiments to harden (process by which a plant becomes tolerant to the effects of such stresses as frost and drought) the species, Hájek and Vicherová subjected them to slow drying, ABA application, and chilling or frost. In the laboratory, *Sphagnum* species that were de-hardened and remained untreated lacked desiccation tolerance. Slow drying, ABA application, and frost induced hardening and desiccation tolerance. The section *Cuspidata* (Figure 44) – aquatic species – did not exhibit hardening. Similar hardening occurs multiple times each year among hollow and lawn species in the field. Hummock and aquatic species, on the other hand, develop their tolerance only in late autumn, a phenomenon that Hájek and Vicherová attributed to frost. Protonemata, however, did not develop desiccation tolerance under any of the hardening treatments. The hummock species exhibit a tradeoff, having greater water-holding capacity to the detriment of their physiological desiccation tolerance.

Figure 44. *Sphagnum cuspidatum*, an aquatic species that does not seem to experience hardening. Photo by Bernd Haynold, through Creative Commons.

*Cratoneuron filicinum* (Figure 40) demonstrates the effects of slow vs rapid drying on a semi-aquatic species (Krochko *et al.* 1978). In rapid drying, the cell contents are very disrupted and become increasingly disorganized over the next 24 hours. In slow drying, only some cells have this appearance while others maintain their cellular integrity. The greater the rate of drying, the more protein synthesis is reduced on rehydration, but it will resume following rapid water loss down to 50% of the fresh weight. On the other hand, respiration does not resume following rapid drying and rewetting.

**Mode of Conduction**

Can the mode of conduction provide a beneficial edge that permits success when faced with limited water? Raven (1999) claims there is a "mechanistically mysterious size limit" for poikilohydric, desiccation-tolerant plants, suggesting an upper limit of 1 m. Anderson and Bourdeau (1955) demonstrated that external water can travel only to a "certain level." Bowen (1933c) and Mankiewicz (1983, 1984a,b, 1987a,b) remind us that this upper limit is imposed by the height to which water can rise by capillarity alone, a distance Hèbant (1977) considers to be only a few centimeters without the addition of other forces. As stated by Mankiewicz, "geometry of bryophytes may be constrained by the cohesive and adhesive forces of water," a statement he was able to confirm by empirical measures of flow rates through bryophyte colonies. However, we are reminded that most bryophytes receive their water from above, hence that capillary limit is of little importance for most of them. Therefore, we might ask, is the endohydric system important for the slow-growing, short bryophyte?

Bowen (1933a,b,c) compared conduction of bryophytes in wet, moist, and dry habitats. External water movement was faster than internal movement in all but two cases [Thamnobryum alopecurum (Figure 50) and Plagiomnium undulatum (Figure 52)]. *Plagiomnium undulatum* has a well-developed internal conducting system and lacks significant capillary channels externally. *Thamnobryum alopecurum*, on the other hand, typically lives where it is constantly wet from splashing or dripping water and seems to lack external conduction, perhaps due to external saturation. However, as the moisture of the habitat increases, the ability of the bryophyte plant to conduct decreases both externally and internally.

All of the taxa Bowen (1933a,b,c) studied had a central strand (Figure 45), varying considerably in relative size. But just how important is that strand in moving water from substrate to plant tissues? If the central strand is important in water movement, should we expect it to be most important in those mosses that suffer frequent drought conditions? In the epiphytic (but pleurocarpous) *Hypnum cupressiforme* var. *filiforme* (Figure 46-Figure 47), the central strand appears only occasionally and is absent in branches. In the boreal forest floor *Rhytidiaedium triquetrus* (Figure 48), the cells are short with numerous transverse walls, suggesting inefficient water movement through walls. Nevertheless, in *Aulacomnium palustre* (Figure 49), internal conduction seems not to exist, despite a "relatively large central strand;" external conduction is rapid, suggesting that other factors, not the central strand, are more important in determining importance of internal versus external conduction.
When Bowen (1933b) compared nine species of moist habitat bryophytes (Figure 53), she found that external conduction likewise predominated in all but *Rhizomnium magnifolium* (Figure 52; as *Mnium punctatum*, but based on her description most likely what is now called *Rhizomnium magnifolium*). *Thamnobryum alopecurum* (Figure 50), apparently erroneously reported in cm instead of mm in her table, has almost no water movement internally or externally (Mägdefrau 1935), but relies instead on the constant humidity of waterfalls and streamsides. Among the dry habitat mosses in the study, only *Plagiomnium undulatum* (Figure 52) exhibits more rapid internal conduction than external conduction.
Based on Bowen's (1931, 1933a,b,c) comparisons, we can derive little satisfaction about the relationship between the central strand and habitat. None of the species lacking a central strand were examined, nor were any extremely xerophytic or aquatic mosses or any liverworts examined. However, external adaptations to movement of water do seem to correlate with habitat, with those mosses from wet habitats having poor conduction capability both internally and externally, relative to taxa from drier habitats (Figure 53). The central strand appears to have only a minor role in conduction, being most useful in those taxa with a well-developed central strand, such as the Mniaceae (Bowen 1933c), and providing almost no value in those taxa with a small strand (Mägdefrau 1935; Zacherl 1956).

Despite Bowen's (1931, 1933a,b,c) small sample size and the presentation of "representative" data rather than means, one can still infer several patterns that indicate water pathway adaptations. The Mniaceae are a good example (Figure 52). There is good external conduction in Mnium hornum (Figure 52), where the leaf insertion is relatively small, but the leaves are strongly overlapping, as are the plants. In the very tomentose Rhizomnium magnifolium (Figure 52), with somewhat overlapping and encircling leaves, external conduction is relatively good, but internal conduction is much better than in Mnium hornum. However, in Plagiomnium undulatum (Figure 52), where the leaves are non-overlapping and the leaf tapers to the equivalent of a petiole at insertion, external conduction is almost non-existent. It is noteworthy that members of this family are particularly difficult to rehydrate for slide preparation, presumably due to thickened cell walls and cuticular substances on the leaves. It is reasonable to expect rapid internal conduction in the Mniaceae because these mosses have well developed central strands of conducting tissue. In Plagiomnium undulatum the central strand occupies up to 2/3 of the stem diameter. Members of the family Mniaceae and Polytrichum commune (Figure 54-Figure 55) are also the only ones examined that have hydroids in the leaves (Bowen 1931, 1933a,b,c). As noted in Aulacomnium palustre (Figure 49), factors other than the size and construction of the central strand are important in determining relative conductance.

In Brachythecium rutabulum (or B. rivulare?) (Figure 56-Figure 57), the slightly decurrent leaf bases form channels that retain capillary films of water. In Entodon rubicundus and Calliergonella cuspidata (Figure 58), internal conduction is appreciable in young tissues, becoming negligible in older stems (Mizushima 1980). Bowen (1933b) attributes this to the changes in hypodermal tissues, which are thin-walled in young stems, becoming thick-walled in older ones. Rather, the epidermis absorbs water and sends it cell-to-cell to the tip of the plant where the young hypodermal cells permit the water to penetrate to the center of the plant where a very thin central strand occupying about 10% of the stem exists. Entry of water into the apex is rapid, as is the external movement to the tip. Campylopus brevipilus (Figure 59) has a central strand of 5-15 cells in diameter. As might be expected in a genus so well adapted to dry habitats, even this more wetland species has little absorption through its stem epidermis and movement of water through the hypodermis is slow, entering primarily at the stem apex. Likewise, little conduction occurs from the base through the central strand.
Figure 53. Comparison of movement of water up the stems in wet, moist, and dry habitat mosses. Note that for *Brachythecium rutabulum*, *Hypnum cupressiforme* var. *filiforme*, and *Rhytiadelphus triquetrus* the internal movement is for 18 hours. (Based on the description of decurrent leaf bases and habitat, *Brachythecium rutabulum* may actually have been *B. rivulare*.) For *Thuidium tamariscinum*, *Hypnum cupressiforme* var. *filiforme*, and *Dicranum scoparium*, the external water reached the tip before one hour. In *Ditrichum flexicaule* and *Anomodon viticulosus* the water reached the tip in 15 minutes. Based on Bowen (1931, 1933a,b,c).

Figure 54. *Polytrichum commune*, a moss with good internal conduction in stem and leaves. Photo by Michael Lüth, with permission.

Figure 55. *Polytrichum commune* leaf cross section showing hydroids (arrow). Photo from Botany website, University of British Columbia, with permission.
Figure 56. *Brachythecium rutabulum*, a moss whose leaf bases create capillary channels. Photo by Janice Glime.

Figure 57. *Brachythecium rutabulum* leaf showing slight decurrency that aids in holding capillary water. Photo by Tom Thekathyil, with permission.

Figure 58. *Calliergonella cuspidata* has mostly internal conduction in young stems but lose it in older stems. Photo by Des Callaghan, with permission.

Figure 59. *Campylopus brevipilus*, a moss in which water enters through the stem apex. Photo by Michael Lüth, with permission.

Could it be that the central strand serves a different function? In an Alaskan black spruce forest, Skré *et al.* (1983) found that endohydic *Polytrichum commune* (Figure 54-Figure 55), which has a well-developed central strand (Figure 60) and considerable internal conduction, suffers less moisture stress than the three ectohydic mosses studied [*Hylocomium splendens* (Figure 61-Figure 62), *Pleurozium schreberi* (Figure 64), and *Sphagnum subsecundum* (Figure 63)] during the summer dry period. *Hylocomium splendens* remained below its water compensation point for nearly 50% of the July measurement period. The rates of water loss and moisture level required to reach field capacity correlate well with the moisture status observed for mosses in the field. This water retention in the endohydic *Polytrichum* supports the suggestion of Skré and coworkers that a major function of the central strand may be water storage.

Figure 60. *Polytrichum commune* stem cross section showing hydrome. Photo from Botany website, University of British Columbia, Canada, with permission.
Osmotic Potential and Turgor

Unlike tracheophytes, whose net photosynthesis decreases when the water potential drops below -1 to -3 bars (Busby & Whitfield 1978), drought-tolerant mosses can resume normal photosynthesis after a drop in water potential to about -1000 bars, a condition found during the dry, hot days of summer in the open (Dilks & Proctor 1979). Even in the shaded forest, the water potential of a moss can drop to -200 to -400 bars. While flowering plants and ferns may have negative photosynthesis at water potentials of -12 to -15 bars, mosses such as the woodland to semi-shaded species *Hylocomium splendens* (Figure 61-Figure 62), *Pleurozium schreberi* (Figure 64), and *Tomentypnum nitens* (Figure 65) can continue net photosynthesis until the water potential falls below -55 to -100 bars (Busby & Whitfield 1978), and *Camptothecium lutescens* (Figure 66) from the United Kingdom can maintain a net positive photosynthesis down to -150 bars (Dilks & Proctor 1979). The drought-intolerant moss *Hookeria lucens* (Figure 67), on the other hand, must maintain 100% humidity and cannot maintain positive photosynthetic gain when the water potential drops below 80 bars (Dilks & Proctor 1979). Yet this highly drought-intolerant moss, relatively speaking, has primary cell walls with pit fields in its stem parenchyma, structures common to tracheids and vessels and permitting lateral transport, suggesting that *Hookeria lucens* may use these cells in internal conduction (Cortella *et al*. 1994).
One adaptation to maintaining water is to increase the osmotic value of the cells. Ochi (1952) compared a number mosses and showed that the highest osmotic values were generally in mosses adapted to xeric conditions. He obtained high values (0.90-0.62) in such tree-trunk and sunny rock dwellers as *Hedwigia ciliata* (Figure 82), *Thamnobryum subseriatum* (=*Thamnobryum sandei* var. *cymbifolium*) (Figure 68), *Myuroclada maximoviczii* (Figure 69), *Thuidium cymbifolium* (Figure 70), *Neckera yezoana*, and *Anomodon giraldii* (Figure 71). Intermediate values characterized those on soil (0.70-0.30), including *Dicranum japonicum* (Figure 37), *Pogonatum inflexum* (Figure 72), *Plagiomnium maximoviczii* (Figure 73), and *Plagiomnium cuspidatum* var. *trichomanes* (Figure 74). In shady, wet, forested areas, Ochi obtained the lowest value (0.26), exemplified by *Plagiomnium vesicatum* (Figure 75) and *Hookeria acutifolia* (Figure 76). Surprisingly, values were highest in older plants and mature portions, not the vital young buds.

**Figure 66.** *Camptothecium lutescens*, a moss that can maintain photosynthesis at very low water potential. Photo by Michael Lüth, with permission.

**Figure 67.** *Hookeria lucens*, showing thin leaves that are very drought-intolerant. Photo by Michael Lüth, with permission.

**Figure 68.** *Thamnobryum subseriatum*, a moss from emergent rocks of streams. Photo by Michael Lüth, with permission.

**Figure 69.** *Myuroclada maximoviczii*, a rock dweller with high osmotic values. Photo by Janice Glime.

**Figure 70.** *Thuidium cymbifolium*, a sunny rock dweller with high osmotic values, with capsules. Photo by Li Zhang, with permission.
Proctor (1999) likewise examined a number of bryophytes to determine their osmotic potential. He found that the leafy ones (mosses and leafy liverworts) have a full turgor osmotic potential of -1.0 to -1.5 MPa, whereas the multistratose thallose liverworts have -0.5 to -1.0 MPa.
The full turgor content of water varies with season, ranging 100-300% in bryophytes from well-drained habitats. But Proctor found that the highest turgor occurs in the new growth. The cell walls are highly extensible in most of the thallose liverworts and such drought-tolerant mosses as *Syntrichia ruralis* var. *arenicola* (Figure 77) and *Racomitrium lanuginosum* (Figure 78), but it is quite low in certain leafy liverworts with very rigid cell walls. Unlike Ochi (1952), Proctor found that variations in water relation parameters seem to bear little relationship to habitat for most bryophytes. He attributed this lack of relationship to the consideration that they are usually only metabolically active when they are fully hydrated.

Some bryophytes can tolerate turgor up to 1400% of their dry mass [*Dumortiera hirsuta* (Figure 79) & *Conocephalum conicum* (Figure 80)] (Proctor et al. 1998). On the other hand, xerophytic mosses such as *Syntrichia ruralis* (Figure 77) and *Andreaea alpina* (Figure 81) reach full turgor at only 110%.

**Figure 77.** *Syntrichia ruralis* var. *arenicola*, a drought-resistant moss with very extensible cell walls. Photo by Michael Lüth, with permission.

**Figure 78.** *Racomitrium lanuginosum*, a drought-resistant moss with very extensible cell walls. Photo by Janice Glime.

**Figure 79.** *Dumortiera hirsuta* showing hairs on edges of thalli and a turgid condition. Photo by Li Zhang, with permission.

**Figure 80.** *Conocephalum conicum*, a liverwort that can tolerate turgor up to 1400% of its dry mass. Photo by Dick Haaksma, with permission.

**Figure 81.** *Andreaea alpina*, a xerophytic moss that can only tolerate turgor up to 110% of dry weight. Photo by Andrew Hodgson, with permission.

### Water Content

Given sufficient water, water content is related to the cell's osmotic potential. Low water content seems to be related to a xeric habitat (Hernández-Garcia et al. 1999), suggesting tolerance rather than the avoidance that might be obtained by maintaining high osmotic potential. In the xeric and mesic pine forests of Tenerife, water content of all mosses tested was <140% of dry mass. *Hedwigia ciliata* (Figure 82), *Grimmia laevigata* (Figure 83), *G. trichophylla* (Figure 84), and *Pterogonium gracile* (Figure 85), the rock dwellers, have the lowest field water content and fastest absorption and water loss rates among the species. *Polytrichum juniperinum* (Figure 86), *Bartramia stricta* (Figure 87), and *Anacolia webbi* (Figure 88) have the highest field water content and slowest water absorption and loss rates. The highest drought tolerance occurs in *H. ciliata*, *B. stricta*, *G. laevigata*, and *G. trichophylla*.
Figure 82. *Hedwigia ciliata*, a very drought-tolerant species. Photo by Michael Lüth, with permission.

Figure 83. *Grimmia laevigata*, a rock-dweller with low water content and rapid water uptake. Photo by Jonathan Sleath, with permission.

Figure 84. *Grimmia trichophylla*, a rock-dweller with low water content and rapid water uptake. Photo by Michael Lüth, with permission.

Figure 85. *Pterogonium gracile*, a rock-dweller with low water content and rapid water uptake. Photo by David Holyoak, with permission.

Figure 86. *Polytrichum juniperinum*, an endohydric moss with high water content and slow water absorption. Photo by Keith Bowman, with permission.

Figure 87. *Bartramia stricta*, a moss with high water content and slow water absorption. Photo by Michael Lüth, with permission.
Nichols (1918) reported that *Sphagnum* (Figure 63) pads, used for bandages in World War I, could absorb up to 22 times their mass (water capacity = 2200%), making them 5-6 times as absorptive as cotton pads. Other bryophytes, as in some South African montane areas, survive on the water they collect from early morning mist in low-lying clouds (Russell 1982), suggesting that these bryophytes may indeed have low water capacities. Furthermore, many bryophyte taxa are tolerant of very low water contents (5-10% of dry mass), resuming photosynthesis upon remoistening (Proctor 1990).

It appears that at least for some bryophytes, it is best to be wet or be very dry. Water pressure in the range of –100 to –200 MPa is best for survival in a dry state (Proctor 2001). Akande (1984, 1985) has examined the effects of the degree of dehydration on Nigerian bryophytes and found that those maintained at 0% humidity for one week and for one month both resumed respiration more quickly than those maintained for the same time period at 32% and 54% (Akande 1984). He found that the leafy liverwort *Mastigolejeunea florea* is less desiccation-tolerant than the two mosses studied, but all three taxa did have individuals that survived at 0%, 32%, and 54% relative humidity at ambient temperature (Akande 1985).

**Water-logging**

Despite their needs for high water content, bryophytes cannot afford to be too wet or they are unable to carry out photosynthesis. Acquiring CO₂ must occur through the leaf surface, and a continuous layer of water interferes with that transfer. Silvola (1991) found that in all the boreal forest mosses he tested except *Polytrichum commune* (Figure 54-55), photosynthesis decreased when the water content exceeded a certain optimal level (see also Williams & Flanagan 1991). It is likely that the leaf lamellae provided air spaces for CO₂ transfer in *P. commune*. Many *Sphagnum* species suffer similarly from water-logging.

**Inducible vs Constitutive Desiccation Tolerance**

As recently as 2011, Green *et al.* reviewed the literature and reported that bryophytes appear to all be constitutive. To support this they cite that no protein synthesis is required upon rehydration before metabolism can commence. Bryophytes furthermore appear to always be protected from desiccation mortality. Further support is the constant presence of high sucrose levels. And the cellular structure is usually maintained during desiccation.

Both constitutive (always present; fully desiccation tolerant) and inducible [produced when drying conditions occur; previously known as modified desiccation-tolerant (Oliver *et al.* 1998)] desiccation tolerance exist among bryophytes (Stark *et al.* 2013). Those with constitutive desiccation tolerance (CDT) are not dependent on the rate of drying to determine their recovery, whereas those that depend on inducible desiccation tolerance (IDT) are. Reduced or no desiccation tolerance following rapid drying is generally an indicator that the plants are IDT plants. Tracheophytes, with the exception of some ferns (Watkins *et al.* 2007), are IDT plants (Oliver *et al.* 1998, 2000), whereas bryophytes are mostly CDT plants (Toldi *et al.* 2009), hence their high ability to survive drying.
Those bryophytes with constitutive desiccation tolerance (CDT) are not dependent on the rate of drying, whereas those with only inducible desiccation tolerance (IDT) are. Therefore, the IDT plants, including IDT bryophytes, are likely to die when exposed to rapid drying. But bryophytes can use both strategies. Stark and coworkers have investigated the inducible protections that permit bryophytes to survive desiccation. Those bryophytes that survive slow drying but not rapid drying provide us with evidence that something happens during that slow drying process, and that happening provides the inducible desiccation tolerance (Stark et al. 2013). Bryophytes also possess constitutive desiccation tolerance, a tolerance that is common among terrestrial bryophytes. For example, the desert moss *Pterygoneurum lamellatum* (Pottiaceae; Figure 7) exhibits both a constitutive and an inducible response. The bryophyte tolerance strategy couples constitutive cellular protection during dehydration with the induction of a recovery/repair mechanism upon rewetting (Oliver et al. 2005; Toldi et al. 2009; Stark & Brinda 2015).

More recently, Stark and Brinda (2015) have found that not only can a desert moss have both inducible and constitutive desiccation tolerance, but it can have each in different parts of the same shoot at the same time or in different stages in the life cycle. Stark and Brinda propose that as the sporophyte grows older, the presence of sugars in the sporophyte facilitates desiccation tolerance. This would help to explain the greater danger of death by desiccation in the early embryonic stage before significant sugar accumulation occurs. At the same time, the early embryo exhibits inducible desiccation tolerance (IDT) and requires slow desiccation, usually not a problem within the protection of the apical gametophyte leaves. As the embryo develops and the seta emerges from these protective leaves, the sporophyte changes from IDT to partially CDT. Stark and Brinda suggest that this evolutionary change resulted from selection pressures of intermittent drying in this exposed sporophyte. This exposed sporophyte tissue is most likely subject to faster rates of desiccation, making an inducible system inadequate to meet the time demands and selecting for the constitutive desiccation tolerance. The presence of a waxy cuticle in the capsules of *Funaria hygrometrica* (Figure 26) provide an example of this CDT (Budke et al. 2011, 2012, 2013).

Stark and Brinda (2015) concluded that once the seta elongation phase reaches the stage of capsule expansion, sucrose imported from the gametophyte (Renault et al. 1992) should be present in the sporophyte, endowing the sporophyte with the raw materials needed to tolerate rapid drying (Stark & Brinda 2015). In *Acaulon muticum* (Figure 92), small vacuoles are present in the placental region of the sporophyte-gametophyte junction (Rushing & Anderson 1996). These abundant vacuoles may be present in the embryonic sporophyte as well, where they could provide protection from water stress in the rapidly growing sporophyte.

Wolkers et al. (2001) had already suggested that a slower rate of drying may permit the proteins and sucrose to interact in a more protective manner. For example, in *Physcomitrella patens* (Figure 21, Figure 90) and *Syntrichia ruralis* (Figure 93), a slow drying treatment induces the production of either ABA (see below) or dehydrin, or both (Werner et al. 1991; Hellwege et al. 1994; Cuming et al. 2007). When ABA is applied to the outside of *Exormotheca holstii* (Hellwege et al. 1994), it elevates sucrose levels and increases protection against rapid drying (see also Pence 1998; Oldenhof et al. 2006). Koster et al. (2010) demonstrated the genetic connection between ABA and the expression of several homologs to stress proteins, including two dehydrin-like proteins. The only problem with this logic is that the natural presence of ABA is still unknown in *Physcomitrella patens* and *Syntrichia ruralis* (Stark & Brinda 2015).
**Hardening**

Hardening is a phenomenon known for flowering plants, but the concept is usually associated with preparation for winter. Beckett et al. (2005) induced desiccation hardening in the moss *Atrichum androgynum* (Figure 11) by reducing the relative water content of apical portions for 1/2 to 3 days, followed by storage fully hydrated for another day. Plants were then desiccated for 16 hours over silica gel, and the recovery of PSII during rehydration was monitored. Hardening affected photosystem II (PSII) before desiccation, decreasing its efficiency, especially at saturating light intensities. Upon rehydration, however, hardened plants recovered their PSII activity more quickly and greatly increased the non-photochemical quenching in the first few hours compared to those plants not subjected to hardening. Beckett et al. concluded that hardening shifts the photosynthetic apparatus from a state of high efficiency to one of less efficiency but having a photoprotected state.

Hardening can confound physiological experiments when comparing desiccation tolerance. Once hardened, the plant is likely to receive the benefits in desiccation resistance for a prolonged period of time, such that a rehydration period of 24-72 hours may not remove that benefit (Bopp & Werner 1993; Stark et al. 2014). Instead, regenerates from fragments (regenerated more than once to eliminate prior hardening) or plants grown from spores may be necessary to create plants that have no prior desiccation experience, hence no hardening (Stark & Brinda 2015).

**Desiccation-induced Changes**

Iljin (1953, 1957) considered that mechanical injury to the protoplast membranes during the drying and rewetting processes is the primary cause of desiccation sensitivity. He considered the tensions that develop in cells during dehydration, pulling protoplasm inward as the vacuoles shrink and cell walls pulling membranes outward, are the primary causes of lethal injuries in drought-sensitive species. Drought-tolerant plants mitigate these tensions by such cellular aspects as reduced cell size, small or absent vacuoles, lack of plasmodesmata, easily deformed cell walls, and reduced osmotic pressure. For example, small cytoplasmic vesicles (vacuoles) are present in such desiccation-tolerant species as *Syntrichia ruralis* (Figure 93), *Neckera crispa* (Figure 94), *Pleurozium schreberi* (Figure 64), and *Triquetrella papillata* (Figure 95) (Oliver & Bewley 1984). But this does not hold true for all species – in the desiccation-tolerant *Ceratodon purpureus* (Figure 96-Figure 98) and *Didymodon vinealis* (Figure 99), the vacuoles are quite large. And the desiccation-intolerant *Cratoneuron filicinum* (Figure 40) does not have large vacuoles. Plasmodesmata (microscopic channels that traverse cell walls of plant and some algal cells, enabling transport and communication between them) likewise do not seem to be related to desiccation-tolerance, but these are difficult to see and often require electron microscopy for viewing.
Henckel and Pronina (1968, 1969, 1973) suggest that those plants that are drought-tolerant are continuously prepared for desiccation, i.e., have constitutive desiccation tolerance. However, this theory likewise did not fit the evidence presented by slow vs rapid drying in bryophytes. Bewley (1979) suggested that instead, three factors are critical to desiccation tolerance:

1. limiting damage during desiccation to a reparable level
2. maintaining physiological integrity in the dry state so that metabolism can be reactivated quickly upon rehydration
3. putting repair mechanisms into effect upon rehydration, in particular to retain or regain integrity of membrane and membrane-bound organelles.

As bryophytes desiccate, a series of changes occurs. In *Physcomitrella patens* (Figure 21, Figure 90), these changes include plasmolysis, chloroplast remodelling, and microtubule depolymerization, as demonstrated by desiccation for more than one month to 10% of fresh weight (Wang *et al*. 2009). Nevertheless, Wang and coworkers found that the membranes retain their integrity. These changes involved 71 responsive proteins. Most of these were involved in metabolism, cytoskeleton, defense, and signaling. But not all changes seem to be that of repair.
or stability. Cytoskeletal protein degradation might cause cytoskeletal disassembly and resulting changes in cell structure. Late embryogenesis abundant proteins (LEA proteins) and reactive oxygen species-scavenging enzymes are among those prominently induced, possibly helping to reduce the damage caused by desiccation. Oliver et al. (2004) likewise found that the LEA proteins were the most abundant transcripts associated with drying tissues. They suggest that the LEA proteins might play a role in recovery from desiccation.

Oliver et al. (2004) took a genetic approach to understanding desiccation tolerance, using the desiccation-tolerant Syntrichia ruralis (Figure 93). They found that the transcriptome (set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA transcribed in a cell) has a diverse population of transcripts that reflects a period of metabolic upheaval in the gametophyte cells. Much of the emphasis in this transcriptome is on the protein synthesis machinery, ion and metabolite transport, and the biosynthesis and repair of membranes. When gametophytes are rehydrated, there is a large number of transcripts that code for enzymes involved in oxidative stress metabolism and phosphorylating activities.

When Pterygoneurum lamellatum (Figure 7) is subjected to very rapid drying, it is severely damaged throughout the entire shoot except the shoot apex (Stark et al. 2013). This damage results in slower growth rates, fewer regenerative shoots, and a damaged photosynthetic system as demonstrated by alterations in fluorescence.

**Cell Contents**

As one might expect, cell contents respond to desiccation stress. In the mosses Bryum argenteum (Figure 100) and Didymodon vinealis (Figure 101) from cryptogamic crusts, the free proline content was significantly greater than in those from a typical (wetter) grassland (Xu et al. 2005).

In the moss Plagiomnium acutum (Figure 103), concentrations of proline, soluble sugar, and reducing sugar all increase noticeably during dehydration, reaching maximum concentration after 12 hours (Li et al. 2009). As the membrane permeability increases, activities of protective enzymes likewise increase, including SOD, CAT, and POD. DNA degrades gradually, with only some of the low molecular weight fragments remaining. Upon rehydration, all of these changes reverse. Physcomitrella patens, like Plagiomnium acutum, accumulates the osmoprotectants altrose, malitol, ascorbic acid, and proline when subjected to drought stress (Erxleben et al. 2012).
Cruz de Carvalho et al. (2015) found that the low water potentials in dehydrating cells of the aquatic moss *Fontinalis antipyretica* (Figure 104) is coupled with osmoregulation due to increase of such soluble materials a soluble sugars and compatible inorganic ions. These increase turgor pressure. In addition to its role as an osmolyte, sucrose stabilizes membranes and proteins through *vitrification*, i.e., by creating glasslike substances. When the moss was dehydrated slowly, the cell walls became more elastic, permitting cell shrinkage that maintained turgor and helped to preserve metabolic functions. However, in rapid drying, there was a loss of turgor and osmotic potential. Although the sucrose content increased, rehydration of the fast-dried samples resulted in 50% loss of sucrose through cell leakage as a result of cell membrane rupture. Slowly dehydrated leaves, on the other hand maintained their sucrose content upon rehydration. The thick mats of long dangling *Fontinalis antipyretica* facilitates slow drying of this species in nature.

Sucrose acts as an osmotic "spacer" in membranes (Werner et al. 1991; Oldenhof et al. 2006; Cruz de Carbalho et al. 2014). This is accompanied by ABA mediation of protein synthesis, strengthening the cellular glasses typical of inducible desiccation tolerance in mosses, as shown in *Physcomitrella patens* (Oldenhof et al. 2006).

**Chloroplast Responses**

Bryophyte chloroplasts undergo ultrastructural changes when undergoing desiccation. Chloroplasts become smaller and more spherical with a less-well defined internal structure (Noailles 1978). The general lamellar structure collapses, with the *thylakoids* (chlorophyll vesicles) becoming dispersed; starch granules are lost. This response is similar to that induced by ABA in experiments related to freezing tolerance (Nagao et al. 2005).

Bryophyte chloroplasts contain *plastoglobuli* (Tucker et al. 1975; Oliver & Bewley 1984) in groups within the stroma. These increase in size and number during dehydration in *Syntrichia ruralis* (Figure 93) (Tucker et al. 1975).

It appears that the chloroplasts may be altered by desiccation in other ways we do not fully understand. I found that I could not extract chlorophyll effectively from dry *Fontinalis* spp. (Figure 104) using acetone unless I rewet them for about 15 seconds first. Tuba (1984) reported a possible decoupling of the chlorophyll from its protein, but later (Tuba 1985) attributed that apparent phenomenon to the separation of upper and lower shoots and the extraction process. In fact, he stated that the chlorophyll *a* and *b* remained unchanged during daily desiccation and early rehydration of *Syntrichia ruralis* (Figure 93). He concluded that the *neoxanthin* (a carotenoid pigment), due to its hydrophilous nature, may be adaptive in binding the LHCP (light-harvesting chlorophyll protein) to the *PS II* chlorophyll core, thus stabilizing the LHCP.

One factor in the protection of chlorophyll against light damage during desiccation is that the pigment *zeaxanthin* can bind to the chlorophyll-containing thylakloid protein (Deltoro et al. 1998; Hebert et al. 2001). On the other hand, loss of chlorophyll fluorescence during drying of pre-darkened mosses suggests that energy dissipation in the desiccated mosses is unrelated to zeaxanthin availability.

Even among the desiccation-tolerant bryophytes, the rate of recovery of chlorophyll fluorescence varies widely upon rehydration (Proctor 2010). For example, some species have high values of Fv/Fm in the early minutes of recovery, accompanied by low absolute values of Fm. But most recovery curves are logistic (S-shaped curve that starts slow, goes up exponentially, than approaches horizontal) for photosynthetic CO2 fixation in the light.

**Photosynthesis**

Lee and Stewart (1971), using *Calliergonella cuspidata* (Figure 58), *Climacium dendroides* (Figure 105), and *Hypnum cupressiforme* (Figure 46-Figure 47), found that the degree of desiccation tolerance correlates with the degree of moisture stress experienced in the habitat. This tolerance is expressed as a rapid recovery of photosynthetic rate in taxa from habitats with severe moisture deficits, whereas those from habitats with no appreciable moisture deficits lose photosynthetic capability more quickly and are slower to recover.

Seel et al. (1992) made similar comparisons using *Syntrichia ruralis* (Figure 93), *Bryum pseudepigitetrium* (Figure 106), and *Dicranella palustris* (Figure 107) from a range of habitats with different water availabilities. All three species become photosynthetically inactive when dried to a water content of 100-200%. But recovery differs. The xeric *Syntrichia ruralis* from sand dunes recovers to its pre-desiccation photosynthetic rates, but its rate of recovery is affected by irradiance during its desiccation. Those mosses from hydric habitats, when rehydrated, have partial resumption of their photosynthetic electron transport if they are dried in the dark, but if they are dried in even low light they did not resume their photosynthetic activity. Their symptoms indicate a lasting photoinhibition of photosynthesis following rehydration. On the other hand, the desiccation-tolerant *Syntrichia ruralis* (Figure 93) experiences significant photoinhibition only when receiving continuous high irradiance (1200 µmol m-2 s-1) while hydrated. But if it is dehydrated while receiving high irradiance it shows less evidence of photoinhibition after rehydrations. Desiccation at low irradiance has no effect following rehydration. Leaf curling reduces photon flux absorption by 50-60% in dry mosses compared to hydrated mosses, although it is...
possible that changes in optical properties of papillae may contribute to that reduction.

Figure 105. *Climacium dendroides*, a species that shows acclimation to its habitat adjusting its tolerance to the moisture stress experienced in the habitat. Photo by Michael Lüth, with permission.

Both thylakoid lipids and chlorophyll reduction coincide with the loss of photosynthesis in dehydrating *Atrichum androgynum* (Figure 11) (Guschina *et al*. 2002).

The desert moss *Syntrichia caninervis* (Figure 12) recovers quickly when shoots are remoistened in the dark (Zhang *et al*. 2011). This is an advantage for this moss that receives much of its moisture from dew, a night-time phenomenon. Its leaf hairs are able to trap the dew (and also fog and raindrops) and direct them to the base of the leaf where it rapidly is absorbed. The chlorophyll fluorescence has a narrow optimum range. The moss seems to experience no damage to its membranes or organelles and reaches 90% of its 30-minute photosynthetic yield within the first minute of rehydration. This permits it to take rapid advantage of small amounts of moisture from fog, dew, snow, and short rainfall events.

**Mitochondria**

The mitochondria [cell organelle that generates most of the cell's supply of ATP (adenosine triphosphate), used as a source of chemical energy] become deformed as they dehydrate, becoming small and rounded (Noailles 1978). Internal cristae may be greatly reduced in size or lost completely.

**Nuclei**

The nuclei seem to suffer little from the effects of desiccation, retaining their normal size (Noailles 1978).

**Vacuoles and Vesicles**

Normal bryophyte cells have one to several large vacuoles (Noailles 1978). During dehydration, these break down to form numerous small vesicles (Oliver & Bewley 1984). It appears that ABA may be involved in this transformation, since the response is similar to that induced by ABA during freezing (Nagao *et al*. 2005). ABA-treated cells have slender chloroplasts, and the quantity of starch grains is reduced in comparison with those of non-treated cells.

**Membranes**

Membranes in general suffer from dehydration, including thylakoids, cristae, and cytoplasmic membranes like endoplasmic reticulum and dictyosomes, resulting in the shrinkage of organelles (Noailles 1978). The chloroplast membrane itself may exhibit clefts (Tucker *et al*. 1975). It is the ability to repair this damage that makes many bryophytes desiccation tolerant (Li *et al*. 2009).

Both desiccation-tolerant and intolerant bryophytes leak electrolytes when rehydrated (Gupta 1976, 1977, 1979), as do dry viable seeds, lichens, pollen grains, fungi, and their spores (Simon 1974, 1978). This leakage lasts only a few minutes except in cases of permanent damage (Oliver & Bewley 1984). Oliver and Bewley (1984) listed amino acids, mono-, di-, and tri-saccharides, sugar alcohols, organic acids, hormones, phenolics, phosphates, and various electrolytes as leaked substances during rehydration, although the leakage often lasts only minutes.

The desert moss *Syntrichia caninervis* (Figure 12) is the dominant species in the Gurbantunggut Desert, a cold desert in Central Asia. Wu *et al*. (2012) investigated the
membrane changes during desiccation of this species. There are no significant changes in electrical conductivity of the rehydration water during dehydration or rehydration. There also appears to be no ultrastructural damage to the membrane during dehydration or rehydration, but there are major changes in cellular ultrastructure. Wu and coworkers suggest three possible explanations for the apparent disruption of the membranes in desiccated state:

1. Adaptive morphological features of the leaf that remain intact permit the leaves to regain membrane integrity rapidly upon rehydration.
2. The moss becomes dormant rapidly, maintaining some level of membrane integrity.
3. Soluble sugars and free proline (constitutive substances) increase rapidly during desiccation, contributing to membrane stabilization.

Plasmolysis

One of the consequences of desiccation can be **plasmolysis** of the cells (shrinkage of protoplast away from cell wall) (Oliver & Bewley 1984). In some cases, very narrow elongate cells seem to resist plasmolysis, perhaps due to the small cell volume and strong adhesion to the cell walls. But plasmolysis can occur in bryophytes and can result in cell damage to both the plasma membrane and the cell wall.

In *Didymodon vinealis* (Figure 99) and *Triquetrella papillata* (Figure 95), the dehydrated cells contract to 50-70% of the original volume (Moore et al. 1982). The cell walls contract, permitting the protoplasm to fill the cell and preventing entry of air into the drying cells.

It appears that at least the liverwort *Sphaerocarpos donnellii* (Figure 108) is able to partially compensate for this plasmolysis damage (Grusak et al. 1980), where both normal and plasmolyzed tissues are composed primarily of hemicellulose and cellulose. But in plasmolyzed cells, labelled C\(^{14}\) is considerably lower than in normal cells. Rather, these cells have higher radioactivity in pectin and hemicellulose and less in cellulose, suggesting a possible mechanism for enhancing wall stability. This transformation would provide numerous sites for cross-linkage between the cellulose microfibrils as walls regenerate.

Liverworts

Liverworts have received surprisingly little attention relative to their drought tolerance strategies. Granted, these plants seem to require higher moisture conditions in general, but their presence as epiphytes in many areas attests to the ability of at least some liverworts to survive long periods of drought, and certainly the thalllose liverworts of flood plains and other seasonal habitats provide another set of highly desiccation tolerant or desiccation avoider species.

Pressel et al. (2009) found that liverworts undergo "profound" cytological changes during dehydration. As in tracheophytes and mosses, these include fragmentation of the vacuole, rounding of chloroplasts and mitochondria with thylakoids, and cristae becoming rearranged but remaining undamaged. Furthermore, chlorophyll fluorescence returns to normal within 24-48 hours during rehydration. And like the mosses, their dehydration and rehydration are associated with the depolymerization and repolymerization of the cortical microtubule cytoskeleton. But unique among the bryophytes is the presence of oil bodies in liverworts, membrane-bound organelles that take on many shapes among the species (Kozlowski 1921; Kis & Pócs 1997). And these cellular inclusions, long considered only for their taxonomic value, seem to have an important role in liverwort recovery from dehydration (Pressel et al. 2009).

Taxonomists have been aware that these oil bodies usually disappear in herbarium specimens, and that they do not reappear upon re-wetting and microscopic observation. But it appears that to see these in herbarium specimens, one must treat the liverworts as nature does – dry them slowly and give them time to recover upon rehydration. It turns out that they remain largely unchanged while they are dry (Pressel et al. 2009), but who observes dry specimens under the microscope? Rather, they become flattened when rehydrated and in the six liverworts tested, they require 48 hours to regain their normal shapes, long after the taxonomist has cleaned the microscope slide. Fast drying causes them to disintegrate upon redrying, along with other liverwort organelles. Pressel et al. interpreted this initial loss of shape upon rewetting to indicate a shift in soluble carbohydrates or other components into the cytosol, suggesting that these may be crucial energy reserves needed for recovery and desiccation tolerance.

Kronestedt (1983) found that there was seasonal variability in the oil bodies of the floating liverwort *Ricciocarpos natans* (Figure 109). But as He et al. (2013) made clear, the function of oil bodies in most liverworts still remains unclear.
Habitat Relations

In their review of lichen and bryophyte desiccation and rehydration, Green et al. (2011) considered that the rate of recovery may relate to the length of the hydrated activity period. They reported that species that hydrate and then dry rapidly (e.g., rock surfaces) recover rapidly. By contrast, those species from habitats that remain wet for a long time recover from dryness more slowly when rehydrated.

Cruz de Carvalho et al. (2014) found that even the aquatic moss *Fontinalis antipyretica* (Figure 104) can survive slow dehydration, during which both dehydration and rehydration proteins are induced. These protein profiles are similar to those of the terrestrial moss *Physcomitrella patens* and *Syntrichia ruralis*. The proteins associated with photosynthesis and the cytoskeleton were reduced during dehydration. In their place, the cells accumulated proteins involved in sugar metabolism and plant defenses. Upon rehydration the protein accumulation patterns for photosynthesis and the cytoskeleton return to normal levels. However, those for sugar accumulation and defense remain high. During fast dehydration, on the other hand, this moss exhibited little change in proteins. Upon rehydration, proteins were leaked. The researchers suggested that bryophytes from contrasting habitats may share common desiccation tolerance mechanisms.

**Summary**

Bryophytes may be desiccation tolerant, surviving dry tissues and beginning photosynthesis upon rehydration, or they may be drought avoiders, using structural adaptations and life cycle stages to escape having a dry vegetative plant. The presence of a central strand does not seem to correlate with the degree of internal conduction, but habitat does.

Life cycles are a major protector against dry seasons, permitting bryophytes to survive as tubers, gemmae, spores, fragments, and buds. These stages are typically timed to coincide with drought seasons. They are likely to be combined with physiological changes, including dormancy, in the plants as they respond to changes in the environment.

Xeric bryophytes are more likely to have greater internal conduction and faster external conduction than mesic and hydric taxa. It is possible that the central strand may serve as a water reservoir in some taxa. Physiologically, some bryophytes can increase the osmotic value of the cells, and they typically have a high water capacity compared to drought-tolerant seed plants. Desiccation tolerance permits some bryophytes to remain dormant in a vegetative state for as many as 23 years.

During drying, chloroplasts undergo ultrastructural changes, mitochondria become deformed, and vacuoles break down to form smaller vesicles. Nuclei seem to remain intact. At least some taxa apparently protect their cell membranes from oxidative destruction. ABA seems to induce the production of H$_2$O$_2$ in light, reduce the loss of K$^+$, and may facilitate the reduction of oxygen release from photosystem II. Despite these adaptations, plasmolysis can occur and membranes can become damaged, requiring repair upon rehydration. Liverworts may have one more trick in their cells—oil bodies that disappear rapidly upon rehydration, apparently converting oils into more usable forms of stored energy that could contribute to repair.

**Acknowledgments**

This chapter has benefitted from the help of Beth Scafone and Medora Burke-Scoll, who helped me tow the line in explaining things without leaving too much to one's imagination, but at the same time not repeating myself. I appreciate Lloyd Stark's generosity in sharing the information he found in reviewing papers and in summarizing the seeming conflicts in the literature regarding *Sphagnum*.

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CHAPTER 7-6
WATER RELATIONS: REHYDRATION AND REPAIR

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CHAPTER 7-6
WATER RELATIONS: REHYDRATION AND REPAIR

Figure 1. *Palustriella commutata* rehydrating in the spring runoff. Photo by Michael Lüth, with permission.

Uniqueness of Bryophytes

As Vitt *et al.* (2014) stated, desiccation tolerance is the ability to survive complete loss of free water, a trait found in many bryophytes. One striking difference between bryophytes and tracheophytes is that if you put a dry bryophyte into water, in most cases you will see an immediate change in turgor, and leaves will spread and take their normal hydrated position – one that presents the greatest surface area to the light and atmospheric CO₂. This is particularly striking in mosses from frequently dry habitats, such as *Hedwigia ciliata* (Figure 2) from rocks or *Syntrichia ruralis* (Figure 3, Figure 21) from open sand. In many mosses, such as *Polytrichum* s.l. (Figure 8, Figure 10) and *Syntrichia*, this ability to spread the leaves when moist and appress them to the stem when dry is the result of enlarged or hyaline leaf base cells (Figure 4) that absorb water easily and swell, forcing the leaf away from the stem.

Figure 2. *Hedwigia ciliata* growing on rock. Photo by Janice Glime.
Bryophytes can look dead, but come back to life when rehydrated. For example, Longton and Schuster (1983) noted that both *Pleurozium schreberi* (Figure 5) and *Bryum argenteum* (Figure 6) can have dark or moribund lower shoot tissues, but new shoots and protonemata can regenerate from them. Clymo and Duckett (1986) made similar observations on *Sphagnum*.

Rehydration in mosses is generally very rapid, but some taxa are rather recalcitrant about getting wet inside. *Polytrichum piliferum* (Figure 8), common on sand in dry, exposed habitats, and *Schistidium apocarpum* (Figure 9), a rock-dweller, can require two hours to become saturated, whereas *Polytrichum juniperinum* (Figure 10), a soil moss with wider ecological amplitude than *P. piliferum*, can become saturated within three minutes (Larson 1981). Larson points out that the surface area to mass ratio is very important in determining the speed of rewetting (Figure 11). The cuticle seems to be another contributing factor in mosses like *Polytrichaceae* and *Mniaceae*. 
Duration Survival

Determining the length of time that bryophytes can survive desiccation can be tricky. Although use of herbarium specimens can provide starting dates, these are stored in the dark, which may differ considerably from survival in the light where chlorophyll can be damaged. And one can never be sure how often the moss was wet for examination, often using up resources for repair without having an opportunity to replace them before being put in the dark again and once again desiccated.

Studies to test viability directly after an assortment of desiccation times are rare, requiring careful record keeping and assurance the conditions remain relatively constant over a lengthy period of time. Specimens must then be rehydrated at intervals, requiring multiple specimens and replication, all collected at the same time from one location.

Ochi (1952) reminds us that even season of collection will affect the degree to which bryophytes can survive desiccation and the length of time they can remain dry and survive, an interpretation reiterated by Kosokawa and Kubota (1957). For example, Dilks and Proctor (1976b) commented that British species of bryophytes tend to have an increased tolerance to drought in spring and summer.

Hoekstra (2005) concluded that small size was not a limiting factor in desiccation survival longevity. Factors such as membrane deterioration during desiccation affect the length of time an organism can survive the desiccation (Koster et al. 2010). Hoekstra (2005) likewise attributed survival to a high level of fatty acid saturation in membranes.

Longevities vary considerably among plants, ranging from a few days in some pollen to decades in some moss spores and even green moss tissue (Hoekstra 2005). In 2000, Alpert (2000) asserted that "some desiccation-tolerant species can survive without water for over ten years." Alpert cited duration periods of adult organisms as 34 years for fungi, 23 years for liverworts, 19 years for mosses, 5 years for ferns and angiosperms, and 1 year for lichens. Hornwort spores can tolerate 21 years of desiccation (Vanderpoorten & Goffinet 2009). Some bryophytes exceed these duration records (Table 1).

Even within a fen, desiccation tolerance can vary widely. When eight fen species were compared, it was the hummock moss species Climacium dendroides (Figure 12), Aulacomnium palustre (Figure 13), and Tomentypnum nitens (Figure 14) that had the highest desiccation survival (>10% of stems after 20 weeks of desiccation). Hamatocaulis vernicosus (Figure 15), Calliergonella cuspidata (Figure 16), and Bryum pseudotriquetrum (Figure 17) had moderate resilience (<10% stem survival after 12 weeks). The lowest survival rates occurred in Campylium stellatum (Figure 18) and Plagiomnium elatum (Figure 19) (~0% survival after 6 weeks).
Figure 12. *Climacium dendroides*, a hummock species with high desiccation survival. Photo by Michael Lüth, with permission.

Figure 13. *Aulacomnium palustre*, a species that has high desiccation tolerance on hummock tops. Photo by Michael Lüth, with permission.

Figure 14. *Tomentypnum nitens*, a species with high desiccation tolerance on hummocks. Photo by Michael Lüth, with permission.

Figure 15. *Hamatocaulis vernicosus*, a species with moderate resilience to desiccation. Photo by Michael Lüth, with permission.

Figure 16. *Calliergonella cuspidata*, a species with moderate resilience to desiccation. Photo by Michael Lüth, with permission.

Figure 17. *Bryum pseudotriquetrum*, a species with moderate resilience to desiccation. Photo by Hermann Schachner, through Creative Commons.

Figure 18. *Campylium stellatum*, a species with poor survival of desiccation. Photo by Michael Lüth, with permission.

Figure 19. *Plagiomnium elatum*, a species with poor survival of desiccation. Photo by Michael Lüth, with permission.
### Table 1. Bryophytes and known desiccation survival times. Based mostly on Stark et al. 2016.

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration Dry</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mosses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andreaea rothii</td>
<td>13 mos</td>
<td>Proctor 1981</td>
</tr>
<tr>
<td>Anisothecium staphylinum</td>
<td>45-48 yr (spores, tubers, or rhizoids in dry soil)</td>
<td>Whitehead 1984</td>
</tr>
<tr>
<td>Anoectantium compactum</td>
<td>19 yr</td>
<td>Malta 1921</td>
</tr>
<tr>
<td>Anomodon longifolius</td>
<td>2 yr</td>
<td>Richardson 1981</td>
</tr>
<tr>
<td>Anomodon viticulosus</td>
<td>45 d</td>
<td>Hinshiri &amp; Proctor 1971</td>
</tr>
<tr>
<td>Archidium ohioense</td>
<td>20 yr</td>
<td>Makinde &amp; Fajuke 2009</td>
</tr>
<tr>
<td>Barbula torquata</td>
<td>18 mos</td>
<td>Moore et al., 1982</td>
</tr>
<tr>
<td>Bryum argenteum</td>
<td>2 yr</td>
<td>Richardson 1981</td>
</tr>
<tr>
<td>Bryum coronatum</td>
<td>20 yr</td>
<td>Makinde &amp; Fajuke 2009</td>
</tr>
<tr>
<td>Dicranella heteromalla</td>
<td>0 d</td>
<td>Streusand &amp; Ikuma 1986</td>
</tr>
<tr>
<td>Dicranoweisia cirrata</td>
<td>2 yr</td>
<td>Richardson 1981</td>
</tr>
<tr>
<td>Fissidens minutifolius</td>
<td>6 yr</td>
<td>Makinde 1993</td>
</tr>
<tr>
<td>Fissidens subglaucissimus</td>
<td>20 yr</td>
<td>Makinde &amp; Fajuke 2009</td>
</tr>
<tr>
<td>Fissidens taxifolius</td>
<td>0 d</td>
<td>Streusand &amp; Ikuma 1986</td>
</tr>
<tr>
<td>Fontinalis flaccida</td>
<td>3 mos</td>
<td>Glim 2015</td>
</tr>
<tr>
<td>Grimmia apocarpa</td>
<td>8 mos</td>
<td>Alpert &amp; Oechel 1987</td>
</tr>
<tr>
<td>Grimmia laevigata</td>
<td>10 mos; 10 yr (shoots), 1 mo (protonema)</td>
<td>Alpert &amp; Oechel 1985; Breuil-Sée 1994; Keever, 1957</td>
</tr>
<tr>
<td>Grimmia muehlenbeckii</td>
<td>1.5 yr</td>
<td>Richardson 1981</td>
</tr>
<tr>
<td>Grimmia pilunata</td>
<td>&lt;7 yr</td>
<td>Segreto et al., 2010</td>
</tr>
<tr>
<td>Grimmia elator</td>
<td>5 yr</td>
<td>Richardson 1981</td>
</tr>
<tr>
<td>Grimmia torquata</td>
<td>&lt;7 yr</td>
<td>Segreto et al., 2010</td>
</tr>
<tr>
<td>Hookeria lucens</td>
<td>~15 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Hylacomium splendens</td>
<td>~160 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Neckera crispa</td>
<td>~160 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Octoblepharum albidum</td>
<td>29 wk (leaves); 20 yr</td>
<td>Egunyomi 1979; Makinde &amp; Fajuke 2009</td>
</tr>
<tr>
<td>Orthotrichum rupestre</td>
<td>9 mos; ~2 yr</td>
<td>Alpert &amp; Oechel 1987; Richardson 1981</td>
</tr>
<tr>
<td>Plagiothecium undulatum</td>
<td>100 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Racomitrium lanuginosum</td>
<td>&gt;239 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Rhytidodiaphlus loreus</td>
<td>&gt;100 d</td>
<td>Dils &amp; Proctor 1974</td>
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<tr>
<td>Scorpiurium cichinatum</td>
<td>~120 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Sphagnum fallax</td>
<td>14 d</td>
<td>Sagot &amp; Rochefort 1996</td>
</tr>
<tr>
<td>Sphagnum fuscum</td>
<td>14 d; 0 d</td>
<td>Sagot &amp; Rochefort 1996; Chipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td>Sphagnum magellanicum</td>
<td>14 d; 0 d</td>
<td>Sagot &amp; Rochefort 1996; Chipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td>Sphagnum [3 spp.]</td>
<td>0 d</td>
<td>Chipperges &amp; Rydin 1998</td>
</tr>
<tr>
<td>Syntrichia caninervis</td>
<td>3 yr; 6 yr</td>
<td>Oliver et al., 1993; Oliver et al., 2005</td>
</tr>
<tr>
<td>Syntrichia norvegica</td>
<td>3 yr</td>
<td>Oliver et al., 1993</td>
</tr>
<tr>
<td>Syntrichia ruralis</td>
<td>3 yr; 14 yr</td>
<td>Oliver et al., 1993; Maheu 1922; Stark et al., 2016</td>
</tr>
<tr>
<td>Tortula muralis</td>
<td>3 yr; 14 yr</td>
<td>Kosnar &amp; Kolar 2009; Glim 2015</td>
</tr>
<tr>
<td>Triquetrella papillata</td>
<td>8 wk</td>
<td>Moore et al., 1982</td>
</tr>
<tr>
<td>13 Antarctic species</td>
<td>&lt;1 yr</td>
<td>Davey 1997</td>
</tr>
<tr>
<td>8 fen spp.</td>
<td>8-20 wk</td>
<td>Manukjanová et al., 2014</td>
</tr>
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<td>protonemal resting cells</td>
<td>49 yr</td>
<td>Bristol 1916</td>
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<table>
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<tr>
<th>Liverworts</th>
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<tbody>
<tr>
<td>Bazzania trilobata</td>
<td>0 d</td>
<td>Sollows et al., 2001</td>
</tr>
<tr>
<td>Marchantia berteroana</td>
<td>&lt;1 yr</td>
<td>Davey 1997</td>
</tr>
<tr>
<td>Oxymitra paleacea</td>
<td>4 yr</td>
<td>Volk 1984</td>
</tr>
<tr>
<td>Plagiochila spinulosa</td>
<td>~30 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>Reboulia hemisphaerica</td>
<td>4 yr</td>
<td>Volk 1984</td>
</tr>
<tr>
<td>Riccia canesens</td>
<td>7 yr</td>
<td>Volk 1984</td>
</tr>
<tr>
<td>Riccia macrocarpa</td>
<td>23 yr</td>
<td>Breuil-Sée 1993</td>
</tr>
<tr>
<td>Riccia macrospora</td>
<td>2 yr</td>
<td>Volk 1984</td>
</tr>
<tr>
<td>Riccia marginata</td>
<td>2 yr</td>
<td>Volk 1984</td>
</tr>
<tr>
<td>Saccogyna viticulosa</td>
<td>~200 d</td>
<td>Dils &amp; Proctor 1974</td>
</tr>
<tr>
<td>13 species of hepcatis</td>
<td>&lt;20 mos</td>
<td>Volk 1984</td>
</tr>
</tbody>
</table>

1 shoots allowed to regenerate only 10–14 d
2 13 species of Sphagnum were shown capable of hardening to DT when partially desiccated at high RHs (Hájek & Vicherová, 2014)
3 in the genera Corsinia, Mannia, Plagiochasma, and Riccia
4 based on visible presence of neutral red stain in vacuoles upon rehydration
The duration of desiccation that plants can survive is dependent on the antioxidant pool present at the time of desiccation (Kranner et al. 2002; Moore et al. 2009). This is because longer periods of desiccation result in greater oxidative damage.

Certain events must occur upon rehydration for the bryophyte to survive (Pressel & Duckett 2010). Using moss protonemata, they determined that cell death will occur if these events do not occur. Slow drying will usually prevent these cell death threats.

This raises the question of desiccation survival under desert conditions, where drying can be quite rapid. For leaves, development will be interrupted, but they seem able to resume (Stark 2005). On the other hand, when sporophyte development is interrupted frequently, the sporophyte seems to fail, with only 9 out of 248 surviving during the 4-year study period. Embryonic abortion accounted for 69% of these, whereas 30% was attributable to herbivory. In the Mojave Desert moss Crossidium crassinerve (Figure 20) required a rain event of at least 2 mm to fully rehydrate. In most cases, the only useful hydration periods occurred in the cooler months of October to April, with a mean hydroperiod of 3.7-4.9 days. Although most dry periods were less than 25 days, Stark recorded them as long as 191 days. In a late winter rain event, the moss patches dried slowly over a period of several days, but during a summer event, the patches were dry in as few as 3 hours.

There are two general strategies that permit drought-tolerant plants to survive periods of desiccation: cellular protection and cellular repair. Those bryophytes that are tolerant of desiccation seem to succeed primarily because of their rapid cellular repair (Oliver et al. 1993). According to Oliver (1991), no novel mRNA (messenger RNA; molecule that carries portion of DNA code to other parts of the cell processing) are recruited or favored for translation during desiccation. Rather, in Syntrichia ruralis (Figure 21), there is a loss of 25 hydration proteins (those present in a normal hydrated state), whereas 74 rehydration proteins are synthesized upon rehydration. This system, rather than protecting the moss from desiccation as in most tracheophytes, prepares bryophytes for repair. This is probably essential because their one-cell-thick leaves remain at full turgor, carrying out photosynthesis, then become desiccated very rapidly before going into a state of water stress and suspended metabolism (Proctor 2000b).

Antarctic mosses can suffer severe desiccation for prolonged periods. Rod Seppelt (Bryonet 2007) relates a story of an Antarctic Grimmia (Figure 22). A student had made a number of attempts at sectioning the dried moss without success. Seppelt suggested wetting the moss first and was amazed to discover, upon examination, that the cells were perfectly intact. When he re-examined the mosses that had been sitting on the lab bench for 15 months, but had been rewet for the sectioning, they had sprouted new shoots!

Resumption of Activity

Upon rehydration, desiccation-tolerant bryophytes generally resume normal activity quickly (Csintalan et al. 1999), whereas the resurrection plants among the tracheophytes in the same habitat take much longer (Peterson et al. 1994; Marschall & Proctor 1999).

Using the moss Anomodon viticulosus (Figure 37) and leafy liverwort Porella platyphylla (Figure 23), both from habitats that dry out frequently, Hinshiri and Proctor (1971) found a consistent pattern of net assimilation upon rehydration. When desiccated up to 22 days at 50% relative humidity in Anomodon viticulosus (Figure 37) and 60 days in Porella platyphylla (Figure 23), the plants recovered in 3-4 hours. However, after longer periods, the initial net assimilation was negative, progressively becoming positive during the next several days. After 70 days, respiration in Anomodon viticulosus is very high in the first 24 hours of rehydration, then drops to normal levels. However, even then recovery is not assured. This negative initial net assimilation explains why frequent desiccation with short periods in which to recover before the next one is usually lethal to the bryophytes. In Polytrichastrum formosum (Figure 28), full recovery requires 24 hours (Duckett et al. 2007).
Deltoro et al. (1998a) compared recovery in seven desiccation-tolerant bryophytes [Figure 23: *Hedwigia ciliata*, *Hypnum cupressiforme*, *Leucodon sciuroides*, *Orthotrichum cupulatum*, *Pleurochaete squarrosa*, *Porella platyphylla* (Figure 23), and *Syntrichia ruralis* (Figure 21)] with that of seven desiccation-intolerant bryophytes [Figure 24: *Cinclidotus aquaticus*, *Philonotis calcarea*, *Lunularia cruciata*, *Conocephalum conicum*, *Platyhypnidium riparioides*, *Barbula bolleana* (Figure 25-Figure 26), *Palustriella commutata* (Figure 1, Figure 27)]. All seven desiccation-tolerant bryophytes experienced full recovery, with many cellular activities back to normal rates within two hours (Deltoro et al. 1998a; Marschall & Proctor 1999). However, those species from the hydric and mesic habitats, the desiccation-intolerant ones, were unable to restore their photochemical activity.
Figure 24. Examples of desiccation-intolerant bryophytes. **Left, top:** *Cinclidotus aquaticus*, **Left, middle:** *Philonotis calcarea*, **Left, bottom:** *Lunularia cruciata*, **Right, top:** *Conocephalum conicum*, **Right, bottom:** *Platyhypnidium riparioides*. Photos by Michael Lüth; *Conocephalum conicum* photo by Janice Glime.

Figure 25. *Barbula bolleana* in a seepage waterfall. Photo by Michael Lüth, with permission.

Figure 26. *Barbula bolleana*, a desiccation-intolerant moss. Photo by Michael Lüth, with permission.
Proctor et al. (2007) used *Polytrichastrum formosum* (Figure 28) to assess recovery from desiccation. In this endohydric moss, the relative water content (RWC) dropped to 40% before it reduced the net CO₂ uptake to zero. It took only 10-30% RWC upon rewetting for the CO₂ uptake to become positive after 9-18 days of desiccation. Net carbon balance returned after 0.3-1 hours. The F_v/F_m (= variable fluorescence / maximum fluorescence) recovery was inhibited in the light by protein-synthesis inhibitors, but had normal recovery in the dark. Without the inhibitors, the F_v/F_m reached ~80% of pre-desiccation levels within ~10 minutes of re-wetting, but it took 24 hours for full recovery.

Even aquatic bryophytes may not die following total desiccation. My experience with boiling *Fontinalis* (Figure 29) and with dead-looking mosses following snowmelt is that seemingly dead bryophytes may have living cells that initiate new growth. The desiccated tissues may not recover, but a few cells may be all that are needed to continue the population. The seemingly drought-intolerant *Fontinalis antipyretica* (Figure 29) is actually drought tolerant, provided it is dried slowly (de Carvalho et al. 2011). This is consistent with its ability to survive late summer drought in the slow streams and vernal pools where it is common because the recession of water is slow and remaining water will permit the slow drying needed.

**Leakage and Membrane Repair**

Dry mosses are essentially inactive. During this time, membranes often become distorted and leaky (Gupta 1977a). Viable tissues may become leaky due to the shock of sudden immersion, whereas injured or dead cells leak due to membrane disruption. Cruz de Carvalho et al. (2015) note that the rupture of membranes results in loss of electrolytes, and that this loss is greatest during rehydration following a rapid drying event. The ability to repair this damage may be an important factor that sets bryophytes apart from tracheophytes.

Upon rehydration, the less tolerant bryophytes initially spend time in repairing membrane damage caused by the dehydration. This is exemplified by the period of 4 to 24 hours that elapse prior to normal photosynthesis and respiration (Peterson & Mayo 1975; Dilks & Proctor 1976b; Proctor 1981). But before that repair occurs, leakage of both photosynthetic and mineral ions can be severe, especially during the first two minutes following addition of water (Bewley 1974; Gupta 1977a). As in tracheophytes, the highly soluble K⁺ is readily leaked during desiccation (Minibayeva & Beckett 2001; Table 2), but in the bryophytes, much of it is retained by cation exchange sites on the cell walls (Bates 1997). Fortunately, these retained ions can be re-absorbed by the cells during early rehydration. Material leaked into a culture medium is taken back into the cell within one hour (Bewley & Krochko 1982). Furthermore, at least in some liverworts, some of the lost photosynthate is resorbed (Noailles 1978).

In *Syntrichia ruralis* (Figure 21), slowly dried plants and undried controls lose only about half as much of electrolytes as do rapidly dried plants (Bewley & Krochko 1982). However, *Cratoneuron filicinum* (Figure 30) suffers more extensive loss under both slow and fast drying regimes and the loss is not reversible. Oliver and Bewley (1984b) interpreted these studies to mean that *Syntrichia ruralis* has membranes that undergo reversible changes during desiccation, but that these changes are incomplete when they are dried quickly. Upon rehydration it requires several minutes for the membranes to revert to their normal integrity. This mechanism to regain membrane integrity apparently is not working in the desiccation-intolerant *Cratoneuron filicinum*. 
Plagiochila natalensis (LL) Beckett (2001). This is possible because, respiration, stopping the leakage (Farrar & Smith 1976; Membrane repair occurs during this period of enhanced respiration during which the cell membrane components and other cellular constituents. Because much repair is needed upon rehydration, it is critical that dry mosses retain the ability to synthesize ATP upon rehydration (Krochko et al. 1979). In Syntrichia ruralis (Figure 21), normal levels of ATP are regained in as little as 30 minutes. On the other hand, the hydrophtic Cratoneuron filicinum (Figure 30) slowly loses ATP after rehydration if the moss has been dried rapidly. Such behavior would prevent this moss from living in the desert, but poses no problem in its streamside habitat. However, Dhindsa (1985) suggested that it may be NADPH that is available immediately upon rehydration, produced by transhydrogenation from NADH during dark NADPH that is available immediately upon rehydration, it is critical that dry mosses retain the ability to synthesize ATP upon rehydration (Krochko et al. 1979). In Syntrichia ruralis (Figure 21), normal levels of ATP are regained in as little as 30 minutes. On the other hand, the hydrophtic Cratoneuron filicinum (Figure 30) slowly loses ATP after rehydration if the moss has been dried rapidly. Such behavior would prevent this moss from living in the desert, but poses no problem in its streamside habitat. However, Dhindsa (1985) suggested that it may be NADPH that is available immediately upon rehydration, produced by transhydrogenation from NADH during dark CO₂ fixation. Thus NADPH could be the important factor in repairing cellular damage by reductive biosynthesis of membrane components and other cellular constituents.

When the membrane first begins repair, there is a period of enhanced respiration during which the cell organelles regain normal appearance (Nоailles 1978). Membrane repair occurs during this period of enhanced respiration, stopping the leakage (Farrar & Smith 1976; Richardson & Nieboer 1980). This is possible because, unlike the case in tracheophytes, protein synthesis begins immediately (Dhindsa & Bewley 1978), undoubtedly because of the conservation of polyribosomes (cluster of ribosomes connected with messenger RNA; play a role in peptide synthesis) in desiccation-tolerant bryophytes. Nothing is known about the role of action potentials in bryophytes and their possible role in membrane repair (Bates 2000), although Trebacz et al. (1994) have shown that Ca²⁺ influx and Cl⁻ efflux in the thallose liverwort Conocephalum conicum (Figure 24) result in depolarization of the cell membranes.

Mechanical damage is probably the primary cause of desiccation damage in cells. Membranes necessarily become contorted and folded during drying and cell shrinkage. In Syntrichia ruralis (Figure 21) pockets or vesicles (membranous spheres involved in transport or storage within cell) form on the endoplasmic reticulum (complex system of membranous stacks involved in membrane production in cell). Oliver and Bewley (1984b) suggested that these vesicles provide membrane material to be used for immediate repair upon rehydration. Other features that can help protect a cell from mechanical damage during dehydration include small cell size, small or no vacuoles, lack of plasmodesmata (tiny, membrane-line channels between adjacent cells), flexible cell walls, and reduced osmotic pressure (Iljin 1953, 1957). However, there is not a strong correlation of these attributes with desiccation-tolerant bryophytes. Bryophytes do have plasmodesmata, but electron microscopy is needed to discern them and few have been thus described; thus we cannot evaluate their correlation.

In support of Iljin’s (1953, 1957) suggestion, some of the largest cells among bryophytes are those of the Hookeriaceae, a family of desiccation-sensitive mosses. And the Pottiaceae (including Syntrichia ruralis) generally have small cells and live in dry places. But the vacuole correlation brings Iljin’s suggested adaptations into question (Table 3), and even the cells of Syntrichia ruralis (Figure 21) shrink but are too rigid to collapse when they dry. One problem in attempting to determine just what happens as the cells dry is that in order to “fix” them for examination, we must partially rehydrate the cells (Oliver & Bewley 1984b). Until another method is forthcoming, we cannot observe what a dry cell looks like.

Table 2. Loss of K⁺ ions during rehydration following desiccation in bryophytes. H = hornwort; LL = leafy liverwort; M = moss; TL = thallose liverwort. Data from Minibayeva and Beckett (2001).

<table>
<thead>
<tr>
<th>Species</th>
<th>H (%)</th>
<th>LL (%)</th>
<th>M (%)</th>
<th>TL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthoceros natalensis</td>
<td>89%</td>
<td>83%</td>
<td>77%</td>
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</tr>
<tr>
<td>Pellia epiphylla</td>
<td>55%</td>
<td>45%</td>
<td>38%</td>
<td>21%</td>
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<tr>
<td>Hookeria lucens</td>
<td>55%</td>
<td>45%</td>
<td>38%</td>
<td>21%</td>
</tr>
<tr>
<td>Dumortiera hirsuta</td>
<td>55%</td>
<td>45%</td>
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<td>21%</td>
</tr>
<tr>
<td>Atrichum androgynum</td>
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<td>45%</td>
<td>38%</td>
<td>21%</td>
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<td>55%</td>
<td>45%</td>
<td>38%</td>
<td>21%</td>
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<td>Plagiochila natalensis</td>
<td>55%</td>
<td>45%</td>
<td>38%</td>
<td>21%</td>
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<tr>
<td>Rhodobryum roseum</td>
<td>55%</td>
<td>45%</td>
<td>38%</td>
<td>21%</td>
</tr>
</tbody>
</table>

Figure 30. Cratoneuron filicinum in hydrated state. Photo by Michael Lüth, with permission.

The leakage problem causes bryophytes to be vulnerable during frequent wetting/drying events. During each rehydration event, the plant must repair its cell membranes, and that requires energy. Frequent events with insufficient recovery time will eventually exhaust the resources within the cells. Because much repair is needed upon rehydration, it is critical that dry mosses retain the ability to synthesize ATP upon rehydration (Krochko et al. 1979). In Syntrichia ruralis (Figure 21), normal levels of ATP are regained in as little as 30 minutes. On the other hand, the hydrophtic Cratoneuron filicinum (Figure 30) slowly loses ATP after rehydration if the moss has been dried rapidly. Such behavior would prevent this moss from living in the desert, but poses no problem in its streamside habitat. However, Dhindsa (1985) suggested that it may be NADPH that is available immediately upon rehydration, produced by transhydrogenation from NADH during dark CO₂ fixation. Thus NADPH could be the important factor in repairing cellular damage by reductive biosynthesis of membrane components and other cellular constituents.

When the membrane first begins repair, there is a period of enhanced respiration during which the cell organelles regain normal appearance (Nоailles 1978). Membrane repair occurs during this period of enhanced respiration, stopping the leakage (Farrar & Smith 1976; Richardson & Nieboer 1980). This is possible because, unlike the case in tracheophytes, protein synthesis begins immediately (Dhindsa & Bewley 1978), undoubtedly because of the conservation of polyribosomes (cluster of ribosomes connected with messenger RNA; play a role in peptide synthesis) in desiccation-tolerant bryophytes. Nothing is known about the role of action potentials in bryophytes and their possible role in membrane repair (Bates 2000), although Trebacz et al. (1994) have shown that Ca²⁺ influx and Cl⁻ efflux in the thallose liverwort Conocephalum conicum (Figure 24) result in depolarization of the cell membranes.

In an interesting contrast to the membrane repair scenario, Singh et al. (1984) concluded that membranes of Syntrichia ruralis (Figure 21) remain intact during desiccation, at least down to 75% relative humidity (-400 bars). The cellular membranes retain their phospholipid bilayers, and during dehydration the cytoplasmic vesicles form layers of membranes under the plasmalemma (cell membrane), appearing to fuse with the surface membrane. They

Table 3. Relative cell and vacuole sizes among bryophytes as listed by Oliver & Bewley (1984b).

<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Vacuole Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desiccation tolerant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceratodon purpureus</td>
<td>small</td>
<td>large</td>
</tr>
<tr>
<td>Syntrichia ruralis</td>
<td>small</td>
<td>small</td>
</tr>
<tr>
<td>Neckera crispa</td>
<td>small</td>
<td>small</td>
</tr>
<tr>
<td>Pleurozium schreberi</td>
<td>long &amp; narrow</td>
<td>small</td>
</tr>
<tr>
<td>Barbula torquata</td>
<td>small</td>
<td>large</td>
</tr>
<tr>
<td>Triquetrella papillata</td>
<td>small</td>
<td>small</td>
</tr>
<tr>
<td>Desiccation sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cratoneuron filicinum</td>
<td>long &amp; narrow</td>
<td>small</td>
</tr>
</tbody>
</table>
concluded that the cellular membranes are conserved and ready to expand upon rehydration. Wu et al. (2013) found a similar conservation of cell membranes in the desert moss *Syntrichia caninervis*.

Based on these various responses of the cell membranes, it is not surprising that Oliver et al. (1993) found that electrolyte leakage alone was not a reliable measure of desiccation tolerance in *Syntrichia ruralis* (Figure 21). Instead, Stewart and Lee (1972) reported that NADP-linked glyceraldehyde phosphate dehydrogenase is affected by desiccation, and Bewley and his coworkers (Bewley 1972, 1973a, b, 1974, 1979, Bewley & Gwozdz 1975) have carefully documented the loss of polyribosomes and their effect on the ability of the cells to synthesize proteins. Oliver et al. (1993) found that comparison of ability to synthesize protein in hydrated and desiccated-rehydrated mosses was the best measure of the capabilities of three *Syntrichia* species to repair damage and thus to exhibit tolerance to desiccation.

**Pulse release** occurs in *Hylocomium splendens* (Figure 32) during rehydration, returning carbon and other nutrients, especially potassium, to the soil (Wilson & Coxson 1999). These mosses are able to concentrate carbon and nutrients from atmospheric sources and return them in concentrated form during these pulse releases caused by rainfall striking damaged membranes.

**Protein Degradation and Ubiquitin**

O'Mahony and Oliver (1999) compared the role of ubiquitin in the grass *Sporobolus stapfianus* and the desiccation-tolerant moss *Syntrichia ruralis* (Figure 21) as a mediator of protein degradation. They found that in *S. stapfianus* the ubiquitin exhibited greater accumulation during drying and rehydration, but that it was hardly detectable in the desiccated tissue. A depletion of ubiquitin monomer levels indicates an increase in protein degradation. In *Syntrichia ruralis*, the ubiquitin transcripts were stable in the dried tissue. The moss contrasted to the grass in that conjugated ubiquitin, indicative of proteins targeted for removal, was detectable in the moss only during slow drying, whereas it was present in all samples of the grass. O'Mahony and Oliver concluded that *S. ruralis* has stable ubiquitin transcripts that rapidly translate during rehydration to permit rapid initiation of cellular repair by degrading targeted proteins, whereas *Sporobolus stapfianus* requires several hours to replace its depleted ubiquitin supply.

**Respiration**

Respiration during recovery can vary considerably among species. Gupta (1977b) found that after 48 hours of desiccation at 0 and 50% relative humidity, rewetting for 32 hours varied in O2 uptake from 2X in *Mnium hornum* (Figure 33) and *Porella platyphylla* (Figure 34) to 6X in *Scapania undulata* (Figure 35). This may in part be due to the presence of many respiring microorganisms that benefit from the leaked cellular contents (Gupta 1977a, b). Methods for measuring recovery processes need to take this microorganism respiration into account.

**Reactive Oxygen Species**

The greatest damage to cells is caused by reactive oxygen species (Kraner et al. 2002; Beckett et al. 2004). Among the bryophytes, Beckett et al. (2004) demonstrated this in desiccated thalli of the liverwort *Dumortiera hirsuta* (Figure 36). In fact, this species produces extracellular superoxide at high rates under normal conditions, but that following mild desiccation stress, it produces considerably
more during rehydration. They postulated that it might have a role in defense against pathogens. Even aquatic mosses like *Fontinalis antipyretica* (Figure 29) has protection from reactive oxygen species. de Carvalho *et al.* (2012) found that when this species was dried slowly and rehydrated, it had a lower production of reactive oxygen species (ROS). This reduced the cellular damage. As it rehydrated, it had an initial high oxygen consumption burst; de Carvalho and coworkers suggested that this may have been due to the burst of ROS production.

**Photosynthesis**

The desert moss *Syntrichia caninervis* (Figure 31) is a dominant soil crust bryophyte in deserts. As such, it has often served as a model for desiccation tolerance. Its photosynthesis recovers quickly following a dehydration-rehydration cycle (Li *et al.* 2010). The recovery occurs in two phases. The initial phase occurs in only three minutes, with a quick increase in maximal quantum efficiency of PS II (Fv/Fm) (photosystem II variable vs maximum fluorescence). In only 0.5 minutes from the onset of rehydration, over 50% of the PS II activities resume, including excitation energy transfer, oxygen evolution, charge separation, and electron transport. The second phase is slower and is dominated by an increase of plastoquinone (PQ; molecule involved in the electron transport chain in the light-dependent reactions of photosynthesis) reduction and accomplishing equilibrium of the energy transport from the inner chlorophyll antenna system to the reaction center of PS II. No de novo chloroplast protein synthesis is needed for this initial recovery of the PS II photochemical activity. The rapid recovery depends on chlorophyll synthesis, quick structural reorganization of PS II, and fast restoration of PS II activity without chloroplast protein synthesis.

Zhang *et al.* (2011) found that in *Syntrichia caninervis* (Figure 31), an ectohydric desert moss, minimum and maximum fluorescence and photosynthetic yield recovered quickly when the shoots were rehydrated in the dark. In fact, this species reached 90% of its 30-minute yield rate within the first minute, a phenomenon that was possible because of the lack of damage to membranes.

In *Syntrichia caninervis* (Figure 31) remoistening elicited rapid recovery of both fluorescence and photosynthetic yield (Fv/Fm) in the dark, reaching within 1 minute 90% of the value attained in 30 minutes (Zhang *et al.* 2011). The optimum moisture level falls in a narrow range, with chlorophyll fluorescence decreasing both above and below that moisture range. In its desert habitat, it is able to use dew, fog, rain, and melting snow as sources of moisture to permit photosynthesis.

At least in some species, rehydration results in an initial period of rapid respiration (Dilks & Proctor 1976b). In several temperate/boreal bryophytes, this rapid period of respiration is followed by a progressive recovery of photosynthesis generally lasting 1-6 hours. *Anomodon viticulosus* (Figure 37), a xerophytic species of well-drained, lightly shaded, base-rich or calcareous rocks and dry stone walls, reached its compensation point (photosynthesis = respiration) within a few minutes of hydration, whereas it required about 4 hours for *Rhytidiodelphus loreus* (Figure 38), a mesophytic forest floor species. For desiccation-tolerant bryophytes such as *Anomodon viticulosus*, *Racomitrium lanuginosum*...
(Figure 39), and *Rhytididelphus loreus*, recovery of photosynthesis upon rehydration is rapid (Proctor & Smirnoff 2000). This rapid recovery necessarily requires pre-existing proteins; *de novo* protein synthesis is generally very limited (Proctor 2001).

Dhindsa (1985) determined that desiccation-tolerant mosses such as *Syntrichia ruralis* (Figure 21) remain active and fix CO$_2$ (dark fixation) at an undiminished rate until tissue losses are about 60% of the initial fresh mass, whereas in the intolerant *Cratoneuron filicinum* (Figure 30) dark fixation of CO$_2$ slowly declines as the moss dehydrates. After that, water stress occurs, the moss rapidly proceeds to suspended metabolism, and CO$_2$ fixation rapidly ceases. Following rehydration, *S. ruralis* immediately begins CO$_2$ fixation, but *C. filicinum* does not. For tracheophytes, this recovery system has been perfected primarily in seeds that return from their suspended metabolism by metabolizing starches to sugars for the rapid supply of energy needed to grow and attain photosynthesis. Even in the desert ephemerals, the return process is slow and the frequency of wetting and drying suffered and survived by some desert bryophytes is unattainable by any tracheophyte (Proctor 2000b, 2001).

Guschina *et al.* (2002) related the rapid recovery to the stress hormone ABA in the mesophytic moss *Atrichum androgynum* (Figure 40). Changes in phosphoglyceride composition due to water stress indicate an activation of phospholipase D and of phosphatidylinositol metabolism. During rehydration, phosphoglyceride composition recovers close to the original levels. Thylakoid lipids and chlorophyll decline during dehydration, accounting for the loss of photosynthesis. Treatment with ABA reduces the overall extent of changes, probably by reducing lipid changes, thus protecting against membrane damage. But can the moss produce its own ABA? And is it inducible?

**Architectural Changes**

We know that many bryophytes, including *Syntrichia ruralis* (Figure 21), undergo multiple architectural changes as they dry (Hamerlynck *et al.* 2000). This results in changes to the surface reflectance. Hamerlynck *et al.* found a sigmoidal (logistic) relationship between the relative humidity and the deviation of the moss mat temperature from its dew point, indicating a slow, then rapid, then slow change in the temperature of the mat, and a concomitant change in its water loss. The conditions of drying affect the ability of this species to use thermal
dissipation of excess light energy, thus affecting potential damage to the chlorophyll.

Breuil-Sée (1994) examined the cell interior upon rehydration of the thallose liverwort *Riccia macrocarpa* (Figure 41) after 25 years of dehydration in a herbarium. Whereas most bryophytes revive to normal metabolism in a few hours, this 25-year-dry bryophyte required nine days. Cytological evidence of its revival included enlargement of nucleoli (sites of ribosome synthesis and assembly in nucleus), evidence for protein synthesis. The dehydrated liverworts had few mitochondria (site in cell that generates most of the ATP) and the chloroplasts lacked starch. Its preparation for desiccation was evidenced in granular cytoplasm with many osmiophilic globules (lipid-containing bodies in chloroplast), especially along the cell wall. Features already known for dry spores and seeds, such as presence of plasmodesmata (microscopic channels which traverse cell walls of plant cells, enabling transport and communication between cells), but absence of dictyosomes [stacks of flat, membrane-bound cavities (cisternae) where proteins are stored and that comprise the Golgi apparatus] and endoplasmic reticulum (ER; interconnected network of flattened, membrane-enclosed sacs or tubes known as cisternae; inner core of cytoplasm and membranes of ER are continuous with outer membrane of nuclear envelope), were evident. The transition of *R. macrocarpa* toward active metabolism upon rewetting was marked by 1) enlargement of nucleolus; 2) important modification of nucleus; 3) amplification of endoplasmic reticulum, Golgi, chloroplasts, mitochondria, and vacuoles; 4) disappearance of lipid reserves; 5) synthesis of starch in chloroplasts; 6) cytoplasm densification.

**Cellular Changes**

Oliver *et al.* (2005) indicated that desiccated cells appear to be intact. Cellular disruption occurs upon rehydration as water is taken up rapidly. Nevertheless, the cellular integrity returns rapidly.

Desert mosses can have remarkable durability to desiccation. Moore *et al.* (1982) found that *Didymodon torquatus* (Figure 42) can survive 18 months of desiccation at a water content of only 5% or less. Nevertheless, after only 24 weeks of desiccation, the photosynthetic and respiratory rate upon rehydration were less than that of fresh (hydrated) materials. What is interesting is that in shorter time periods this species returned to control levels within one hour of rewetting. *Triquetrella papillata* (Figure 43), however, had a shorter survival time. In both species, the integrity of the organelles was maintained during short periods of desiccation, but that integrity diminished progressively with time. Net photosynthesis was delayed, apparently due to the disappearance of chloroplast and mitochondrial membranes and loss of internal structure.

The protonemata are important survival structures in some habitats and for some species. Pressel and Duckett (2010) found that in their experiments the protonemata could survive slow, but not fast drying. During dehydration, the cell experiences vacuolar fragmentation, reorganization of the endomembranes, changes in cell wall thickness, changes in the morphology of plastids and mitochondria, and a controlled dismantling of the cytoskeleton. These events cannot occur during fast drying. Externally applied abscisic acid mimicked the effects of slow drying, permitting the protonemata to survive.

**Figure 42.** *Didymodon torquatus* dry, a species that can survive extreme desiccation for 18 months. Photo from Canberra Nature Map, through Creative Commons.

**Figure 43.** *Triquetrella papillata* dry, a species that survives a short period of drought. Photo by David Tng, with permission.
Despite this degradation with time, Breuil-Sée (1994) found that the thallose liverwort *Riccia macrocarpa* revived after 23 years of drying. Upon rehydration, the endoplasmic reticulum became extended and the nucleolar volume increased, but these events were not observed until day 9.

**Leptoid Recovery**

Pressel (2006) pointed out the lack of study on the behavior of leptoid cells following rehydration. Using the endohydric moss *Polytrichastrum formosum*, she documented that desiccation cause dramatic changes in leptoid tissues. The endoplasmic microtubules disappear; the nucleus, mitochondria, and plastids become rounded and longitudinal alignment of the organelles disappears. Cytoplasmic polarity is at least partly retained. Instead of the prominent stacks of endoplasmic reticulum that characterize the hydrated state, the membranous tubules are arranged at right angles to the main cellular axis. The cytoplasm of the leptoids is filled with small vacuoles. The plasmalemma deposits ingrowths of cell wall material, forming labyrinthine extensions. The plasmodesmata of apical meristematic and stem parenchyma cells seem unaffected by dehydration, but in the leptoids they become plugged with electron-opaque material. Starch is depleted in the parenchyma cells adjoining the leptoids. In control plants, the cellular structure is completely re-established in 12-24 hours, but this is not the case in cells treated with oryzalin, a microtubule-disrupting drug. Pressel concluded that the microtubular cytoskeleton is key in the rapid re-establishment of the cytoplasmic architecture of leptoids during rehydration.

**Chloroplast Recovery**

Proctor *et al.* (2007) found that thylakoids, grana, and mitochondrial cristae of *Polytrichastrum formosum* (Figure 28) remain intact during drying and re-wetting. Nevertheless, the form of organelles changes quite noticeably. Chloroplasts lose their prominent lobes, becoming rounded when desiccated. They require ~24 hours to return to their normal shape. Photosynthesis likewise requires 24 hours for full recovery, but is independent of protein synthesis. It appears that the physical structure of the chloroplast remains the same, but that the spatial relationships among the components is altered during dehydration. Proctor *et al.* concluded that the cytoskeleton has a significant role in the bryophyte desiccation response.

Wood and coworkers may have a partial answer to the recovery of the chloroplasts following desiccation (Wood & Oliver 1999; Wood *et al.* 1999; Zeng & Wood 2000; Zeng *et al.* 2002). There is a change in gene expression during rehydration of *Syntrichia ruralis* (Figure 21), suggesting that new proteins are being made. It appears that some of these proteins may account for the rapid chlorophyll recovery. We now understand that the moss prepares for its desiccation and rehydration events by altering gene expression in response to desiccation, then altering translational controls as it rehydrates. When the drying rate has been slow, mRNPs (messenger ribonucleoprotein particles) are formed in the drying plants, and within these particles they sequester rehydrin mRNA (mRNA transcripts used during rehydration). It appears that one of these rehydrins may be responsible for the production of antioxidants during rehydration (Oliver *et al.* 1997). It is the production of these mRNPs that makes slow dehydration so important to the recovery (Oliver 1996). If the moss is dried rapidly, it must make these when it rehydrates.

Wood and coworkers (1999) supported this discovery that *Syntrichia ruralis* (Figure 21) has an active recovery mechanism that is induced by rehydration. It makes a set of polypeptides that are not present at any time except during rehydration. These polypeptides were products of a large number of as yet unidentified plant genes and 71% of these are unknown in other plant phyla.

Among these are most likely the cDNA Rp115 identified by Zeng and Wood in 2000 and which is conserved as mRNA in desiccated gametophytes, and two additional cDNA units (*Elipa* and *Elipb*), both of which have significant similarity to Early Light-Inducible Proteins (ELIP; Zeng *et al.* 2002). The ELIP group (coded by Elip genes) includes over 100 stress-inducible proteins (Heddad & Adamska 2002). They are produced in response to light stress and accumulate in photosynthetic membranes where they have a photoprotective function. They are closely related to the light-harvesting chlorophyll *a/b*-binding antenna proteins of photosystems I and II. Because of the response of *Elipa* genes to slow desiccation, rapid desiccation/rehydration, salinity, ABA, and rehydration in high light, and the response of *Elipb* genes to ABA or rehydration in high light, Zeng *et al.* (2002) suggested that ELIPs and ELIPb provide an adaptive response to the photodamage that is likely to occur within a moss chloroplast during desiccation, most likely playing an important role in protecting and/or repairing the photosynthetic apparatus.

In support of this hypothesis, Hutin and coworkers (2003) found that when they suppressed this rapid accumulation of ELIPs during high-light stress in a mutant of the flowering plant *Arabidopsis thaliana*, the leaves became bleached and cells suffered extensive photooxidative damage, but when the plant was permitted to accumulate ELIPs before the stress, they exhibited normal phototolerance. Hence, it appears that they do indeed perform a photoprotective function, either by binding the chlorophylls that are released during turnover of the pigment-binding proteins or by stabilizing the proper assembly of those proteins when they are being subjected to high-light stress.

Lüttge *et al.* (2008) found that the three poikilohydric species *Campylopus savannarum, Rhacocarpus fontinaloides,* and *Ptychomitrium vaginatum* achieved photo-oxidative protection in their light-adapted state. This was accomplished by a reduction of chlorophyll fluorescence to near zero. When rewet, they have a very fast recovery in the first 5 minutes, but require more than 80 minutes to reach an equilibrium. Even though they occupy different niches on their rock outcrop habitat, they had similar recovery kinetics, with only their photosynthetic capacity differing slightly.

**Photodamage**

For the most desiccation-tolerant mosses, those from xeric (dry) habitats, fluorescence (emission of light of longer wavelength due to absorbance of light from outside source) levels upon rehydration indicate that the
photosynthetic apparatus is fully functional, unlike that of mosses from hydric (wet) and mesic (moderate) habitats (Delto et al. 1998a; Marshall & Proctor 1999).

Photoinhibition (inhibition of photosynthesis by light) is a well-known consequence of desiccation because the light quenching is greatly diminished or absent. Only the desiccation-tolerant bryophytes exhibited photo-quenching at low water content in these experiments. Delto and coworkers (1998a, b) suggest that this loss of photosynthetic capability in mesophytic bryophytes might be not only a consequence of photoinhibition, but also a result of membrane damage, as indicated by the large K⁺ leakage. In desiccation-tolerant taxa, they suggest, the ability to enhance the dissipation of thermal energy during dehydration might permit them to take advantage of the erratic water supply in places like the desert and decrease the problems of photodamage during the dehydration stage, thus permitting them to recover quickly.

Measuring Damage

Records of survivability may sometimes be misleading. For example, Makinde and Fajude (2009) reported survival based on microscopic views of vacuoles as soon as the cells were hydrated without any verification by regeneration, a true test for survival.

Not only do different species respond differently, but leaves and cells vary on the same plant. Streusand and Ikuma (1986) suggested a protocol that requires a large number of cells counted in a given leaf, a large number of leaves, and a large number of shoots. They considered 10 cells in 6 areas of each of 6 leaves per shoot on 10 shoots to be adequate and it provided a near perfect correlation with shoot survival in experiments with different desiccation protocols.

Factors Affecting Recovery

Temperature

In the dry state, plants are much more resilient at temperature extremes than are hydrated plants. As Alpert (2000) pointed out, some can survive as low as -272°C or as high as 100°C. He raises two questions regarding survival of desiccation: What are the mechanisms by which plants tolerate desiccation and why are desiccation-tolerant plants not more ecologically widespread? In general, they seem to require protection from oxidants and from loss of configuration of the macromolecules during their dehydration period.

Drying Speed

Many studies have indicated that drying speed is important to successful recovery from desiccation (Krochko et al. 1978; Schonbeck & Bewley 1981a; Greenwood & Stark 2014). This varies, based on inducible vs constitutive desiccation tolerance responses. Those that are harmed by rapid drying, but that recover after slow drying, are able to use an inducible system (one that develops in response to desiccation) to protect them against desiccation effects. The slower timing is required for that inducible system to prepare. This system is more likely to be effective in aquatic or wet-habitat species, as demonstrated by the semi-aquatic Cratoneuron filicinum (Figure 30). In this species, rapid drying results in considerable disruption of the cell contents, whereas following slow drying some cells are able to maintain their cellular organization and integrity. Protein synthesis is reduced upon rehydration under both very slow and rapid drying, but these effects are reversible down to a water loss of 50% of fresh weight. Unlike the observations of Dilks and Proctor (1976b) on several terrestrial boreal/temperate bryophytes, respiration does not occur when the moss is rewet after rapid drying.

Even in such xerophytic taxa as Syntrichia ruralis (Figure 21), rapid drying causes visible injury, reduced total chlorophyll, reduction in chlorophyll a:b ratio, greatly enhanced electrolyte loss, and consequent inhibition of gross photosynthesis (Schonbeck & Bewley 1981a). Partial desiccation for 1-3 hours before rapid drying will eliminate this injury, suggesting that the moss requires time to prepare for its recovery. When Syntrichia ruralis and hydrophytic Cratoneuron filicinum (Figure 30) are dried rapidly, the chloroplasts and mitochondria swell and lose their integrity upon rewetting (Krochko et al. 1978, 1979), but S. ruralis regains normal appearance within 24 hours, whereas C. filicinum loses its cell contents and shows considerable cell degradation. However, if the cells are dried more slowly (e.g. 12 hours at 75% RH), both species recover within 24 hours. Dhinda and Bewley (1978) attribute the ability of Syntrichia ruralis to survive this swelling of organelles to their ability to synthesize or retain sufficiently the enzymes needed for repair.

Hamerlynck et al. (2002) later found that Syntrichia ruralis (Figure 21) grown in high light intensity has greater desiccation tolerance than plants grown in the shade, but that those plants growing in the shade may benefit from their longer periods of metabolic activity and greater acquisition of resources, permitting them to adjust sufficiently to canopy openings and other disturbances.

Proctor (2003) subjected both desiccation-tolerant and moderately desiccation-tolerant species to drying for various periods up to 240 days. The more desiccation-tolerant species (Grimmia pulvinata, Syntrichia ruralis, Andreaea rothii, Racotritium lanuginosum, R. aquaticum, Leucodon sciuroides, Pleurochaete squarrosoa, Uloa crispa) had their best long-term survival (>30-120 days) at ~100 to -200 MPa (20-45% r.h.). The moderately desiccation-tolerant Anomodon viticulosus, Porella platyphylla, and P. obtusata survived best at the highest humidity used, -41 MPa (74% r.h.). The lower humidities would speed desiccation and only the most tolerant could survive.

Greenwood and Stark (2014) determined that when Fv/Fm are less than 0.1, Physcomitrella patens fails to regenerate. The Fv/Fm fluorescence is the standard measurement for stress in plants, testing whether or not plant stress affects photosystem II in a dark adapted state. Fv refers to fluorescence in its variable state; Fm is maximum fluorescence. They used a process of drying that permitted as long as 284 hours for drying and found a significant increase over results obtained using salt solutions to create desired moisture conditions. Survival rates and chlorophyll fluorescence both improved and tissue regeneration time was shortened, demonstrating a much greater desiccation tolerance than was previously known for this species.
Frequency of Dehydration/Rehydration

Upon rehydration, it requires time to repair membranes and regain the energy lost. Oliver and Bewley (1984a) have demonstrated that in some mosses the first 24 hours are spent in repair, and it is only after that period that there is a net photosynthetic gain. For this reason, frequent short sequences of desiccation can be devastating to many species, whereas the same moss can endure long periods of desiccation. For example, Didymodon vinealis (Figure 44) (Moore et al. 1982) recovered completely within one hour of rewetting after 18 months of desiccation at less than 5% relative water content. However, following short periods of desiccation, the integrity of the organelles was progressively lost, including membrane loss from chloroplasts and mitochondria. Repairing this damage resulted in delays in net photosynthetic gain.

Figure 44. Didymodon vinealis, a moss that is able to recover within one hour of hydration after 18 months of desiccation. Photo by Jan-Peter Frahm, with permission.

Dilks and Proctor (1976b) likewise promoted the understanding that frequency of desiccation can be more important than duration. Using 6 days wet – 1 day dry conditions compared to 1 day wet – 6 days dry, 1 day wet – 1 day dry, and 7 days wet – 7 days dry for a period of 18 weeks, they showed that Hylocomium splendens (Figure 32) grew equally well in continuous moist conditions and in 6 days wet – 1 day dry (32% relative humidity). However, there was little or no growth among the other treatments. In Rhytidiadelphus loreus (Figure 45), growth was best in continuously hydrated mosses, then 6 wet – 1 dry day mosses, then 7 wet – 7 dry day mosses. There was essentially no growth in the other treatments. Responses by Syntrichia ruralis (syn. Tortula ruraliformis; Figure 21) were so variable that they could not be interpreted. However, Dilks and Proctor were able to conclude that 63 wet-dry cycles were not harmful, but that constant moist conditions were harmful in this highly desiccation-tolerant moss. Rhytidiadelphus loreus, unlike the other mosses, showed a hardening effect (process of increasing resistance to stress factor), indicating less effect from drought as more droughts occurred. Syntrichia ruralis is always drought-ready so hardening is not discernible.

To test the impact of intermittent desiccation on reproductive success of xerophytic mosses, Mishler and Newton (1988) measured the success of germination of both fragments and spores of four Syntrichia species [S. ruralis (Figure 21), S. princeps (Figure 46), S. norvegica (Figure 47), S. laevipila (Figure 48)] in continuous versus intermittent moisture. Only S. princeps fragments did slightly better under the intermittent moisture conditions, as did its spore germination. In all other species, the continuous hydration seemed beneficial to the spores. Establishment success was quite different. None of the spore-derived protonemata gave rise to stems (Mishler & Newton 1988). Fragments, however, produced numerous stems both from protonemata and directly from the fragments, independent of the hydration conditions. Most likely some other physiological or environmental cue was missing for the spore-derived protonemata.

Figure 45. Rhytidiadelphus loreus, a moss that undergoes drought hardening. Photo by Michael Lüth, with permission.

Figure 46. Syntrichia princeps, a moss that has better germination of spores and fragments under intermittent moisture than under continuous moisture. Photo by Jan-Peter Frahm, with permission.
In other species, high resistance is attained after several short exposures to drought (Clausen 1952; Abel 1956; Patterson 1964; Dilks & Proctor 1976a, b). We know that Syntrichia ruralis (Figure 21) is capable of drought hardening (Schonbeck & Bewley 1981b). When subjected to daily episodes of desiccation and rehydration, it develops a greater desiccation tolerance. However, the wet-dry cycle may be of less importance for boreal forest mosses. Hanslin and coworkers (2001) exposed Dicranum majus (Figure 49) and Rhytididiadelphus loreus (Figure 38) to various watering regimes and found that responses, while differing greatly, lacked any consistent pattern. However, the relative growth rate increased with the length of the wet-dry cycle, provided the total number of wet and dry days remained equal, suggesting that these taxa probably would be unable to take advantage of night-time dew accompanied by day-time drought, but they are adapted to the more weekly or monthly wet-dry cycles typical of the boreal forest.

Davey (1997) showed that Antarctic hydric mosses are susceptible to damage by frequent wetting and drying, but that was not the case for the mesic and xeric mosses, which seemingly were adapted to frequent wet/dry cycles. All the mosses suffered a greater loss of photosynthetic rate as the duration of the dehydration periods increased. Davey suggested that mosses from the drier habitats were adapted to use short periods of rehydration. This is consistent with the use of late night/early morning moisture from clouds in xeric African montane sites and other habitats where nighttime dew is the major source of water. Csintalan and coworkers (2000) supported this concept with their work on Syntrichia ruralis (Figure 21) in dry grasslands. They found that the moss absorbed progressive amounts of water through the night, permitting it to obtain about 1.5 hours of net photosynthetic gain immediately after dawn. Although this gain on many days may not be enough to offset the carbon loss during the remainder of the day, it does contribute to the overall carbon gain and may permit the moss to gain on a yearly scale when added to those occasions when more dew or moisture is available.

Oliver et al. (1993) proposed a three-part strategy of tolerance that is based on carbon balance, damage limitation, and cellular repair. To support this they used protein synthesis following desiccation/rehydration in three desiccation-tolerant moss species: Syntrichia caninervis (Figure 31), S. ruralis (Figure 21), and S. norvegica (Figure 47). Using this as a measure of repair, they ranked the tolerance of these species as $S. \text{caninervis} > S. \text{ruralis} > S. \text{norvegica}$. 

**Carbon Balance**

The bottom line in the dehydration/rehydration cycle over the course of the lifetime of the bryophyte is carbon gain (Alpert 2000). Short-term rehydration events can use more carbon in repair processes than can be gained from photosynthesis once everything is working properly. For those species that can regain photosynthetic activity within the first minute, an array of water sources becomes available, including dew and fog in addition to rain and snow. These may be the same species that experience rapid drying because of a desert-like habitat. For these, constitutive desiccation tolerance is important. This strategy may include structural adaptations that slow drying and cellular mechanisms that preserve the integrity of the cellular organelles. But as demonstrated in the desert moss Pterygoneurum lamellatum (Figure 50), tolerance to slow drying can be inducible (Stark et al. 2013).
Membranes and stabilize the cell. More questions!

Photosynthetic activity seems little affected and disrupted initially. Nevertheless, the cell soon regains its integrity. LEA proteins proliferate, but their role is unknown, perhaps functioning to restructure the membranes and stabilize the cell. More questions!

Imlications

It appears that characteristics suggested for tracheophytes to permit them to survive desiccation (Iljin 1953, 1957) do not apply well to bryophytes. Rather, Oliver and Bewley (1984b) suggested that tolerant species must do three things to survive drying: (1) limit damage to a level that can be repaired; (2) maintain physiological integrity of the cell so metabolism can quickly reanimate during rehydration; (3) put repair mechanisms into effect upon rehydration, especially to regain integrity of membranes.

Many questions remain to be answered in understanding the recovery process in bryophytes. When studying the grass Sporobolus stapfianus, Neale et al. (2000) found that Elip genes were expressed differently in tissues that were desiccation tolerant than in those that were desiccation sensitive and suggested that there are unique gene regulatory processes occurring as desiccation ensues, permitting different drought-responsive genes to be expressed at different stages during water loss. Since these genes have been identified in bryophytes, it is likely that Zeng et al. (2002) are correct in their suggestion of a photoprotective role during the dehydration state of bryophytes.

As summarized by Oliver et al. (2005), desiccation tolerance is a primitive trait, a necessary trait for invasion of land. In bryophytes, two aspects permit their survival: constitutive cellular protection and effective recovery/repair mechanism. (To this we must add inducible tolerance in at least some bryophytes.) But upon recovery, the cells behave like any container of light-weight objects that suddenly gets an influx of water, being disrupted initially. Nevertheless, the cell soon regains its integrity. Photosynthetic activity seems little affected and recovers quickly. LEA proteins proliferate, but their role is unknown, perhaps functioning to restructure the membranes and stabilize the cell. More questions!

Summary

Desiccation tolerance most likely originated in the early land bryophytes in their colonization of land. Yet, they remain almost unique in their ability to tolerate desiccation in the vegetative state. Bryophyte gametophytes recover from desiccation by the actions of numerous rehydration proteins, including rehydrons, and rapid membrane repair. The rapidity is dependent upon slow dehydration that gives the bryophyte time to make mRNPs and is provided by a rehydration-inducible recovery mechanism in which new proteins are synthesized rapidly (Oliver 1996). The rapid recovery is complemented by enlargement of the nucleolus, amplification of the endoplasmic reticulum, Golgi, chloroplasts, mitochondria, and vacuoles, disappearance of lipid reserves, and synthesis of starch in chloroplasts during rewetting.

Photosynthesis resumes almost immediately, reaching normal levels within 24 hours, indicating the readiness of the chloroplasts. Because of the resources needed for recovery, short periods of hydration between frequent drying periods deplete resources and are more harmful than long dry periods, issuing foreboding for moss gardeners.

Acknowledgments

This chapter has benefitted from the help of Beth Scafone and Medora Burke-Scoll, who helped me tow the line in explaining things without leaving too much to one's imagination, but at the same time not repeating myself.

Literature Cited


Zhang, J., Zhang, Y.-M., Downing, A., Wu, N., and Zhang, B.-C.  
# CHAPTER 7-7

## WATER RELATIONS: BIOCHEMICAL ADAPTATIONS TO DRYING

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CHAPTER 7-7
WATER RELATIONS:
BIOCHEMICAL ADAPTATIONS TO DRYING

Figure 1. Grimmia affinis drying on a rock. Photo by Michael Lüth, with permission.

The biochemistry of bryophytes is still a relatively young field. This is true of the biochemical level of response of bryophytes to desiccation stress. This chapter will attempt to portray what we know and how that biochemistry relates to the habitats of the bryophytes. But at this early stage in our studies, few species have been studied in detail, leaving much of the discussion incomplete or even somewhat ambiguous.

Membrane Chemistry

Since membrane damage is a common response to desiccation stress, Guschina et al. (2002) examined lipid composition of membranes in Atrichum androgynum (Figure 19) during desiccation in an effort to understand the role of the stress hormone ABA. Drought stress causes changes in the phosphoglyceride composition of the membranes. Reduction of thylakoid lipids, resulting in chlorophyll damage, causes a loss in photosynthesis as a result of desiccation, as already demonstrated in tracheophytes. Guschina et al. found that application of ABA reduced the extent of these membrane lipid changes.

Some plants may take advantage of the leakage through damaged membranes to rid cells of protectants used during dehydration. Working with canopy liverworts in the tropical rainforest of Guadeloupe, Coxson and coworkers (1992) found that for Frullania atrata, exposure to simulated wetting/drying resulted in production of substantial glucose, erythritol, glycerol, and sucrose. They suggest that whereas these sugars may help this liverwort survive severe desiccation, the liverwort subsequently releases them into throughfall upon rewetting.

Robinson et al. (2000) suggest that sugars may indeed help some mosses survive desiccation. They found stachyose, an oligosaccharide known for its role in desiccation tolerance of seeds, in Bryum pseudotriquetrum (Figure 2), but not in Ceratodon purpureus (Figure 3; most tolerant) or Schistidium antarcticum (Figure 4; least tolerant). This is another example showing that not all bryophytes have the same adaptations to desiccation.
ABA Role

The stress hormone ABA (abscisic acid) is present in many groups of organisms, including animals and bacteria as well as plants (Hartung 2010; Takezawa et al. 2011). This ability to protect against abiotic stress may have been one of the most critical attributes permitting plants to move to land.

Using immunoassay, Hartung and coworkers (1987, 1994) demonstrated the presence of ABA in all Bryopsida, Anthocerotophyta, and Marchantiopsida tested. They were able to extract more ABA from the hornwort Phaeoceros grown under slightly drier areas than from those in wetter areas. Furthermore, they have shown that the sporophyte of Phaeoceros laevis (Figure 5) produces ABA in response to stress and that the sporophyte guard cells close in response to ABA, much as in tracheophytes. This is in sharp contrast to the findings of Duckett and Ligrone (2004). They were unable to find any response to ABA or to moisture changes in the stomata of Phaeoceros.

In bryophytes, this hormone occurs in Physcomitrella patens (Figure 6) where it has a major role in dehydration stress tolerance (Takezawa et al. 2011). To determine the genetic response of bryophytes to water stress, Cuming et al. (2007) used the lab moss Physcomitrella patens. These plants were subjected to ABA as well as osmotic, salt, and drought stress. The response of the protonema differed from that of the gametophore, with 130 genes in the protonema responding to dehydration. Of these, 56 were induced by ABA, but only 10 genes by osmotic stress and 8 by salt stress. Another 51 genes were induced by more than one of these treatments. Many of the ABA and drought-responsive genes were homologues of those expressed during seed development, supporting the assertions of Fisher (2008) discussed in Chapter 7-5. As seen by Wang et al. (2009) during dehydration, many of the ABA- and drought-responsive genes include genes for LEA proteins.
Werner et al. (1991) found that even protonemata produce ABA in response to slow drying, as shown in Funaria hygrometrica, and as in mature plants, it imparts drought tolerance. But it does not inhibit water loss. Rather, it appears to induce synthesis of new proteins that impart drought tolerance.

In Cyanobacteria and algae, the few studies on stress-induced ABA production indicate that the excess is released to the external medium (Hartung 2010). Taking an evolutionary approach, Hartung demonstrated that organisms that start to colonize terrestrial habitats increase their ABA production in response to even mild drought stress. Such signals seem to initiate the production of terrestrial organs, perhaps explaining the change from aquatic to terrestrial forms of Riccia fluitans (Figure 7; see below). In bryophytes, stomata respond to ABA. The levels of ABA in sporophytes of hornworts and mosses that have stomata is especially high, although the regulatory role of the ABA seems ambiguous. Fungi release ABA, and these hormones may interact with the bryophytes through mycorrhizal associations or just through their presence in the environment.

One of the unusual abilities of ABA is to cause the conversion of the aquatic forms of the thallose liverworts Riccia fluitans (Figure 7) and Ricciocarpos natans (Figure 8) into their terrestrial forms (Hellwege et al. 1992; Hartung et al. 1994). This conversion results in plants with greater volume, hence a smaller surface area to volume ratio, making them somewhat less vulnerable to desiccation.

Liverworts use lunularic acid where other plants use ABA as a dormancy hormone and, apparently, to help prepare them for drying, as shown in Lunularia cruciata (Figure 9) (Schwabe 1990). When subjected to long days, their drought resistance increases (Figure 10), as does their lunularic acid content.
ABA, liverworts seem to be fully responsive to ABA. Pence (1998) found that ABA was necessary for the cryopreservation of some liverworts such as *Riccia fluitans* (Figure 7) and *Marchantia polymorpha* (Figure 21), preventing desiccation damage, but it had little effect on the leafy liverwort *Plagiochila* (Figure 11).

Burch and Wilkinson (2002) used ABA and sucrose to increase the success of cryopreservation of the moss *Ditrichum cornubicum* (Figure 12) protonemata. We also know that application of ABA increases the desiccation tolerance of the mesophytic moss *Atrichum undulatum* (Figure 13) (Beckett *et al.* 2000). Using *Atrichum androgynum* (Figure 19), Guschina *et al.* (2002) demonstrated phosphoglyceride composition changes during water stress. ABA treatment reduces the overall extent of these changes, possibly by reducing membrane damage by reducing the lipid changes.

Figure 11. *Plagiochila asplenioides* near a stream in Wales. Photo by Janice Glime.

Figure 12. *Ditrichum cornubicum*, a moss that survives cryopreservation with the help of ABA and sucrose. Photo by David T. Holyoak, with permission.

The Afromontane understory moss *Atrichum androgynum* (Figure 19) recovers its CO$_2$ fixation more slowly than recovery of photosystem II activity following 16 hours of desiccation, then rehydration (Mayaba *et al.* 2001). Pretreatment with ABA increases the recovery rate of both of these activities and doubles the non-photochemical quenching, hence reducing reactive oxygen species. Mayaba and coworkers suggest that this may partly explain the desiccation hardening process in this species. Plants pretreated with ABA, unlike untreated plants, experience a significant increase in soluble sugars that could promote the vitrification (transformation into a glassy substance) of the cytoplasm. This could, in turn, protect the membranes during desiccation. ABA has only a slight effect on the starch concentrations during desiccation. ABA furthermore has no effect on chlorophyll breakdown.

But how does this relate to preventing the oxidative damage? Beckett and coworkers (2000) suggested that ABA pretreatment may act by reducing the energy transfer between light-harvesting chlorophyll II and photosystem II. This could harden the moss to desiccation stress by reducing the production of reactive oxygen at the site of photosystem II. Experiments indicated that photosystem II photosynthesis recovers faster in the pre-treated plants. ABA may play another role as well. One of the most serious consequences of desiccation is loss of membrane integrity, causing membranes to become leaky (Bewley 1979). Beckett (1999) found that application of ABA could reduce the loss of K$^+$ from *Atrichum androgynum* (Figure 19) in much the same manner as partial dehydration treatment prior to desiccation. The response is similar to that obtained by reducing the relative water content to 0.6 for three days, which reduces the K$^+$ loss by 15-20%. This seems to be the ideal combination because using less humid air or more time does not decrease the K$^+$ loss further. This species, and probably most, experiences drought hardening (process of increasing resistance to drought; see Chapter 7-5) as the dry season progresses, as indicated by the loss of 80% of its intracellular K$^+$ at the beginning of the dry season, but less than 25% by the end of that season (Beckett & Hoddinott 1997).

Abscisic acid (ABA) has already been noted to have an important role in desiccation tolerance. Werner *et al.* (1991) found that slowly dried protonemata of *Funaria hygrometrica* survived desiccation, but rapidly ones did not. The slowly dried mosses experienced a six-fold increase in abscisic acid during drying. If ABA is added to the protonemata at an appropriate concentration, the ABA mediates drought tolerance, apparently by inducing the synthesis of new proteins.
Sucrose

De Cruz et al. (2014, 2015) found that desiccated cells of the aquatic moss *Fontinalis antipyretica* lose 50% of their sucrose through leakage when the cells are rehydrated. Fast dehydration results in higher sucrose accumulation, but it is not enough to induce desiccation tolerance. The increase in soluble sugars helps in osmoregulation during the decreasing turgor pressure of the cells. In addition to serving as an osmolyte, sucrose in bryophytes helps to stabilize membranes and proteins through *vitrification* (process of forming glasslike substances). In *Fontinalis antipyretica* desiccation tolerance requires slow dehydration, suggesting that high sucrose content does not act alone to create desiccation tolerance.

Protection from Oxidation

Just what is it that varies among the bryophytes that dry out, become metabolically inactive, and then revive? What physiological mechanism protects, or fails to protect them? How can photosynthesis achieve its maximum rate within 30 seconds upon receiving rain or dew in some desiccated species (Anderson 1980)? Proctor (1990) and Alpert (2000) suggest that in drought-hardening the cell must protect itself from oxidative damage, as well as loss of configuration of macromolecules, and this protection depends on the intensity and duration of desiccation.

Minibayeva and Beckett (2001) noted that drought-sensitive bryophytes can release an oxidative burst (respiratory burst; rapid release of reactive oxygen species – superoxide radical and hydrogen peroxide) in response to rehydration. These bursts developed best in the hornwort and two thalloid liverworts tested (Minibayeva & Beckett 2001). A similar oxygen burst is, however, almost absent in all the mosses tested as well as a leafy liverwort and desiccation-tolerant lichens.

Oxidative Damage

Kramer et al. (2002) examined the "resurrection plants" – those plants that can survive desiccation – to determine what permits them to survive. They found that in a woody plant desiccation can trigger increases in zeaxanthin and redox shifts of the antioxidants glutathione and ascorbate to their oxidized forms. New ascorbate and glutathione were produced upon rehydration and the oxidized forms from the dehydration event changed back to reduced forms. Using lichens, Kramer et al. (2008) further demonstrated that reactive oxygen species can damage nearly every molecule in living cells. These included nucleic acids, proteins, and lipids.

The absence of oxidative bursts in mosses lends support to the hypothesis that mosses protect themselves from the damage such highly reactive oxidative bursts can cause during rehydration. Shiono et al. (2000) found that in testing the liverwort *Marchantia paleacea* subsp. *diperta* (Figure 15), the moss *Barbula unguiculata* (Figure 16), and the hornwort *Anthoceros punctatus* (Figure 17), the liverwort differed from the other two in its isozyme patterns for *superoxide dismutase*. This enzyme is known for its ability to maintain safe levels of the highly reactive oxides that are produced during cell stress, including effects of desiccation.
But some bryophytes produce high quantities of oxides even when they are not stressed, and some bryophytes produce them at extremely high rates. For example, *Anthoceros natalensis* exceeds 1000 µmol g⁻¹ dry mass h⁻¹, whereas excised tracheophyte roots produce only about 1% of that amount (Minibayeva *et al.* 1998). These data do not present a consistent pattern that permits us to interpret the role of oxidative bursts or superoxide dismutase in protecting bryophyte cells that undergo desiccation. Instead, the high oxidative responses in some species may be one to the presence of invading pathogens (see below).

Mayaba *et al.* (2002) later found that *Atrichum androgynum* (Figure 19) from the Afromontane understory displays an oxidative burst of hydrogen peroxide (H₂O₂), not superoxides, during rehydration, with maximum rates during the first 15 minutes (Figure 20). The moss even produces peroxide during times when dehydration is insufficient to cause K⁺ leakage. Using polyethylene glycol to induce desiccation causes the moss to produce significant amounts of H₂O₂. Mayaba and coworkers suggest that peroxidases might be responsible for the production of H₂O₂. They determined that ABA and light influenced the rate of production of peroxide.

This peroxidase system would have several advantages. Peroxidases oxidize phenolics to quinones and generate peroxide (H₂O₂). Peroxide, a well-known antibacterial agent for cleaning cuts and wounds, can itself help to kill invading organisms. Furthermore, peroxide releases free radicals that increase polymerization of phenolics into lignin-like substances. In tracheophytes,
these substances are known to reinforce the cell wall and contain the pathogens. They may have similar roles in bryophytes.

The thallose liverwort *Marchantia polymorpha* (Figure 21) contains a peroxidase that has been characterized as a glycoprotein that is different from any known tracheophyte peroxidase (Hirata et al. 2000). Hirata and coworkers demonstrated that this peroxidase is able to perform oxidative polymerization of **lunularin**, the liverwort counterpart of ABA.

![Figure 21. Marchantia polymorpha, a thallose liverwort that produces a peroxidase with a glycoprotein that differs from those in tracheophytes. Photo by David T. Holyoak, with permission.](image)

Other known constituents also influence the activity of peroxidases. Seel et al. (1992a) examined the effects of desiccation on **superoxide dismutase** (enzyme that destroys highly reactive superoxides by converting them into peroxide and O$_2$) activity in *Syntrichia ruralis* var. *arenicola* (=*Tortula ruraiiformis*; Figure 22), a desiccation-tolerant moss, and *Dicranella palustris* (Figure 23), a flush moss with limited desiccation tolerance. Activity of this enzyme is known to enhance membrane integrity (Dhindsa & Matowe 1981; Dhindsa et al. 1981; Gong et al. 1997). *Syntrichia ruralis* var. *arenicola* has higher superoxide dismutase activity in both the hydrated and desiccated states than does *D. palustris* (Seel et al. 1992a). But effects on the activities of peroxidase or ascorbic peroxidase do not seem to be related to hydration state. Nevertheless, both species become depleted of the anti-oxidant ascorbic acid when desiccated. From these experiments, Seel and coworkers deduced that antioxidants may be more important than removal of chloroplastic peroxide in endowing desiccation tolerance. Using different methods, Seel and coworkers (1992b) found a greater lipid peroxidation in *D. palustris* than in *S. ruralis* var. *arenicola* following desiccation. Calcium also seems to play a role by increasing superoxide dismutase activity, thus enhancing membrane integrity (Gong et al. 1997).

Proctor et al. (2007) used the endohydric moss *Polytrichastrum formosum* (Figure 30) to try to resolve conflicting implications between physiological and cytological evidence regarding desiccation recovery in bryophytes. They found that protein synthesis inhibitors cause rapid decline of photosynthetic recovery in the light, but not in the dark. Rapid recovery of respiration and photosynthesis indicates that systems are conserved intact during the dehydration and rehydration, an indication that is consistent with the physical evidence that thylakoids and cristae do remain intact during the dehydration-rehydration process. Microbodies that are closely associated with chloroplasts remain unchanged during the dehydration-rehydration process and play an important role in removal of the superoxide radicals (Duckett & Renzaglia 1988; Smirnoff 1993; Minibayeva & Beckett 2001; Mayaba et al. 2002). The prominence of these microbodies in leaves of *Syntrichia ruralis* (Figure 23) (Robertson 1991) and *Polytrichastrum formosum* may be associated with the desiccation tolerance of these two species (Proctor et al. 2007).

![Figure 22. Syntrichia ruralis var. arenicola, a desiccation-tolerant moss. Photo by Michael Lüth, with permission.](image)

**Glutathione**

Glutathione (GSH) is important in protecting plants from environmental stresses like oxidative stress and pathogens (Bruns et al. 2001; Burritt 2008). More recent studies have used glutathione to measure drought stress. Activities of the enzymes glutathione reductase, glutathione peroxidase, and glutathione S-transferase increase during slow drying and likewise during rehydration following rapid drying of the drought-tolerant moss *Syntrichia ruralis* (Figure 22) (Dhindsa 1991).
On the other hand, the activity of the enzymes malate dehydrogenase exhibit little change during either dehydration or rehydration. Treatment of the moss tissues with cycloheximide, actinomycin D, or cordycepin suppresses the increased activities of glutathione reductase and glutathione S-transferase, but has a much lower effect on glutathione peroxidase. At the same time, the percentage of total glutathione as oxidized glutathione increases. This increase is correlated positively with levels of lipid peroxidation and solute leakage, but is correlated negatively with the rate of protein synthesis. The oxidized glutathione level serves as a good indicator of oxidation stress and suggests that oxidized glutathione may mediate the drought-stress-induced inhibition of protein synthesis.

In addition to protection from oxidative damage, glutathione may help to protect the bryophyte cells from heavy metal damage following rehydration (Saxena & Saxena 2012). Although it is likely that this benefit has not had any evolutionary selection advantage for very long, current pollution conditions often deposit heavy metals that accumulate while the bryophytes are dry. These could gain entry into the cells along with the resorption of needed cell electrolytes during rehydration and before membrane repair is completed. Bruns et al. (2001) have demonstrated a protective detoxification role of glutathione against heavy metals in the aquatic moss Fontinalis antipyretica (Figure 18), Leinenweber et al. (2009) in the terrestrial moss Thuidium sp. (Figure 24), and Saxena and Saxena (2012) in the moist forest moss Sphagnum squarrosum (Figure 25).

Figure 25. *Sphagnum squarrosum*, a species that is able to use glutathione as protection against heavy metals. Photo by J. C. Schou, with permission.

Gupta (1977) reported the oxidative burst in bryophytes as an "artifact." He found that *Dicranella palustris* (Figure 23; a wet-habitat moss) and *Scapania undulata* (Figure 26; an aquatic leafy liverwort) had a large number of microorganisms present following dehydration and rehydration. This is a reasonable expectation when membranes are damaged and both electrolytes and organic compounds are able to leak from the cells, especially upon rewetting. Furthermore, the respiratory oxygen uptake increased to about 6X that of controls of *S. undulata*, 2.5X for *Dicranella palustris*, and 2X for *Porella platyphylla* (Figure 27) and *Mnium hornum* (Figure 28). Little increase occurred in *Syntrichia ruralis* (Figure 22), the most desiccation-tolerant species. But it appears that the respiratory increases were due to the adhering microorganisms, not to the bryophytes. Such respiratory increase could indicate injury to the bryophytes, but it cannot be a useful tool to measure survivorship or metabolic recovery of the bryophytes. These microorganism growths indicate the potential importance of oxidative bursts that can help to protect the bryophyte cells from invasion from these potentially harmful organisms.

Beckett *et al.* (2004) demonstrated that the liverwort *Dumontiera hirsuta* (Figure 29) produced extracellular superoxide at high rates even under normal, unstressed circumstances. Nevertheless, production increased extensively during rehydration, but not during desiccation. It appears that peroxides produce the superoxide, but little H₂O₂ seems to be present in the cell. However, indications are that the concentrations of peroxides are rapidly reduced by the liverwort. Beckett and coworkers likewise suggested a role in protection against bacteria and fungi. Lehtonen *et al.* (2012) verified the importance of such defense-related genes, stimulation of phytoalexin (substance produced by plant tissues in response to contact with a parasite and that specifically inhibits growth of that parasite) biosynthesis, and promotion of hypersensitive response (HR; mechanism to prevent spread of infection by microbial pathogens, causing rapid death of cells in local region surrounding infection).
oxidative bursts in response to a fungal elicitor (chiton) in the moss *Physcomitrella patens* (Figure 14).

**Figure 26.** *Scapania undulata*, a species in which microbial respiration/oxygen uptake increases by a factor of 6 following rehydration. Photo by Hermann Schachner, through Creative Commons.

Figure 26. *Scapania undulata*, a species in which microbial respiration/oxygen uptake increases by a factor of 6 following rehydration. Photo by Hermann Schachner, through Creative Commons.

Figure 27. *Porella platyphylla*, a desiccation-tolerant leafy liverwort on tree bark; a species in which microbial respiration/oxygen uptake increases by a factor of 2 following rehydration. Photo by Michael Lüth, with permission.

**Figure 27.** *Porella platyphylla*, a desiccation-tolerant leafy liverwort on tree bark; a species in which microbial respiration/oxygen uptake increases by a factor of 2 following rehydration. Photo by Michael Lüth, with permission.

**Figure 28.** *Mnium hornum*, a species in which microbial respiration/oxygen uptake increases by a factor of 2 following rehydration. Photo by Des Callaghan, with permission.

**Figure 28.** *Mnium hornum*, a species in which microbial respiration/oxygen uptake increases by a factor of 2 following rehydration. Photo by Des Callaghan, with permission.

**Figure 29.** *Dumortieria hirsuta*, a thallose liverwort that produces extracellular superoxide at high rates even under normal circumstances. Photo by Michael Lüth, with permission.

White and Torres (2010) suggested that endophytes in plants may protect the plants from oxidative damage by the production of antioxidants, thus possibly protecting them against other forms of stress, including desiccation. It appears that this protective role of endophytes (fungi) has not been explored in bryophytes.

**Shoot Tips – Variable Tolerance within Plants**

Some moss shoot tips may have a rehydration potential not afforded the rest of the plant. In *Polytrichastrum formosum* (Figure 30), desiccation in the shoot tips induces the rapid resorption of starch grains in plastids of the meristematic cells without any major thylakoid disorganization (Hallet *et al.* 1987). In the adult leaves, however, the starch grains are preserved. Upon rehydration, the plastid ultrastructure of the apex is entirely restored and new starch inclusions appear in less than 4 hours. Little work has been done to relate the resistance of various parts of the bryophyte plants to differences in biochemistry.

**Figure 30.** *Polytrichastrum formosum*, a moss where desiccation of the apices causes rapid resorption of starch grains in plastids of the apical meristematic cells. Photo by Des Callaghan, with permission.

**Figure 30.** *Polytrichastrum formosum*, a moss where desiccation of the apices causes rapid resorption of starch grains in plastids of the apical meristematic cells. Photo by Des Callaghan, with permission.

**The Genes**

While the physiologists are attempting to find substances that affect desiccation tolerance and recovery rates, the geneticists are attempting to identify genes and
the biochemical pathways they affect. Chen and coworkers (2002), working with the desiccation-tolerant model system in *Syntrichia ruralis* (Figure 22), found a new polypeptide, known as ALDH21A1, that is less than 30% identical to known ALDH proteins. Data suggest that this new aldehyde dehydrogenase plays an important role in the detoxification of aldehydes generated in response to desiccation and may represent a unique stress tolerance mechanism among eukaryotes. Could it be this aldehyde dehydrogenase, perhaps coupled with ABA, that explains why Hamerlynck and coworkers (2002) found *Syntrichia ruralis* to be homoiochlorous (maintaining constant chlorophyll concentration) in its response to desiccation? Growing in the sun endows these plants with a greater desiccation tolerance than that experienced by shade-grown plants of the same species.

To fit these pieces together requires a great deal of speculation because our knowledge is still too meager. However, let’s look at what we know about these pieces and see if we can develop a hypothetical story (Figure 31).

![Summary](image)

**Summary**

Membranes become leaky during desiccation. Some mosses protect their membranes with sugars such as stachyose, glucose, erythritol, glycerol, and sucrose.

ABA increases the stress tolerance of bryophytes and is known to turn on the promoters of stress tolerance genes. Hence, it is important in controlling transcription. That is consistent with the conclusions of several authors who have determined that drought tolerance in bryophytes evokes control of gene transcription. We also know that peroxidases destroy H2O2 (peroxide), which is harmful to plants. We know that H2O2 is responsible for lipid damage of membranes and that lipid peroxidation and increased membrane permeability correlate with the decrease of superoxide dismutase (Dhindsa *et al.* 1981). And we know that superoxide dismutase controls oxygen toxicity by converting the superoxide radical to less dangerous forms (Michael Potter of Andrew McCammon's group at the University of California, San Diego). Since *Syntrichia ruralis* var. *arenicola* has a higher concentration of superoxide dismutase than the less desiccation-tolerant *Dicranella palustris*, we can then hypothesize that the superoxide dismutase is an important contributor to drought tolerance in bryophytes. Perhaps it is one of the 74 proteins produced in response to desiccation stress. Glutathione may help to protect the cells from excessive oxides, but it may have a more important role in protecting against pathogenic microorganisms while they are vulnerable with damaged membranes.

Shoot tips seem able to survive better than other parts of some mosses, but we know nothing about any differences in their biochemistry. New genetic studies are making it possible to learn more about the functions of various compounds in the cells.

**Acknowledgments**

This chapter, in an early version, has benefitted from the help of Beth Scafone and Medora Burke-Scoll, who helped me to the line in explaining things without leaving too much to one's imagination, but at the same time not repeating myself.

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CHAPTER 7-8
WATER RELATIONS: HABITATS

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CHAPTER 7-8
WATER RELATIONS: HABITATS

Figure 1. *Bryum caespiticium* at a high elevation where winds and ice crystals contribute to desiccation, but where at other times fog can maintain moisture without rain. Photo by Michael Lüth, with permission.

**Habitat Relations**

Proctor (2014) summarized the importance of water relations for bryophytes in their invasion of land. He pointed out that the poikilohydric strategy is optimal at smaller scales, *i.e.*, bryophytes. Microhabitat and habitat structure are important in conferring the hydration state of bryophytes, and drought sensitivity varies according to species (Irmscher 1912). Norris (1990) found that *Braunfelsia* disappeared from some areas of tropical rain forests in Papua New Guinea following disturbance to the forest because of the increased dehydration frequency and the admission of greater wind movement. In the Mediterranean area in the southern and southeastern Iberian Peninsula, Varo and coworkers (1992) found that as the climate has become drier and warmer the bryophyte taxa have changed, with leafy liverworts and pleurocarpous mosses diminishing and *Sphaerocarpos* (Figure 2) and acrocarpous mosses becoming more prominent. In central Sweden, greater numbers of bryophytes occur in spruce forests on more moist north-facing slopes, whereas vascular plants are more abundant on the exposed south-facing slopes (Söderström 1981).

Figure 2. *Sphaerocarpos micheli*, member of a genus that becomes more prominent as the climate dries. Photo by Michael Lüth, with permission.
Bryologists learn inductively through field experience that certain bryophytes are characteristic of dry habitats and others of wet habitats. Actual studies that correlate these conditions with species are less common than descriptive observations, with a number of these being relative to water level in peatlands. Bates et al. (2004) used canonical correspondence analysis (CCA) to develop a more rigorous approach to these relationships by sampling epiphytes along a transect across southern Britain from southwest to northeast. With climate, presence of water courses, and forest cover contributing to the analysis, they determined that *Frullania tamarisci* (Figure 3), *Metzgeria temperata* (Figure 4), *Microlejeunea ulicina* (Figure 5), *Neckera pumila* (Figure 6), and *Hypnum andoi* (Figure 7) were restricted to habitats with high moisture availability. On the other hand, *Syntrichia ruralis* (Figure 39), *Grimmia pulvinata* (Figure 8), *Tortula muralis* (Figure 9), and *Aulacomnium androgynum* (Figure 10) only occurred as epiphytes in locations with low moisture. They did not sample these species in other habitats.
Figure 8. *Grimmia pulvinata*, a cushion moss that can only survive as an epiphyte in areas that have high moisture. Photo by Barry Stewart, with permission.

Figure 9. *Tortula muralis* habitat on a wall. This moss is unable to live as an epiphyte unless the habitat has low moisture levels. Photo by Janice Glime.

Figure 10. *Aulacomnium androgynum*, a moss that can only survive as an epiphyte in areas that have high moisture. Photo by Jan-Peter Frahm, with permission.

In mature black spruce forests of central Alaska, the endohydric *Polytrichum commune* (Figure 11) is able to avoid moisture stress more so than such ectohydric taxa as *Hylocomium splendens* (Figure 12; Skré *et al.* 1983). The latter species remains below its compensation point for water for nearly 50% of the July growing season.

Figure 11. *Polytrichum commune*, an endohydric moss that is able to avoid moisture stress in black spruce forests more readily than ectohydric taxa. Photo by Michael Lüth, with permission.

Figure 12. *Hylocomium splendens*, an ectohydric moss. Photo by Janice Glime.

Open expanses of urban areas are notoriously devoid of extensive bryophyte cover, even on trees where taxa are already xerophytically adapted. Hébrard and Rolando (1985) found that when comparing four holm-oak thickets in France, species composition correlated more with plot exposure than with thicket age, suggesting that desiccation, light, and temperature may be most influential. Sheard (1968) likewise found a correlation between the prevailing north wind and the pattern of moss-lichen heath on Jan Mayen Island.

Among the most significant climatic stress inducers for mosses are high temperatures, frost, and drought (Longton 1979). Dry mosses are typically much more heat resistant than wet mosses. For example, Nörr (1974) found that eight European mosses reach lethal limits at 42-51°C when turgid, but survive to 85-110°C when dry. Lange (1955) found similar dry survival of mosses from 70-110°C. Temperature relationships will be discussed more thoroughly in the chapter on temperature.

These relationships also exemplify that, although bryophytes are able to survive on rocky and shallow substrates with little water, they are unable to compete with the tracheophytes in areas where there is sufficient soil, light, and moisture for the tracheophytes to root. But at the extremes, bryophytes may have an advantage. Therefore, it is fitting to conclude our attempt to understand the water stresses of bryophytes by comparing them at the two extremes, the aquatic and the arid habitats.
Using electrolyte leakage as an indication of desiccation stress, Šinžar-Sekulić et al. (2005) compared the desiccation tolerance of three mosses from different moisture regimes. *Thamnobryum alopecurum* (Figure 13), a moss of open, vertical limestone cliffs, has the highest degree of desiccation tolerance among these three. *Anomodon viticulosus* (Figure 14), a moss of limestone rocks in the forest, releases electrolytes under desiccation, causing pronounced changes in the cells. The aquatic moss *Platyhypnidium riparioides* (Figure 15) suffers irreversible change following desiccation. It is likely that speed of drying plays a role for the latter species because its frequency on emergent rock habitats suggests that it should be adapted to slow drying. Nevertheless, it seems to live where it stays moist even during periods of low water levels.

Among the hornworts (*Anthocerotophyta*) little information exists on desiccation tolerance. Some are drought avoiders, producing special structures that survive periods of desiccation (Vitt et al. 2014). These, occurring on hornworts of seasonally dry localities, include abundant swollen, marginal or apical tubers on the thalli (*Phaeoceros* spp.; Figure 16-Figure 17) or long-stalked, subterranean ventral tubers (*Phymatoceros*; Figure 18). Both of these special tubers form as the sporophytes mature and persist in the soil crust or soil bank after the vegetative thallus has deteriorated. Rainfall causes these tubers to germinate and form new plants (Crandall-Stotler et al. 2006). Hartung et al. (1994) found that these tubers contain large amounts of ABA, a hormone known to induce desiccation tolerance in bryophytes (Pence et al. 2005). These tubers can survive at least nine months of dryness and still germinate (Vitt et al. 2014).
Other hornworts take advantage of short life cycles. For example, rapid spore release in *Notothylas* (Figure 19), coupled with the ability to survive many years dry (Renzaglia *et al.* 2009) permit this genus to avoid drought conditions.

Liverworts are known for loving damp habitats, but they contain their xerophytic members as well. Seppelt (pers. comm. 1999) relayed to me that these include species surviving in as little as 150 mm of rainfall per year (*Fossombronia* (Figure 20), *Asterella* (Figure 21), *Plagiochasma* (Figure 22)). At somewhat higher levels (200 mm), such taxa as *Lethocolea* (Figure 23), *Cephaloziella* (Figure 24), *Riella* (Figure 66), *Enigmella*, and *Gongylanthus* (Figure 25) appear. *Enigmella* is ephemeral in its vegetative phase, but its reproductive structures are well suited to their environment. Some taxa survive drought by having a shortened life cycle, e.g. *Riccia cavernosa* in the Arctic (Seppelt & Laursen 1999).
Wagner and Titus (1984) compared desiccation tolerance of the hummock species *Sphagnum nemoreum* (Figure 26) to that of the hollow species *S. fallax* (Figure 27). The hollow species is more desiccation tolerant than the hummock species. It has both a higher number of plants surviving and a better recovery of its photosynthetic rate. However, its ability to recover decreases as the desiccation periods are lengthened or the water content is decreased. Despite being close to the water, *S. fallax* apparently dries more frequently and for longer periods of time than does *S. nemoreum*. The latter species, instead, is able to remain moist in the field by holding more water when the habitat dries.

Schipperges and Rydin (1998) compared the responses of photosynthetic CO\(_2\) exchange in five species of *Sphagnum* in response to tissue water content. These species ranged in microhabitat from hummock top (*S. fuscum*; Figure 28), hummock mid to top (*S. papillosum* (Figure 29) & *S. magellanicum* (Figure 30)), wet areas of ombrotrophic bogs and ditches (*S. balticum*; Figure 31), to submerged (*S. cuspidatum*; Figure 32). Laboratory experiments using infrared gas analysis (IRGA) measured recovery of net photosynthesis after several long-lasting desiccation/rehydration events. One important structural adaptation that emerged is the importance of contact between capitula and basal parts of the mosses; if the

**Peatlands**

Peatlands provide a good ecosystem for comparing adaptations for differences in moisture regimes by habitat. But the dominant moss, *Sphagnum*, has unusual structural adaptations that can complicate this analysis.
 capitula were isolated from the water table, they were unable to recover from complete desiccation (<10-20% of compensation point water content; 15°C for 2-4 days). It is interesting that they found no relationship between recovery of net photosynthesis and wetness of the natural habitat. Rather, those species that live under regularly drying conditions are able to avoid death by themselves avoiding drying out, using high capillarity or a dense growth form such as that of *S. fuscum* (Figure 28).

Figure 28. *Sphagnum fuscum*, a hummock top species. Photo by Michael Lüth, with permission.

Figure 29. *Sphagnum papillosum*, a species of mid to top of hummocks. Photo by Michael Lüth, with permission.

Figure 30. *Sphagnum magellanicum*, a species of mid to top of hummocks. Photo by Michael Lüth, with permission.

Figure 31. *Sphagnum balticum*, a species of wet areas of bogs and ditches. Photo by Michael Lüth, with permission.

Figure 32. *Sphagnum cuspidatum*, a submerged species. Photo by Jan-Peter Frahm, with permission.

Hájek and Vicherová (2013) concluded that *Sphagnum* species have inducible desiccation tolerance. These species are generally desiccation intolerant, instead using mechanisms to avoid internal desiccation, as noted by Schipperges and Rydin (1998). Hájek and Vicherová tested the inducible nature of their tolerance by using various bryophyte species, including *Sphagnum*. They hardened the bryophytes by slow drying, ABA application, and chilling or frost. Both chilling and frost can create desiccating conditions by drawing water from the cells through the hygroscopic nature of ice crystals, much like the effects of freezer burn in your freezer. Presuming that the tolerance was inducible, they monitored the seasonal changes in desiccation tolerance of bog bryophytes. Among these, *Sphagnum* species in hollows and lawns developed desiccation tolerance several times during the year as a response to reduced precipitation and lowered water table. The hummock and aquatic species developed this tolerance only in the autumn, possibly responding to frost. Following initial de-hardening in the lab, untreated *Sphagnum* shoots lacked desiccation tolerance. On the other hand, all hardening treatments except chilling induced desiccation tolerance in all groups except those in section *Cuspidata* (Figure 32), a submerged species. They suggest that lack of adequate desiccation tolerance may prevent *Sphagnum* establishment in the drier habitats that are otherwise suitable. Those species that avoid desiccation typically do so by forming compact hummocks – or living submerged. Thus, hummock species invest their
resources in water retention, avoiding desiccation, but have a lower ability to develop desiccation tolerance.

Peatlands typically have moisture gradients, and Hettenbergerova et al. (2013) took advantage of this gradient to compare species richness relative to water availability. They were fortunate to have a system that graded from a spring fen to a semi-dry grassland in the Czech and Slovak Republics. They found that the number of species of tracheophytes tended to increase toward the lower moisture values. The species richness had a negative correlation with the N:P biomass ratio, whereas the percentage of endangered species had a positive correlation. These relationships for bryophytes differed markedly from those of the tracheophytes. Instead, bryophyte species richness decreased linearly toward the dry end of the transects, and there was no correlation with any of the nutrient measurements (N, P, K, C, Ca). Furthermore, the bryophytes exhibited a very high percentage of specialists in fen plots.

Sagot and Rochefort (1996) were concerned about the effects of desiccation on regeneration. They found that fragments of Sphagnum fallax (Figure 27), S. fuscum (Figure 28), and S. magellanicum (Figure 30) could survive 14 days without water when air dried at 20°C, relative humidity ~60%, but regeneration was delayed. Sphagnum fallax and S. magellanicum survived better than did S. fuscum.

Aquatic Habitats

The aquatic bryophytes are distributed worldwide, but they seem to be more common in temperate than in tropical areas. Aquatic species are classified as obligate aquatics, having little or no tolerance to drought conditions, facultative aquatics, having some degree of tolerance to desiccation and xerophytic conditions, and semi-aquatic emergents (Vitt & Glime 1984), being in locations where they are partly in the water and partly out of it, but usually moist (Figure 33).

Figure 33. Fontinalis novae-angliae submerged and Plagiochila porelloides on the rock above the water in a New Hampshire stream. The P. porelloides is subject to intermittent flooding but can become dry when the stream level is low in mid and late summer. Photo by Janice Glime.

Rehydration in aquatic mosses is much like that of tracheophytes. Whereas many mosses are able to protect their ribosomes during dehydration (Bewley 1974), permitting rapid recovery of protein synthesis and respiration upon rehydration, aquatic bryophytes are not. Instead, irreversible ribosome damage occurs (Krupa 1977). For example, Cratoneuron (Figure 34), a semi-aquatic moss, loses ATP during rapid drying, and with its damaged ribosomes it is unable to replace it upon rehydration (Bewley & Gwozdz 1975). Aquatic mosses typically suffer membrane damage during desiccation, but xeric (dry habitat) mosses often do not (Brown & Buck 1979). Thus, in aquatic mosses, rehydration results in loss of nutrients.

Figure 34. Cratoneuron filicinum, a moss known to lose ATP during rapid drying. Photo by Ivanov, with permission.

Even such obligate aquatic mosses as Fontinalis are subject to periods low water when they are exposed above water. Carvalho et al. (2011) found that the aquatic moss Fontinalis antipyretica (Figure 65) demonstrates desiccation tolerance. Laboratory experiments can be misleading because this species requires slow drying in order to survive, supporting the hypothesis of induced desiccation tolerance. In fact, Cruz de Carvalho et al. (2011) concluded that the protein profiles following rehydration were similar to those of the terrestrial mosses Physcomitrella patens and Syntrichia ruralis (Figure 39). They concluded that desiccation tolerance mechanisms were similar regardless of habitat.

Arid Habitats

Contrary to the popular concept that mosses must grow in wet places, a number of species are xerophytic, that is, adapted to places like the dry, hot desert. In such habitats, some mosses are able to absorb water from dew and night air, permitting brief photosynthesis during the early hours of morning. They dry again each day, cycling on a 24-hour wet-dry cycle (Kappen et al. 1979). Where the sun reaches the mosses directly, as on the south-facing slopes in North American deserts, the temperature can increase by as much as 20°C in the first 30 minutes of daylight, thus providing too short a period for the moss to gain photosynthetic energy before drying out (Nash et al. 1977). In such locations the mosses are restricted to the north-facing slopes. The biomass is quite small, less than 2 g m⁻², but at least 18 different species are able to survive, the most common being tuft-forming taxa such as Syntrichia ruralis (Figure 39), Grimmia laevigata (Figure 36), and Bryum caespiticium (Figure 1).

One advantage of having sufficient moisture in the desert habitat is that it can provide evaporative cooling. But that does not seem to be the case in all situations. In the Mojave Desert, Nevada, USA, Crossidium
crossinervium (Figure 62) experienced temperatures above ambient, independent of the state of hydration (Stark 2005). During cooler months, the moss patch exhibited a temperature lower than ambient, again with state of hydration failing to play a role. The periods of hydration were essentially restricted to the cooler months of October to April with hydration lasting 3.7-4.9 days. The longest dry period was 191 days during the measurement period. In late winter, drying was slow, lasting several days, but in the summer the mosses were dry in as little as three hours.

Peatland bryophytes are not the only ones that practice avoidance and tolerance. These practices are also common among bryophytes that live in some of the most harsh moisture conditions on the planet. One mechanism is to go dormant during the dry periods, surviving as spores, gemmae, and probably in some cases protonemata (Vitt et al. 2014). Such an escape strategy is advantageous to bryophytes that lack a physiological tolerance to desiccation in the leafy gametophore (Figure 35). Liverworts have fewer genera with an escape strategy, but many thallose liverworts have tubers or other means, especially Riccia, to survive (see Figure 69); many leafy liverworts have gemmae.

In the Sonoran Desert of North America, Alpert (1979) found that an overnight storm provided 85% of the saturated water contents, available at 6 a.m., for Bryum capillare (Figure 37), Grimmia spp. (Figure 36), Syntrichia spp. (Figure 39), and Weissia controversa (Figure 38). By 9 a.m., eleven of the twelve species investigated had only 2 g water per g of plant dry mass, and by 3 p.m., only 0.5 g remained. By 5 p.m., less than 0.1 g per gram of plant remained, resulting in only about 9 hours of water available from that rare storm. Richardson (1981) points out that it is not damage by drought that eliminates many species from the desert, but the very short time available for photosynthesis.

One adaptation that permits some mosses to tolerate frequent dehydration/rehydration cycles is that those xeric mosses with undamaged membranes are able to retain ions by binding them to the cell wall (Brown & Buck 1979). Another adaptation in the desert moss is that rapid water loss, typical of the desert, can result in a retention of 50% of the polysomes, whereas slow drying can completely deplete them. Fortunately, in drought-tolerant mosses like Syntrichia ruralis (Figure 39), the polysomes can be strongly rebuilt after two hours of rehydration (Oliver & Bewley 1984b), but the process continues for a longer
period of time in those that were dried rapidly. RNA synthesis likewise requires six hours after rapid drying and only two hours after slow drying to reach the level of that in non-dried control mosses (Oliver & Bewley 1984a).

One unclear factor in this story is the role of nitrite. Nitrite accumulates during slow dehydration of *Syntrichia ruralis* (Figure 39), but not when desiccation is rapid (Mahan et al. 1998). Upon rehydration, the nitrite in the slowly-dried moss declines and reaches normal levels within one hour. Mahan and coworkers considered that the nitrite might provide a nitrogen source for the nitrogen metabolism needed during rehydration. On the other hand, Brown and Mahmood (1996) determined that nitrite apparently causes considerable membrane damage in the mesophytic *Mnium hornum* (Figure 40); thus we need further research to understand the conditions under which it is detrimental vs adaptive.

Marschall (1998) examined the activity of nitrate reductase during desiccation and rehydration of nine bryophytes and concluded that there was no difference in the proportional decrease in nitrogen reductase activity between desiccation-tolerant and non-tolerant bryophyte taxa. Eight of these bryophytes did exhibit detectable nitrate reductase (NR) activity. Pretreatment with KNO₃ did affect the increase in NR activity between these two types, with the desiccation-tolerant *Syntrichia ruralis* var. *arenicola* (Figure 39) increasing activity by a factor of 3 while the desiccation-intolerant *Dicranum majus* (Figure 41) and *Hookeria lucens* (Figure 42) had a 6-fold increase in nitrate reductase activity. Following rehydration, *Syntrichia ruralis* (Figure 39) exhibited a marked decline in NR activity during the first hour, whereas the epiphytic/saxicolous *Porella platyphylla* (Figure 43) maintained a relatively constant low level in the light but increasing NR activity in the dark. While we might assume that these physiological differences relate to survival, it is too early to explain just how this is accomplished.

Proctor (1982) considers such structures as papillae to be adaptive in ensuring that the moss does not spend a long period of time in a semi-dry state, during which it is likely to lose more carbon by respiration than it gains by photosynthesis. He notes that the papilla systems, so common on xerophytic leaves, are often separated by regions where the capillary continuity is broken at high water potentials, causing the leaf to have either an abundant water supply, or none. Such discontinuities could be amplified if the leaf rolls as it dries and bends away from the discontinuity. Vanderpoorten and Engels (2002) considered papillae so important as to be one of only four life history traits contributing to predictability of species occurrence in a particular environment on a regional scale. Nevertheless, experiments on the role of papillae in conserving water have mostly failed (Frey & Kürschner 1991).
Few bryophytes approach the succulent or sclerophyll strategies known in tracheophytes (Grime 1977), although one might argue for succulence in the Marchantiales. Plants with numerous or large papillae take on the appearance of sclerophylls, and for many years we assumed that papillae functioned to prevent the loss of water. However, as Frey and Kürschner (1991) pointed out, tests to validate that theory have failed. Nevertheless, while it appears that the papillose mosses do not slow down water loss, the papillae may have a function in water uptake (Crandall-Stotler & Bozzola 1991). As discussed earlier with leaf strategies (see Chapter 7-4a of this volume), papillae in *Andreaeobryum macrosporum* (Figure 44) are constructed in such a way that they provide a channel for the uptake of water.

Alpert (1979, 1982, 1985, 1988) investigated five species of poikilohydric mosses (those that depend on external conditions to regulate their water content): *Schistidium apocarpum* (Figure 45), *Grimmia laevigata* (Figure 36), *Hedwigia ciliata* (Figure 46), *Orthotrichum rupestre* (Figure 47), and *Syntrichia ruralis* var. *crinata* (see Figure 39). These bryophytes are characterized by short cushions of tufted growth, except for *Hedwigia ciliata*. The latter moss has a whitened appearance due to numerous papillae, and its leaves are closely appressed to the stem when dry. When wet, the leaves spread broadly, causing it to look sufficiently different from its dry state that it causes many bryologists to stop and puzzle over its identity.

Alpert (1979, 1982, 1985, 1988) found that these five mosses were able to colonize unoccupied, stressful boulder habitats, but that they were intolerant of competition or of disturbance beyond their normal desiccation regime. They grew in particular microclimatic niches on the rock substrata and were unable to occupy the most xeric conditions within the same macroclimate, although laboratory studies indicated that they can tolerate both temperatures and droughts that exceed those of the habitats they occupy. Alpert showed through transplant experiments that they could indeed occupy additional locations, suggesting that dispersal and establishment impose limits on their distribution.
The aspect and angle of slope had strong influences on the evaporation stress experienced by these mosses (Alpert 1979, 1982, 1985, 1988). Mosses growing under rock overhangs should experience the least water stress by late day, but do not regain as much moisture as those at 15° and 75° slopes (Figure 48). As expected, mosses at the tops of boulders had the greatest peaks of evaporation stress. Alpert’s work illustrates the importance of 24-hour measurements in comparing potential evaporative stresses of different microsites.

Aspect separated the evaporation stresses even more clearly (Figure 49), with evaporation stress on the east side peaking at about 10:00 hours and at most other aspects peaking at about 12:00 hours (Alpert 1982). Stress on the west side peaked last, at 14:00 hours, but with a lower peak than at the other aspects. The north, as might be expected, had the least daily variation. Although daily evaporation potential was high, a brief nighttime rainfall of no more than 5 mm was sufficient to rewet the moss for several days (Figure 50, Alpert 1982).

Open, exposed soils in temperate climates are arid for bryophytes because of their insignificant soil penetration by which to obtain water. In these habitats, the mosses Barbula (Figure 51), Syntrichia (Figure 39), and the thalllose liverwort Riccia (Figure 52) are able to survive (Schofield 1985). The two mosses are both papillose and able to roll their leaves and contort them as they dry. The Riccia thallus usually has inrolled margins and a thick cuticle; Frey and Kürschner (1991) have demonstrated that thallus and leaf inrolling correlate with increasing aridity, suggesting a protective role. Ceratodon (Figure 53), Funaria (Figure 54), and Cephaloziella (Figure 55) seem to lack any structural adaptations to their sometimes dry habitats, although Ceratodon does have crispate leaves and rolled margins.

In cryptogamic crusts of arid regions, bryophytes are important in holding water, retaining several times their volume after rainfall (Mücher et al. 1988; Rivera-Aquilar et al. 2005). This leads to higher germination rates of seed plants compared to areas with no crust (Mücher et al. 1988; Rivera-Aquilar et al. 2005; Serpe et al. 2006).
A comparison of mosses from a variety of habitats in Israel revealed varying degrees of drought tolerance that related well to their habitats (Di Nola et al. 1983). The desert mosses *Tortula brevissima* (Figure 56) and *Trichostomopsis aaronis* exhibited rapid return of metabolic activity after prolonged drying and were able to resume photosynthesis without new chlorophyll synthesis. The Mediterranean moss *Barbula fallax* (Figure 57) behaved similarly to the desert mosses, but *Homalothecium aureum* (Figure 58) and *Didymodon tophaceus* (Figure 59), more mesic mosses, had slow recovery after desiccation. *Mniobryum* sp. (Figure 60) had almost no drought tolerance and was killed by the prolonged drying.

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Figure 51. *Barbula convoluta* var. *commutata*, an ectohydric moss growing on rock over little or no soil. Michael Lüth, with permission.

Figure 52. *Riccia nigrella*, a thallose liverwort surviving on dry soil. Photo by Michael Lüth, with permission.

Figure 53. *Ceratodon purpureus* on rocky soil, a moss that has few structural adaptations to such a dry habitat. Photo by Janice Glime.

Figure 54. *Funaria hygrometrica*, a moss with no noticeable xerophytic adaptations, living on sand and rocks. Photo by Michael Lüth, with permission.

Figure 55. *Cephalozia stellulifera*, a leafy liverwort that seems to lack structural adaptations to this rock habitat. Photo by David T. Holyoak, with permission.

Figure 56. *Tortula brevissima*, a desert moss that rapidly returns its metabolic activity upon rehydration. Photo by Michael Lüth, with permission.
Longton (1988b) pointed out the importance of dispersal among desert bryophytes, since many of them are drought avoiders. Since sexual reproduction will occur infrequently, he contends that desert mosses should be acrocarpous perennial stayers with long-lived, desiccation-tolerant gametophytes, small spores, and long setae. The annual taxa are ephemeral (short-lived) mosses and liverworts that can develop rapidly after a rain because their dormancy is accomplished by large spores; their capsules are often immersed, presumably shortening the time required to mature and preserving moisture. The perennial shuttle species are mostly thallose liverworts that have both desiccation-tolerant gametophytes and large spores. Fugitives generally stay only one to two years while the habitat remains suitable at a site and produce small spores that permit them to be dispersed easily.

The short duration of the life cycle is one of the advantages provided to many desert bryophytes. In the southwestern desert habitat (USA), the desiccation-tolerant 

**Syntrichia ruralis** (Figure 39) requires a year to reach maturity, producing new innovations in midwinter and growing slowly through spring (Mishler & Oliver 1991). In late summer, it lengthens rapidly, completing its growth by midwinter. Female gametangia are initiated in midwinter and terminate the growth of these innovations. However, the female gametangia are present during the next 6 to 9 months on these innovations, ultimately disappearing some time between June and August. In the New Mexico populations observed by Mishler and Oliver, there were no male gametangia, and thus no sporophytes produced. Consequently, this plant must propagate entirely by vegetative means.

In the Negev Desert, southern Israel, the dioicous moss **Bryum dunense** takes advantage of fog and dew prior to the first winter rain to initiate its reproductive organs (Herrnstadt & Kidron 2005). The sporophytes are most common in partially shaded habitats and appear following the winter rains. This reproduction is supplemented by the typically more reliable reproduction through bulbils in the partially shaded and exposed habitats, whereas secondary protonemata are most abundant in the shaded habitats.

Alpert and Oechel (1985) hypothesized that even the xerophytic mosses cannot live in the most xeric habitats due to their inability to maintain a positive carbon balance. **Grimmia laevigata** lives under the xeric conditions of rocks and boulders. When subjected to such extreme conditions of long, severe drought and extreme temperatures, this moss supported the hypothesis.

### Sporophyte Damage

The leafy gametophyte is not the only generation affected by desiccation. For desert mosses, too little rainfall in early sporophyte development can also be a problem. In the Mojave Desert, Nevada, USA, the moss **Syntrichia inermis** (Figure 61) experienced 66% abortion of sporophytes due to a reduced winter-spring rainfall...
But unusually heavy rains in the summer likewise resulted in an increase in sporophyte abortion from 9 to 43%. Stark suggested that the summer abortions may have been the result of membrane damage resulting from rapid drying as well as from high temperatures while hydrated. *Crossidium crassinervium* (Figure 62) experienced similar sporophyte abortions in the same desert (Stark 2005).

**Syntrichia caninervis** (Figure 64) from approximately 25% cover to less than 2% in just one growing season. The addition of small precipitation events resulted in a negative carbon balance; larger events are able to maintain carbon balance. The loss of moss cover changed the nitrogen cycling, reducing soil fertility. On the other hand, increased temperature had no effect.

**Grimmia orbicularis** (Figure 63) can suffer sporophyte abortion as a result of poor timing. It was summer rainstorms in the Mojave Desert that led to the demise of ~50% of the sporophytes, mostly in the seta elongation phase (Stark 2001). As in *Syntrichia inermis* (Figure 61), Stark suggests that the abortion resulted from stresses caused by wet-dry cycles during summer heat, a time when the moss would normally be dry and have arrested metabolism. This may have been complicated by the premature seta elongation that resulted in more exposure during the remainder of the summer or that set in motion the physiology for capsule maturation at a time when insufficient nutrients were available.

The arid and semi-arid lands occupy approximately 40% of the land on planet Earth (Reed *et al.* 2012). Climate change that changes annual rainfall could result in profound mortality of bryophytes growing there. An increase of rainfall frequency, resulting in only a 1.2 mm increase in summer rainfall, reduced the moss cover of *Syntrichia caninervis* (Figure 64). Within only one minute, it recovers 90% of its photosynthetic yield (Zhang *et al.* 2011). In fact, this species can use moisture from dew and fog, collected and directed into the leaf by its hair points (Tao & Zhang 2012). In addition to the collection effect of the hair tips, mosses from dry habitats have high osmotic values that enable them to absorb water vapor from the air. These attributes seem almost contradictory to the
loss of cover due to a minor increase in rainfall events in the desert, but a short daytime rainfall is quite different from the prolonged moisture available from fog or dew at night. Daytime moisture from a short rainfall lasts for a very short time, apparently insufficient to recover the lost energy before high temperatures and evaporation shut it down. Yet this leaves the question of rebuilding energy at night. It suggests that it is the cellular changes that use up one readily available form of energy but do not permit rebuilding it, whereas the hydrated cells from dew are fully functional and ready for photosynthesis with the first light of day – there should be no delay at all. At this time the moss is still cool from the night and evaporation should be slower.

**Desiccation from Salt**

Salt pans and regions of salt spray, when not under water, can be the most arid conditions of all. Few bryophytes are adapted to this regime, although some species of *Fontinalis* (Figure 65) can tolerate brackish (somewhat salty, often from a mix of fresh and salt water) waters. The liverworts *Riella helicophylla* (Figure 66), *R. numidica*, and *Carpos* (?) are among the few (Schofield 1985).

**Flood Plains**

The flood plain habitat is one of extremes. For part of the year the inhabitants are under water, but once the water recedes the habitat can become extremely dry. This regime requires different adaptations from other kinds of dry habitats.

A number of thallose liverworts exhibit drought or desiccation tolerance (Figure 69) and are able to live in these alternating habitats (Bischler 1998; Wood 2007). Such genera include fairly small liverworts with thick thalli and short life cycles: *Corsinia* (Figure 67), *Cronisia*, *Exormotheca* (Figure 68), *Monocarpus*, *Riccia* (Figure 52), and *Targionia* (Figure 70). But larger thalli in the *Aytoniaceae* [*Asterella* (Figure 71), *Mannia* (Figure 72), *Reboulia* (Figure 73)] also exhibit desiccation tolerance (Vitt *et al.* 2014). Much of this tolerance may be structural. For example, these genera typically roll their edges to avoid desiccation. Their pegged rhizoids serve as water conduits and help them to resist desiccation in periodically dry habitats by providing capillary spaces [e.g. *Mannia*, *Plagiochasma* (Figure 74), *Targionia*] (Duckett *et al.* 2014). On the other hand, liverworts from moist habitats tend to be drought intolerant (Figure 69). These include genera with thin thalli such as *Pellia* (Figure 75), *Fossombronia* (Figure 76), *Moerckia* (Figure 77), *Pallavicinia* (Figure 78), and *Symphyogyna* (Figure 79). Likewise, the primitive genera of *Haplomitrium* (*Haplomitriidae*; Figure 80), *Treubia* (*Treubiidae*; Figure 81), and *Apotreubia* (*Treubiidae*) all grow on constantly moist soil and are drought intolerant (Wood 2007).
Figure 69. Comparison of liverwort genera with desiccation tolerance vs those with avoidance. Many species of *Riccia* are able to use the escape strategy by going dormant to avoid desiccation. From Vitt *et al.* 2014.

Figure 70. *Targionia lorbeeriana*, member of a genus with thick thalli and short life cycles. Note the black marsupia visible from the ventral side of the thalli. Photo by Michael Lüth, with permission.

Figure 71. *Asterella saccata*, a large thallus with desiccation tolerance. Photo by Jan-Peter Frahm, with permission.

Figure 72. *Mannia fragrans*, a large thallus with desiccation tolerance. Note how it rolls as it dries. Photo by Michael Lüth, with permission.

Figure 73. *Reboulia hemisphaerica*, a large thallus with desiccation tolerance. Photo by Michael Lüth, with permission.

Figure 74. *Plagiochasma appendiculatum*, a large thallus with desiccation tolerance. Photo by Michael Lüth, with permission.
Figure 75. *Pellia epiphylla*, member of a genus with thin thalli that are desiccation intolerant. Photo by David T. Holyoak, with permission.

Figure 76. *Fossombronia caespitiformis*, member of a genus with thin thalli that are desiccation intolerant. Photo by Des Callaghan, with permission.

Figure 77. *Moerckia blyttii*, member of a genus with thin thalli that are desiccation intolerant. Photo by Michael Lüth, with permission.

Figure 78. *Pallavicinia lyellii*, member of a genus with thin thalli that are desiccation intolerant. Photo by Jan-Peter Frahm, with permission.

Figure 79. *Symphyogyna brasiliensis* female plant, member of a genus with thin thalli that are desiccation intolerant. Photo by George J. Shepherd, through Creative Commons.

Figure 80. *Haplomitrium hookeri*, a desiccation-intolerant bryophyte of damp or wet habitats. Photo by Janice Glime.
Volk (1984) elucidated the behavior of the genus *Riccia* in these flood plain circumstances in Namibia. These are seasonally very dry habitats and members of the genus *Riccia* are very common. In this genus, the dorsal (upper) surface is unable to take in water. The ventral surface can absorb water through capillary action among rhizoids and scales, and in some cases hairs. During the dry period, they roll their thalli, exposing these rhizoids and scales from the ventral surface. This rolling thus facilitates the uptake of water from rainfall when it first occurs. These perennial species are very drought resistant and can survive up to seven years with no new water input. They are able to endure heat to greater than 80°C when dry, whereas temperatures above 50°C injure wet plants. They are unable to compete with other plants, but annual species survive by producing large numbers of spores. Perennial species produce fewer spores and survive primarily by going dormant.

**Arctic and Antarctic**

Kennedy (1993) asked "What limits the presence, distribution, and abundance of life in Antarctica?" To this question he answered that isolation restricts arrival and the paradigm has been that the extreme cold limits survival. But he challenges the latter tenet, suggesting that instead it is moisture that limits the organisms on vertical, horizontal, and temporal scales. Gradients in meltwater, seepage, and upwelling create moisture differences on a continental scale.

Antarctic communities, in particular, experience physiological extremes in water availability and bryophytes must survive both desiccation and submergence (Wasley et al. 2006). Growth rate is slow (mean for 17 yrs was 3.7 g dw m⁻² y⁻¹) (Kanda 1986), providing limited opportunity to compensate for losses during drying. Exposure accounts for the loss of bryophyte flora in many circumstances. This can be particularly true in Arctic climates. Flock (1978) found that acrocarpous mosses dominated in areas with only light snow cover, but in areas with deep snow the pleurocarpous mosses were more abundant. The reason for this is unclear, but one might hypothesize that pleurocarpous mosses are less likely to suffer apical damage from the heavy snow, and even if they do, they usually have numerous growing points to permit their continuation. In this seasonally arid climate, primarily soil moisture and slope account for the distribution of moss communities (LaFarge-England 1989).

At Wilkes Land, Antarctica, colonies of *Bryum algens* with a dense tomentum of rhizoids held significantly more water than those with sparse rhizoids (Lewis Smith 1988). In *Schistidium antarcticum*, the dense shoot arrangement facilitates its high water-holding capacity in the turf form, whereas when this species has less densely packed shoots and thicker cell walls in xeric cushions it maintains a lower water content. On the other hand, the loss of water was much faster in the turf form and the tomentose form of *Bryum algens*, but this relationship was reversed when it was expressed as a percentage of the initial water content. The mosses take several times longer to reach minimal water conditions when compared to the lichens.

Some mosses form large mounds on the Antarctic terrain. Robinson et al. (2000) were able to demonstrate the relationship of desiccation tolerance to habitat in three of these moss species. *Schistidium antarcticum* (Figure 82), limited to relatively wet sites, had the least ability to sustain photosynthesis during desiccation. The worldwide *Ceratodon purpureus* (Figure 83) had the most and inhabited the driest sites. Intermediate in tolerance was *Bryum pseudotriquetrum* (Figure 84), which occupied intermediate habitats and exhibited the greatest plasticity of the three. These responses fit their typical habitat distribution, with *Ceratodon purpureus* being common in the driest sites and *Schistidium antarcticum* living in relatively wet sites. Following desiccation, *Bryum pseudotriquetrum* contains stachyose, a soluble carbohydrate known to provide desiccation tolerance to seeds.

**Figure 81.** *Treubia* sp, a desiccation-intolerant bryophyte of damp or wet habitats. Photo by Jan-Peter Frahm, with permission.

**Figure 82.** *Schistidium antarcticum*, a moss limited to relatively wet sites. Photo by Rod Seppelt, with permission.

By contrast, *Ceratodon purpureus* has poor survival when it is submerged (Wasley et al. 2006). The wet habitat *Schistidium antarcticum*, on the other hand, has high submersion tolerance. *Bryum pseudotriquetrum* (Figure 84) is an intermediate species that is able to co-exist with both of these species and has flexible responses.

Davey (1999) summed up the Antarctic situation by stating that mosses from hydric habitats had lower carbohydrate and higher protein, nitrogen, and phosphorus content than species from drier habitats, suggesting that the
constant flushing provided nutrients. This emphasizes another aspect of the importance of both water and physical factors in the success of Antarctic mosses.

Figure 83. *Ceratodon purpureus*, the moss with the greatest ability to sustain photosynthesis during desiccation in the Antarctic study of Robinson *et al.* (2000). Photo Rod Seppelt, with permission.

Are the responses of Antarctic species different from those of other habitats? Apparently not very. Davey (1997) examined effects of various desiccation regimes on photosynthesis of 14 bryophyte species. Using testing intervals of 6 months and 12 months of desiccation, Davey found that the photosynthetic rate decreased as the length of dehydration period increased in all these species. The xeric species had greater retention of photosynthetic rate than did the hydric species, but even the hydric species retained some photosynthesis. Repeated cycles of wet/dry do more harm than continuous dehydration to the hydrophytic species, but the mesophytic and xerophytic species show the opposite response, suggesting that the mesophytic and xerophytic species were able to recover better during short periods of hydration. As the season progresses from spring to autumn, the percentage loss of photosynthetic rate following dehydration/rehydration increases, and this change is most evident in the hydrophytic species. At the same time, it appears that the long winters with concomitant water stress have driven these species to similar adaptations to those of some desert species.

Longton (1988a) concluded that phenotypic plasticity, opportunistic responses in CO$_2$ exchange, and a poikilohydric water strategy endowed the polar bryophytes with their considerable frost and desiccation tolerance. But he was quick to point out that this plasticity was not unique to polar bryophytes, but rather was common among bryophytes in general. To really understand polar adaptations we need to do physiological studies on the *endemic* (restricted to a certain area) species.

Bryophytes and water level are intimately related in the Arctic. Where the water table is maintained above the bryophyte surface, marshes develop. Where the water table is high above the permafrost, but remains below the bryophyte surface, fens develop. These moss tundras normally have no standing water and water courses are able to move through them from below the surface, maintaining the fen status. The standing water level is thus the primary factor determining the species alliances in that area. Some species complexes, such as that of the *Cataspodium nigritum* community (Figure 85), require a temporary period of desiccation to subsist (Vanderpuye *et al.* 2002).

Figure 85. *Cataspodium nigritum* exhibiting its fen community where temporary desiccation is required. Photo by Michael Lüth, with permission.

The role of bryophytes in mediating water in the sub-Arctic is crucial for making climate models that adequately predict the effects of climate change. Using *Sphagnum fuscum* and *Polytrichum piliferum*, Street *et al.* (2012) demonstrated that the model does not adequately predict the effect of turf water content on their primary productivity fluxes.

In subarctic mires, water balance often determines which species will dominate (Sonesson *et al.* 2002). When the codominant mosses *Sphagnum fuscum* and *Dicranum elongatum* were subjected to increased precipitation, both species increased their growth rate, up to 5 mm per day. *Sphagnum fuscum* had a 50% higher response in growth compared to *Dicranum elongatum*, a species of drier habitats than those of *S. fuscum*. In winter, the responses were affected by the neighboring plants. *Sphagnum fuscum* grew better when it was next to *D. elongatum*, but *D. elongatum* also did better when next more *D. elongatum*.

In the Arctic tundra, Rixen and Mulder (2005) found that high moss species diversity increased productivity, especially in low-density plots, when the plots were...
watered regularly. Furthermore, moisture retention was greater in plots with high species richness. Furthermore, plant height was greater in mixed cultures than in single-species cultures. Likewise, 10 out of 12 species grew better in mixture than in monoculture when the density was high and droughts were short. It is interesting that this is the opposite of the relationships found in temperate moss communities.

As suggested by the Antarctic species discussed above, growth form is important in these cold environments that are frequently subjected to water stress. In the subalpine habitat, Nakatsubo (1994) found that large cushions and compact mats were the most common among the xerophytic species. The mesophytic species of the coniferous forest, by contrast, were smooth mats, wefts, and tall turfs. The relation between evaporation rate per basal area of the moss and dry weight per basal area of the colony correlated closely with the growth form. Nakatsubo concluded that the difference in the evaporation rate per weight between the xerophytic and mesophytic species was largely due to the difference in dry weight per basal area of the colony, and that the growth forms of the xerophytic species were suitable for increasing dry weight per basal area of the colony without increasing surface roughness. Increasing surface roughness would lead to an increase in evaporation rate due to increased exposed surface area and increased air turbulence.

**Forest Floor**

The forest floor would seem to be the most straightforward and familiar habitat for most of us who have lived our lives in the temperate zone and who hunt mosses. But water relations in this habitat are not so simple. Bryophytes may actually deprive the trees of water in several ways.

In her collections of water samples under moss mats and without moss mats in a Jack pine forest (*Pinus banksiana*), Scafone (unpublished data) found that there were many occasions when 1-2 cm of water accumulated in the collectors with no moss, but the collectors under the moss mats were dry. This means the soil does not receive any of the throughfall during short or light rainfall events where there is a substantial moss mat on the surface. Such a cover is common in boreal and pine forests, depriving upper fine roots of much needed moisture.

But it appears that mosses can even derive their moisture at night from the soil. Carleton and Dunham (2003) accounted for moisture available to mosses during dry summer weather by explaining nocturnal cooling on the forest floor. Cooling of the soil surface at night was sufficient to bring the moss to dew point, reversing the daytime temperature gradient in the forest floor organic profile. By using a vapor barrier for comparison, they determined that the soil provides an upward movement of water at night that permits moss shoots to survive summer "dry-downs." This happens most noticeably in late summer when organic layers have accumulated the most warmth.

**Temperate Epiphytes**

Epiphytes are subjected to feast or famine for their water needs. In the growing season, they can get flooded by stem flow and may grow best on the side of the tree that gets better stem flow. In the winter they often remain exposed, unprotected by snow, and subject to the harsh, dry winds.

Trynoski and Glime (1982) demonstrated the apparent role of winter when they mapped the locations of epiphytic bryophytes in a northern deciduous forest in the Keweenaw Peninsula of Michigan, USA. The highest cover at the base was on the north side of the tree, but contrary to popular belief, midway between the base and breast height it was greatest on the south side. They attributed this southern location to the drying winds from the north and a safe haven in the space between the tree trunk and the snow that provided a moist microcosm where sufficient sun could penetrate through the snow to permit photosynthesis in winter.

Where winters are not in a constant state of snow cover, they may afford a better growing season for epiphytes due to cooler temperatures and fewer dry days. In British woods, Pitkin (1975) found that most of the growth of the epiphytes *Hypnum cupressiforme* (Figure 86) and *Platygyrium repens* (Figure 87) occurred in autumn and winter. A similar pattern of growth was found for epiphytes at a second location, and growth periods in both locations corresponded with greater moisture. When summers were wet, the winter growth did not increase proportionally to the summer increase. In wetter climates, temperature and day length have greater importance in determining growth rates.
Wu et al. (1987) found that epiphyllous liverworts in southeast China required about two hours of direct light and ten hours of diffuse light in winter, with light, temperature, and humidity being the primary factors to control their distribution.

**Tropics, Rainforests, and Cloud Forests**

Cloud forest (Figure 88) and rainforest bryophytes can experience a wide range of water status in a single 24-hour period (Zotz et al. 1997). In a submontane tropical rain forest in Panama, both low and high water content limited carbon gain significantly on a daily basis for bryophytes exhibiting a variety of life forms. More than half of the daily carbon gain (mean 2.9 mg C per g plant) is lost through respiration at night.

![Figure 88. *Macromitrium* habitat on a tree in a New Zealand cloud forest. Photo by Vita Plasek, with permission.](image)

Although we are beginning to understand the broad aspects of tropical bryophyte ecology, understanding of their physiology has been hampered by taxonomic difficulties and remoteness of the study site from most of the research labs. Hence, only a few studies exist on their desiccation tolerance (e.g. Renner 1933; Biebl 1964a, b).

Johnson and Kokila (1970) reviewed desiccation responses in primitive photosynthetic organisms and surmised that in the algae, accumulation of fat in cells, thickening of the cell walls, and accumulation of mucilage can facilitate desiccation resistance. Other characters that correlate with resistance in some algae include resistance to plasmolysis in a hypertonic solution, rigid and viscous protoplasm, and more abundant granules. But in the mosses *Bryum* (Figure 1) and *Mnium* (Figure 40) the viscosity decreased during drying. Hence, Johnson and Kokila considered how applicable these attributes might be to the desiccation tolerance of tropical bryophytes. They examined ten species that represented a wide range of habitats and exhibited a number of structural adaptations that might contribute to survival of drought.

Some species exhibit damage near the tips, with damage spreading slowly to the lower leaves, and others experience more apparent damage near the base (Johnson & Kokila 1970). The species they studied fell into two groups that mostly coincided with this pattern of damage progression:

**Low resistance to desiccation:**
- *Calympers moluccense* (*
- *Fissidens crassinervis*
- *Leucobryum sanctum* (*
- *Semibarbula orientalis* (*
- *Syrrhopodon loreus* (*

**High resistance to desiccation:**
- *Bryum coronatum* (Figure 89)
- *Leucophanes octoblepharioides* (Figure 93)
- *Neckeropsis lepineana* (Figure 94)
- *Paraleucobryum longifolium* (Figure 95)
- *Pelekium velatum*

![Figure 89. *Bryum coronatum* surviving on an exposed rock. Photo by Michael Lüth, with permission.](image)

In the tropics, epiphytes can experience long periods of drought during the dry season. Salazar Allen (1985) found that the genus *Leucophanes* (Figure 90) survives the drought by an unusual life form strategy. *Leucophanes* is an acrocarpous moss that may be branched or unbranched and that forms turfs. The unusual feature is that leaf-tip gemmae germinate on the parent plant to form a new layer of gametophores (Figure 91). In many bryophytes, there seems to be an inhibitory substance that prevents such occurrences (see interaction chapter). However, in *Leucophanes*, this seems to be an important adaptation for water retention. Lacking subterminal innovations, *Leucophanes* benefits from the thicker turf where the numerous stems can protect each other from drying out. It is my guess that if the tips were to become so dry that they would die in an unusually dry year, there would be at least some lower (older) stems with enough life remaining to re-establish the colony. If not, surely some of the gemmae would survive. In any event, this habit of germination of gemmae within the parent colony provides *Leucophanes* with a dense turf that could resist drying.
Among those adapted for drying, with little damage down to 10% humidity, *Leucophanes octoblepharioides* (Figure 93) has abundant leuco cysts that serve as a water reservoir (Johnson & Kokila 1970). The costa is thickened and prevents the leaf from collapsing. *Paraleucobryum longifolium* (Figure 95), a species of exposed situations in the hill forest, has a thickened costa and thick-walled lamina cells. *Pelekium velatum* (Figure 92) uses a different strategy with very small leaves pressed against the stem and with papillose cells. *Neckerosis lepineana* (Figure 94) holds its secondary branches at an angle to the tree trunk in a way that subjects it to drying.

Those species with low desiccation resistance are damaged at humidity of 63% and are likely to die at 10% (71-94% of cells damaged) (Johnson & Kokila 1970). These species live in habitats that have near saturation humidity levels. Three of these species live on the ground where the humidity is constantly and exceedingly high. Two are *corticolous* (growing on bark) species [*Calympetes moluccense* (Figure 96) and *Syrrhopodon loreus*) that live on the wettest side of the tree in areas that are constantly wet due to runoff.
Figure 95. *Paraleucobryum longifolium* on rock, a species adapted for drying by a thickened costa and thick-walled lamina cells. Photo by Janice Glime.

Figure 96. Saturated *Calymperes* sp. *Calymperes moluccense* lives on the wettest sides of trees in humid areas and is desiccation intolerant. Photo by Niels Klazenga, with permission.

Pardow and Lakatos (2013) explored the desiccation tolerance of epiphytic bryophytes from contrasting microsites in tropical lowland forests of French Guiana. Canopy species are well adapted, as indicated by the recovery of chlorophyll fluorescence, with 13 of the 18 species maintaining more than 75% of their photosynthetic capacity after 9 days at 43% relative humidity. On the other hand, understory species were sensitive to desiccation and were only able to withstand a reduction to 75% relative humidity. The bryophytes were able to reanimate by reaching equilibration with water vapor as their only moisture source.

Pardow *et al.* (2012) noted the importance of lowland cloud forests in the Guianas as a site for high epiphytic bryophyte diversity. This area is subject to frequent early morning fog events that provide moisture for the bryophytes. The growth forms were those that could take greatest advantage of this cloud moisture: tail, weft, and pendent (Figure 97).

Romanski *et al.* (2011) likewise studied epiphytes, in this case in the lower montane (2400 m) rainforest of Peru. A single tree of *Weinmannia* supported 110 bryophyte species (77 hepatics, 1 hornwort, 32 mosses). They divided the tree into Johansson zones (lower trunk, upper trunk, mid-crown, mid-inner crown, outer crown) and found the greatest species richness and abundance on the upper trunk and large branches of the mid-crown. Exposure to light and desiccation appeared to account for the bryophyte distribution, but more research is necessary to tease out these relationships.

Atala *et al.* (2013) expressed concern that dendroid mosses with conducting tissues likewise lacked study. They examined desiccation tolerance in the *Dendroligotrichum dendroides* (Figure 98) from Chile, where it grows in the understory of temperate forests. They tested plants from two contrasting moisture conditions and found that both populations exhibited desiccation tolerance. But the responses were not equal. Those from the northern population lost water more slowly and recovered the PSII Fv/Fm to higher values when compared to the southern population. They suggested that exposure to summer droughts in the northern population could contribute to differences in their response.

Figure 97. *Papillaria*, a pendent moss in the cloud forest at Mt. Budawang, Australia. Photo by Peter Woodard, through Public Domain.

Figure 98. *Dendroligotrichum dendroides*, a Chilean species with desiccation tolerance. Photo by Felipe Osorio-Zúñiga, with permission.
Epiphytes

Epiphytes in most habitats have sharply contrasting moisture conditions. When it rains, they can be in a river of water rolling down the tree trunks. But when the rain stops, they are elevated where there is more access to wind and drying can be rapid.

These conditions are not so severe in a cloud forest due to the moisture in the clouds. Bryophytes are able to use such moisture and some are even adapted to collect it by providing fine wirelike structures, expressed as such structures as thin awns or pendent growth forms.

In two Venezuelan cloud forests, León-Vargas et al. (2006) the rainfall averages only 20 mm or less in January and February, 200 mm or more in August to October, and variable year-round. Continuous 100% relative humidity occurred 8.5% to 52.2% of the time. Humidity increased at night. Although these cloud forests are among the most ideal for epiphytic bryophytes, even they can have short periods droughts at any time of year. They noted that the pendent life form was probably important in harvesting the moisture from the air in these forests. All of the six pendent bryophyte species survived at least a few days of desiccation.

Pendent Mosses

Pendent mosses (those that hang down; Figure 99-Figure 100) often suffer desiccation, with little surrounding them to help hold in the water. *Floribundaria floribunda* (Figure 99) and *Pilotrichella ampullacea* (Figure 100) from Uganda humid tropical forests survive partly by avoidance, holding large quantities of external capillary water, with *Pilotrichella ampullacea* holding approximately twice as much as *Floribundaria floribunda* (Proctor 2002). Both species were able to recover from 11 months of dry storage at 5°C, although they required several days to recover, with *F. floribunda* recovering more slowly and less completely. Following 20 hours of air drying, *P. ampullacea* achieved a positive carbon balance within 30-60 minutes after rewetting.

León-Vargas et al. (2006) studied the epiphytes in the Venezuelan cloud forests. The rainfall there drops to an average of 20 mm or less in January and February and increases to 200 mm or more from August to October. Nevertheless, the longest recorded dry period was only 143 hours. Nighttime humidities of 90% relative humidity were common, with 100% for significant periods, creating cloud water deposition in about 50% of the nights. Although these cloud forests are among the most ideal for epiphytic bryophytes, even they can have short periods droughts at any time of year. They noted that the pendent life form was probably important in harvesting the moisture from the air in these forests. All six species of pendent bryophytes survived for at least a few days of desiccation; these recovered better from high than from low humidities.

Altitude Differences in the Tropics

In the tropics, altitude can have a strong effect on both biomass and diversity among bryophytes (Bader et al. 2013). The lowlands are characterized by low abundance and low species richness. These could be a consequence of short daily periods of suitable light, temperature, and moisture and nighttime high respiration due to high temperatures. Moisture regimes are quite different, with lowland forests having more concentrated but less frequent precipitation than montane cloud forests. they furthermore have sunny mornings that cause rapid drying. The high levels of moisture in high altitude cloud forests is manifest in a high diversity and cover by bryophytes (Figure 101). But both lowland and montane species are able to survive more than 80 days of dry periods, far exceeding the duration of lowland tropical dry periods.
Summary

Because of their small size, bryophytes are able to occupy microsites in otherwise unfavorable habitats. Their ability to recover from dehydration typically correlates with habitat, with aquatic bryophytes having little ability to tolerate dehydration and resume photosynthesis, whereas dry habitat bryophytes can withstand extended periods of desiccation. In aquatic bryophytes, ribosomes can be damaged irreversibly and membranes are more likely to be damaged than in dry habitat taxa. On the other hand, there is no difference in nitrogen reductase activity between dry and wet habitat bryophytes.

Peatland bryophytes (Sphagnum) of lawns and hollows are typically desiccation tolerant, benefitting from inducible tolerance. Those of hummocks generally are intolerant but are desiccation resistant. Submersed species rely on the water of their habitat and have little tolerance for desiccation.

Aquatic bryophytes have poor desiccation tolerance, especially with rapid drying, but usually benefit from slow drying and sometimes can survive considerable dry periods.

A number of bryophytes are xerophytic. Their life cycle is typically short and the strategy is adapted to the short periods of rainfall. But in some arid habitats, nighttime dew is the only source of water for bryophytes. Hair points gather the dew and facilitate its uptake. In others, cooling of soil can bring bryophytes to dew point and draw water upward from the soil. The real limiting factor is carbon balance. If the bryophyte loses too much carbon by respiration and experiences a hydrated state for which the duration is too short to recover it, the bryophyte will perish. Rapid repair and recovery of photosynthesis permit these bryophytes to take advantage of short periods of hydration. The rapid daytime drying makes constitutive desiccation tolerance essential for survival where short daytime storms are common. But at least some of these bryophytes also have inducible desiccation tolerance. Some use an escape strategy of desiccation-tolerant gemmae, spores, and protonemata that help these bryophytes succeed in habitats with extensive dry periods. Nitrite and nitrate reductase both seem to be involved in recovery, but more research is need to determine the mechanisms involved. Leaf rolling and papillae seem to provide a protective role, perhaps by reducing light damage of dry cells and facilitating water uptake, but their role in water retention remains to be demonstrated. Compact growth form is also important.

Flood plains have extremes of habitat and require special strategies to weather these. Many of the bryophytes adapted to these extremes are species of the thalllose liverwort Riccia. This genus is able to go dormant when it is dry with a variety of strategies, including rolling the thallus, surviving as tubers, having hairs on the surface. Some have small thalli with short life cycles; others have thick thalli that survive the desiccation.

In the Arctic and Antarctic, frost can be a desiccant. Water height above permafrost determines existence of fens, where bryophytes are emergent, and marshes exist where the water table is high above the bryophyte surface. Arctic bryophytes suffer from exposure that creates desiccating conditions. Aspect and angle of slope play important roles in speed and frequency of drying. Acrocarpous mosses do better in areas of light snow cover, whereas pleurocarpous mosses suffer less apical damage from heavy snow. In the Antarctic, the longer the dry period, the lower the subsequent photosynthetic rate, especially among hydrophytic species.

On the forest floor, bryophytes may sequester all the water from a brief rainfall (1-2 cm). In the dry summer, bryophytes may derive moisture from the soil during the cooling temperatures.

Temperate epiphytes may take advantage of cooler temperatures of winter for maximum growth.

In the tropics, carbon balance can, as in the desert, be a problem. When the mosses are hydrated at higher temperatures, respiration loss exceeds photosynthetic gain. This is generally not a problem at higher altitudes in the cloud forests; bryophytes are abundant on nearly every substrate there. Physiology is poorly known for tropical bryophytes, but it appears that they have similar adaptations to those of other locales with similar moisture conditions such as thick costa and thick cell walls. Some (Leucophanes) have leaf-tip gemmae that germinate and layer the colony, making a thick turf.

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Literature Cited


CHAPTER 7-9
WATER RELATIONS: WINTER PHYSIOLOGY

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Freezing tolerance must necessarily be coupled with drought tolerance and therefore this chapter would be incomplete without a discussion of winter effects. While other plants are dormant and have either lost their leaves, died back to ground level, or adapted in other ways to prevent damage from heavy snow and loss of water due to ice crystals, many bryophytes remain exposed, ready to have photosynthesis whenever light, water, and temperatures permit (Figure 1). Here we will examine the conditions related to their winter water relations. Temperature relations will be covered in a different chapter.

Problems in Winter

Bryophytes do have problems to deal with in winter. These include damage to their DNA and photosynthetic tissue (chlorophyll) from the UV light, temperature stress, cellular freezing and structural damage, and desiccation damage due to ice crystals. Alberdi et al. (2002) consider that adaptations to cold include high resistance to light stress, high freezing resistance, and high photosynthetic capacity at low temperatures. To this list I must add the ability to regain hydration quickly upon thawing. Most of these topics will be discussed elsewhere in chapters that deal with that particular physiological parameter. This chapter will examine the winter water relations.

The all-important water, whether as fog or rain or dew, is suddenly no longer liquid, but solid. Not only does this present problems for obtaining water, but it also means that hygroscopic ice crystals can draw water from the bryophyte cells.

But not all bryophytes suffer from the problem of ice damage. The thallose liverwort Ricciocarpos natans (Figure 2-Figure 4) can spend the winter encased in ice and can tolerate temperatures to -30°C (Frahm 2006). Frahm suggested that it was able to survive this frozen condition because it has no water vacuoles, thus providing no free internal water to form crystals that could destroy its
membranes. Rod Seppelt (Bryonet discussion 14 November 1997) also noted an absence of vacuoles in Antarctic mosses. Both *Ricciocarpos natans* and *Riccia fluitans* (Figure 5-Figure 6) are common in Arctic streams, so we might expect them to have this absence of vacuoles. I find it interesting that no one seems to have reported either presence or absence of vacuoles in *R. natans* (based on literature search and question posed on Bryonet in April 2015). Rather, lipids and starch bodies may help in their winter tolerance (Rod Seppelt, Bryonet discussion 14 November 1997).

**Figure 2.** *Ricciocarpos natans* in ice. Photo by Jan-Peter Frahm, with permission.

**Figure 3.** *Ricciocarpos natans* thallus, a species with lipids and starch bodies that may help it survive winter. Photo by Norbert Stapper, with permission.

**Figure 4.** *Ricciocarpos natans* section showing the many chambers packed with small chlorophylllose cells. Photo by Norbert Stapper, with permission.

**Figure 5.** *Riccia fluitans* with pearling, a species that survives freezing. Photo by Christian Fischer, with permission.

**Figure 6.** *Riccia fluitans* cross section showing large air chambers that help it to float. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

**Frost Damage**

Those venues of green in the spring attest to the survival of bryophytes through the winter, subjected to frost before snow cover arrives and subsisting at near 0°C under the snow. But few studies give us specifics on what species survive and which ones are damaged.

Fletcher (1982) had the opportunity to document the frost responses of a number of species in cultivation. Among the winter survivors, reaching temperatures as low as -3°C, are species from New Zealand [*Papillaria crocea* (Figure 7), *Hypopterygium* spp. (Figure 8), *Rhizogonium bifarium* (see Figure 9), *Cyathophorum bulbosum* (Figure 10), *Eriopus brownii*], South Africa [*Hypopterygium* sp.], Australia [*Gigaspermum repens* (Figure 11), *Goniomitrium acuminatum* subsp. *enerve* (=*Goniobryum enerve*; Figure 12)], and Florida, USA [*Rhizogonium spiniforme* (Figure 13)]. Even the delicate-looking *Takakia lepideozioides* (Figure 14-Figure 15) remains healthy. As we might expect, the widespread mosses *Sphagnum* spp. (Figure 16) and *Mnium* spp. [probably *Plagiomnium* since no *Mnium* species are present in New Zealand (NZOR 2015); Figure 17] survive the frost. On the other hand, *Haplomitrium hookeri* (Figure 18) from New Zealand and *H. mnioides* (Figure 19) from Japan had no healthy plants remaining after an exposure to -3°C, despite their ability to survive and grow in the winters in their native habitats.
Figure 7. *Papillaria crocea*, a winter survivor in NZ. Photo by Janice Glime.

Figure 8. *Hypopterygium didictyon*, a genus that can withstand temperatures to -3°C. Photo by Juan Larrain, with permission.

Figure 9. *Rhizogonium novae-hollandiae*. *Rhizogonium bifarium* survives temperatures as low as -3°C in New Zealand. Photo by Niels Klazenga, with permission.

Figure 10. *Cyathophorum bulbosum* from Tasmania, a moss that survives freezing. Photo by Vita Plasek, with permission.

Figure 11. *Gigaspermum repens* with capsules, a moss that tolerates freezing in Australia. Photo by David Tng, with permission.
Figure 12. *Goniumitrion acuminatum* subsp. *enerve* with capsules, a moss that tolerates freezing in Australia. Photo by David Tng, with permission.

Figure 13. *Rhizogonium spiniforme* with capsule, a moss that tolerates frost. Photo by Janice Glime.

Figure 14. *Takakia lepidozioides* in its native habitat in Japan. This moss species remains healthy through the Hokkaido winters. Photo from the Digital Herbarium of the University of Hiroshima, with permission.

Figure 15. *Takakia lepidozioides*, a winter survivor. Photo from the Digital Herbarium of University of Hiroshima, with permission.

Figure 16. *Sphagnum cristatum*, a New Zealand species that survives in winter there. Photo by Janice Glime.
Experiences with freezing in *Plagiomnium undulatum* (Figure 20) may help us to understand some of these differential responses (Hudson & Brustkern 1965). If this moss is cooled slowly, it experiences extracellular freezing; this prevents the intracellular freezing that could be fatal. Following that experience, the leaves can be cooled down to -30°C without injury. The slow freezing prevents the formation of extensive extracellular ice. Young shoots, however, cannot withstand temperatures below -12°C.

Among the thallose liverworts, *Lunularia* (Figure 21), *Pellia* (Figure 22), *Preissia* (Figure 23-Figure 24), *Riccardia* (Figure 25), *Riccia* (Figure 26), and *Marchantia polymorpha* (Figure 27), all survive frost (Fletcher 1982) and remain healthy. On the other hand, the thallose liverworts *Moerckia blyttii* (Figure 28-Figure 29), *Symphogyna* sp. (Figure 30), *Corsinia coreandra* (Figure 31-Figure 32), and *Asterella* (Figure 33) all can become severely bleached when subjected to frost. *Dumortiera hirsuta* (Figure 34-Figure 35) doesn't die, but it becomes blackened. Similarly, *Fossombronia* (Figure 36) and *Anthocerotophyta* experience decay, but for them the decay is a normal winter occurrence; growth resumes in the spring. In the greenhouse, which reaches -5.5°C, *Asterella* and *Monoclea forsteri* (Figure 37) are blackened by frost, whereas *Marchantia* spp, *Dumortiera hirsuta, Anthoceros punctatus* (Figure 38), and *Phaeoceros laevis* (Figure 39) remain healthy in the same greenhouse.
Figure 22. *Pellia endiviifolia* males with reddish antheridal cavities & females in center, a species that survives freezing. Photo by David Holyoak, with permission.

Figure 23. *Preissia quadrata* with archegoniophore, member of a genus that survives freezing. Photo by Michael Lüth, with permission.

Figure 24. *Preissia quadrata* thallus section showing several globose oil bodies that may help it to survive desiccation and freezing. Photo by Kristian Peters, with permission.

Figure 25. *Riccardia* sp, a thallus that survives freezing. Photo by Niels Klazenga, with permission.

Figure 26. *Riccia beyrichiana*, a genus that is able to survive frost – and desiccation. Photo by Jan-Peter Frahm, with permission.

Figure 27. *Marchantia polymorpha* with gemmae cups, a species that survives frost. Photo by Michael Lüth, with permission.
Figure 28. *Moerckia blyttii*, a liverwort that is sensitive to frost, becoming bleached. Photo by Michael Lüth, with permission.

Figure 29. *Moerckia blyttii* habitat. Photo by Michael Lüth, with permission.

Figure 30. *Symphyogyna podophylla*, a liverwort genus in which one species is sensitive to frost and becomes bleached. Photo by Andras Keszei, with permission.

Figure 31. *Corsinia coriandrina*, a thallose liverwort that is sensitive to frost under some conditions. Note bleached tissues, especially in the bottom center. Photo by Michael Lüth, with permission.

Figure 32. *Corsinia coriandrina* in its habitat on a ledge, a thallose liverwort that is sensitive to frost under some conditions. Photo by Michael Lüth, with permission.

Figure 33. *Asterella lindenbergiana*, a thallose liverwort that is sensitive to frost. Photo by Michael Lüth, with permission.
Figure 34. *Dumortiera hirsuta*, a thallose liverwort that is sensitive to frost and becomes blackened, but doesn't die. Photo by Michael Lüth, with permission.

Figure 35. *Dumortiera hirsuta* habitat. Photo by Michael Lüth, with permission.

Figure 36. *Fossombronia angustata*, a species in which frost causes decay, a normal winter occurrence. Note the patches of colorless plants. Photo by Michael Lüth, with permission.

Figure 37. *Monoclea forsteri*, a species that is blackened by frost. Photo by Jan-Peter Frahm, with permission.

Figure 38. *Anthoceros punctatus*, a species that survives to -5.5°C. Photo by Jonathan Sleath, with permission.

Figure 39. *Phaeoceros laevis* with capsules, a species that remains healthy to -5.5°C. Photo by Michael Lüth, with permission.

*Corsinia coreandra* (Figure 31-Figure 32) is a puzzle. It is a xerophyte, but in cultivation frost causes it to become bleached (Fletcher 1982). At the same time in the same garden as the cultivation containers, it remains healthy on an exposed wall top and likewise remains healthy in the greenhouse that goes down to -5.5°C.

Much of what we know about cold tolerance has come from Antarctic studies. The Antarctic continent has only
2% of its land free from ice (Seppelt & Ochyra 2008). These areas are dominated by bryophytes (24 species of mosses; 1 liverwort), lichens, and algae.

We have learned that macromolecular substances (ice-active substances or IASs) can modify the shape of the growing ice crystals (Raymond & Fritsen 2001). These semipurified substances from *Bryum* sp. (Figure 40) from the Antarctic contain both protein and carbohydrate. The substances lose most of their recrystallization ability by heat treatment. Raymond and Fritsen suggest that these substances might increase freezing tolerance by preventing ice recrystallization.

Figure 40. *Bryum pseudotriquetrum* in Antarctica, a species in which protein and carbohydrate might increase freezing tolerance by preventing ice recrystallization. Photo by Catherine Beard, with permission.

Some bryophytes thrive in habitats where they regularly get exposed to sub-zero temperatures. *Bryoxidium norvegicum* (Figure 41) is such a species (Shirasaki 1984). In Japan, *B. norvegicum* subsp. *japonicum* lives in an altitudinal range of 80-2350 m, being most abundant in districts where deep snow covers the ground for a long period. But it does not grow where the snow is, but rather grows on the vertical sides of overhanging rocks in ravines. Hence, it survives winter without the protection of snow, but it is sheltered by the rocks from the cold, desiccating winds.

Figure 41. *Bryoxidium norvegicum*, a species that grows on vertical surfaces where it is exposed to sub-zero temperatures without snow cover in winter. Photo by Bob Klips, with permission.

**Ice Crystals**

Ice crystals can cause plant tissues to dry out. Ice crystals are very hygroscopic and thus their presence can result in water being drawn out of tissues. But they also gather water from the atmosphere. Moffett *et al.* (2009) suggest that these ice crystals can sequester water that becomes available when they melt. Because bryophytes are able to absorb water through their leaves, this water can be immediately available and provide rapid rehydration.

Rod Seppelt (pers. comm. 7 April 2015) does not consider it to be unusual that *Ricciocarpos natans* (Figure 2-Figure 4) and *Riccia fluitans* (Figure 5) can be encased in ice or survive under a layer of snow. As he points out, temperatures within the ice are not typically very cold. As an example, he cites putting a pot of water 80 cm under the Alaskan snow overnight. The air temperature that night dipped to -22°C, but the pot of water remained unfrozen.

Of course dehydration caused by freezing can have other consequences. Dependence on the symbiont *Nostoc* is interrupted and nitrogen fixation is significantly reduced in winter due to dehydration resulting from freezing in the epiphytic leafy liverwort *Porella* (Figure 42-Figure 43) in Oregon, USA (De Gezelle 2003).

Figure 42. *Porella cordeana* in one of its vertical habitats where the symbiotic Cyanobacterium *Nostoc* provides it with needed nitrogen. Photo by Michael Lüth, with permission.

Figure 43. *Porella cordeana* on a vertical substrate, a species that suffers in winter from diminished nitrogen fixation by its symbiont. Photo by Michael Lüth, with permission.
Desiccating Conditions

If you have ever gone out in early spring in areas where there is snow cover all winter, bryophytes provide a refreshing green cover on the newly emergent ground. This fresh green color requires the presence of water to rehydrate the tissues. But where does it come from?

In many temperate regions, spring brings rain, hence making rehydration an easy task. But in some regions, my own home in the Keweenaw Peninsula of Michigan included, snowmelt is followed by drought, and this is exacerbated along roads by the sand and salt that was used to provide traction for vehicles during winter ice and snow.

Nevertheless, in northern habitats, snowmelt can provide water for a considerable time. In the Cairngorm Mountains, Scotland, *Kiaeria starkei* (Figure 44) is immediately ready for photosynthetic activity when its own snow cover disappears (Woolgrove & Woodin 1996). It has just spent its winter at temperatures of 0°C to slightly above, but with no light penetration while the snow depth is greater than 50 cm. When the snow disappears from it, its tissue chlorophyll recovers rapidly to 250% of its winter low and within two weeks its carbohydrate concentrations increase by 60%. This moss has nitrate reductase activity and is able to take advantage of pollutant nitrate, accumulated by the snow, that becomes available as the snow melts.

One advantage for bryophytes is that they have a low temperature compensation point. Hence, snowbed bryophytes such as *Anthelia juratzkana* (Figure 45-Figure 47) and *Polytrichastrum sexangulare* (Figure 48-Figure 49) can maintain photosynthesis at low temperatures with a lower temperature compensation point of about -4 to -5°C. Furthermore, *A. juratzkana* can survive in the dark under cold, wet conditions for nine months with no effect on its photosynthetic capability. This makes *A. juratzkana* well adapted to grow in the border zone along permanent snow patches. However, the net photosynthesis is reduced due to an increase in respiration rate. *Polytrichastrum sexangulare*, on the other hand, does not tolerate this border regime as well as does *A. juratzkana*.
In tracheophytes, freezing can cause ice to form within cells, potentially causing membrane damage and subsequent loss of cell constituents. Lenne et al. (2010) write "A dehydrating moss gathers no ice." Using the widespread moss *Ceratodon purpureus* (Figure 50-Figure 51), they demonstrated that no ice accumulates in the cells during freezing. But external ice does induce desiccation. The effects of this desiccation depend on the cell type. Water-filled hydroid cells *cavitate* like tracheophyte xylem cells, becoming *embolized* (blocked, in this case by ice) at -4°C. Parenchyma cells of the inner cortex of the stem exhibit *cytorrhysis* (complete and irreversible collapse of a plant cell wall due to loss of water through osmosis), losing 20% of their original volume at -20°C *nadir temperature* (lowest temperature of a cycle). It is puzzling that chlorophyll fluorescence shows no evidence of damage after thawing from a -20°C event, especially since the sugar concentrations are insufficient to confer freeze tolerance in these conditions (see below). Furthermore, ice nucleation occurs in hydrated tissues at ~12°C. The answer to this puzzle seems to lie in the desiccation itself. No damage occurs to those desiccated mosses at -20°C. The very desiccating nature of ice crystals appears to be the mechanism that prepares the moss for the low temperatures.

This desiccation relationship is supported in the Antarctic moss *Polytrichum juniperinum* (Figure 52) wherein repeated freeze-thaw cycles cause a greater reduction in photosynthesis than constant freezing for the same time period (Kennedy 1993). This is much like the effect of repeated dehydration/rehydration that causes a net carbon loss. This is supported by the observation that freeze-thaw cycles every 12 hours cause more damage than those every 24 or 48 hours. Most of the damage occurs during the first cycle with little occurring during subsequent cycles. Kennedy found that at 10°C the gross CO₂ flux is directly proportional to moss water content between 0.3 and 3.5 g g⁻¹ dry mass. Mosses with a low water content withstand freeze-thaw cycles to sub-zero temperatures better than do samples with a high water content. Kennedy suggests that on Signy Island in the Antarctic the populations of *Polytrichum juniperinum* may be limited in distribution by sub-zero temperatures and freeze-thaw cycles at times when snow cover is insufficient to provide insulation.
Davey (1997) examined Antarctic bryophytes and demonstrated the importance of water. The photosynthetic rate decreased as the length of the dehydration period increased in all bryophytes examined. The photosynthetic capacity is affected by stress, and Davey found that both desiccation and winter freezing caused a loss of photosynthetic capacity. But the base level of photosynthetic capacity is able to survive both. Furthermore, frequent dehydration and rehydration cycles cause a loss of photosynthetic rate that is greater than that in continuous dehydration. Davey hypothesized that water availability is an important contributor to the distribution of bryophytes in the Antarctic, where winter-like weather can occur on almost any day of the year.

Barker et al. (2005) found bleaching in *Syntrichia caninervis* (Figure 53) during winter in the Mojave Desert, USA. They attributed this loss of green color to frequent rain events during warmer months that year, citing appearance of chlorosis just after that. This is consistent with the effects of frequent dehydration-rehydration events seen by Davey (1997). Under this regime, particularly for short, light rainfall events, the plants do not have enough time to repair membranes before they become dehydrated again, thus losing energy with each mild rainfall event. This leaves them with diminished color for the winter, a condition hopefully to be repaired in the spring.

*Crossidium crassinervium* (Figure 54), from the Mojave Desert, benefits from late winter rain because it permits the moss to dry slowly (several days), whereas in summer the moss dries in as little as 3 hours (Stark 2005). The winter months of October to April constitute the hydrated period for this species, with hydration periods lasting 3.7-4.9 days.

Desiccation Tolerance

Desiccation tolerance is seasonal, probably in most bryophytes. Only the moss *Andreaea rothii* (Figure 55-Figure 56) failed to show seasonal variation in net assimilation following 24 hours of remoistening, compared to clear seasonal differences in the leafy liverwort *Plagiochila spinulosa* (Figure 57-Figure 58) and mosses *Hylocomium splendens* (Figure 59), *Scorpiurium circinatum* (Figure 60), *Syntichia ruralis* (Figure 61-Figure 62), and *Racomitrium aquaticum* (Figure 63-Figure 64) (Dilks & Proctor 1976). Those with seasonal variation usually had low desiccation tolerance in autumn and winter and greater tolerance in spring and summer. *Hylocomium splendens* differed in having relatively high tolerance in January (winter), with little change from then until July.
Figure 56. *Andreaea rothii* in a typical vertical rock habitat where snow does not accumulate. Photo by Michael Lüth, with permission.

Figure 57. *Plagiochila spinulosa* in a soil bank habitat where it exhibits seasonal differences in photosynthesis. Photo by Michael Lüth, with permission.

Figure 58. *Plagiochila spinulosa*, a leafy liverwort that has seasonal differences in its hydrated photosynthetic rate. Photo by Michael Lüth, with permission.

Figure 59. *Hylocomium splendens* with clinging snow, a moss that exhibits seasonal differences in photosynthesis. Photo by Michael Lüth, with permission.

Figure 60. *Scorpiurium circcinatum*, a moss that exhibits seasonal differences in photosynthesis. Photo by Michael Lüth, with permission.

Figure 61. *Syntrichia ruralis* habitat in a cliff splash zone. Photo courtesy of Betsy St Pierre.
Burch (2003) noted that some mosses are able to survive cryopreservation (preservation at low temperatures) with no prior treatment. She suggested that their natural desiccation tolerance already gave them adequate protection during cryopreservation. What is it about freezing that actually kills or damages the bryophytes? Crystals can damage the membranes, but isn't the real damage ultimately desiccation damage? For example, 90-100% of the protonemata of the desiccation-tolerant *Bryum rubens* (Figure 65) survived freezing, whereas only 30% of those encapsulated and 20% non-encapsulated *Ditrichum cornubicum* (Figure 66) protonemata, with limited desiccation tolerance, survived freezing. These two species each had slightly better survival numbers after 18 days of desiccation with no freezing. *Cyclodictyon laete-virens* (Figure 67), a desiccation-intolerant species, did not survive desiccation or freezing. In *D. cornubicum*, pretreatment with sucrose or ABA in the medium caused a reduction in growth rate of the protonemata, but these compounds resulted in a high level of protection against tissue damage in both dehydration and freezing – 100% regeneration of pretreated plants after thawing compared to 53% of controls (Burch & Wilkinson 2002). Sucrose plus ABA gave the best results.
Syntrichia ruralis (Figure 62) is one of the model organisms for studying desiccation tolerance. When subject to slow freezing at 3°C decrease in temperature per hour to -30°C, hydrated Syntrichia ruralis suffers only temporary metabolic changes, and these are reversible (Malek & Bewley 1978). Malek and Bewley attributed the changes to desiccation tolerance resulting from extracellular ice formation. When this same moss is subject to rapid freezing in liquid nitrogen and rapid thawing in 20°C water, all aspects of its metabolism deteriorate. Ribosomes, proteins, and ATP levels decrease and protein synthesis activity is rapidly lost. Malek and Bewley suggest that these problems are the result of intracellular ice crystals. Changing the freezing rate to 60°C per hour – a slower rate than in liquid N, but still a rapid rate – only reduces the levels of ATP and protein synthesis. The polyribosomes (protein-synthesizing apparatus) remain intact and active 24 hours after the freeze-thaw cycle. Segreto et al. (2010) reported that all species cryopreserved in situ regenerated mostly through budding; the number of regenerating samples correlates positively to desiccation tolerance and show higher frost tolerance than previously thought. Herbarium samples up to 7 years old of the most desiccation-tolerant species regenerate by protonemata; shoot tips regenerate better than small plant fragments.

Desiccation tolerance can be an antagonistic (one species benefits at the expense of another) interaction. Sphagnum fuscum (Figure 68) and Dicranum elongatum (Figure 69) dominate a subarctic mire (Sonesson et al. 2002). In winter, Sphagnum fuscum growth increased when Dicranum elongatum was its immediate neighbor, but D. elongatum grew better when it grew with other members of its own species. Neither increased temperature nor UV-B radiation affected these relationships, implying that moisture relations were probably important.

Ice-nucleating Proteins

Ice-nucleating proteins can help to create desiccating conditions and prevent cell freezing. These proteins are small structures that become surrounded by ice, but the water does not crystallize. The principle has been used by orange growers to prevent desiccation of the fruits during winter freezing events. Small nucleating bacteria are able to accomplish desiccation protection by out-competing the larger nucleating bacteria. These small species are sprayed on oranges to protect them. Such proteins or bacteria form centers for ice formation on the outsides of cells, providing a protective covering (Zachariassen & Kristiansen 2000).

Clouds use nucleation centers to create their precipitation (Ahern et al. 2007). Bacteria have been known from clouds for a long time. Clouds may be an ideal habitat for these bacteria to live and thrive. Ahern and coworkers found 100 OTUs (operational taxonomic units – used when species cannot be named) among 256 clones from clouds. Half of these were identified as bacteria from psychrophilic terrestrial habitats (habitats where low-temperature-tolerant organisms can live). Among these bacteria, a mix of fluorescent Pseudomonas species dominate and some are known ice nucleators. But none of the cultures demonstrated the ice-nucleation gene. Rather, 55% of the isolates from cloud and rain samples had significant biosurfactant activity. Surfactants influence droplet size and are important in lowering the critical supersaturations necessary for activating aerosols into cloud condensation nuclei. Such bacteria facilitate water scavenging and counteract desiccation. Could they perform such functions in some bryophytes?
In *Sphagnum capillifolium* (Figure 70-Figure 71) the chlorophyllous (containing chlorophyll) cells exhibit extended freezing cytorrhysis immediately after ice nucleation at -1.1°C in water (Buchner & Neuner 2010). This cytorrhysis is exhibited as cell shrinkage that appears within only 2 seconds. And the shrinkage is significant – 82%, with chloroplast diameter reduction from 8.9 to 3.8 μm. This is accompanied by a sudden rise in chlorophyll fluorescence. On the other hand, frost damage occurs at a much lower temperature (LT50 at -16.1°C) (LT50 = median time until death after exposure of organism to toxic substance or stressful condition). The ice-nucleation temperature of -1.1°C is likewise the temperature threshold of PS II. Surprisingly, the LT50 for freezing in *S. capillifolium* is higher than that in most tracheophytes in the European Alps in the summer.

**Atmospheric Source**

One big question in this story is the source of the nucleating proteins. Until recently, bacteria seemed to be the only organic source of nucleating proteins (Möhler *et al*. 2008). But only a few bacteria, the pseudomonads, seem capable of this role (Lindow 1983; Ahern *et al*. 2007). This notion has been challenged by the research of Kieft and coworkers (Kieft 1988; Kieft & Ahmadjian 1989; Kieft & Ruscetti 1990) and more recently by Moffett *et al*. (2009).

Bauer *et al*. (2002) supported their challenge and reported that both bacteria and fungal spores contribute to the organic content of cloud water. In fact, the fungal spores in clouds of the Austrian Alps contribute 1.5% of the organic content, whereas the bacteria contribute only 0.01%. Although Pouleur and coworkers did not discuss the roles of these groups in nucleation, their study (Pouleur *et al*. 1992) suggests that slime molds might also provide nucleating proteins.

**Hyphomycetous fungi** (*Fusarium* spp.; Figure 72) were also added to the list of organisms providing nucleating proteins to clouds (Pouleur *et al*. 1992). We also know that the fungal partner of at least some lichens contribute nucleating proteins (Kieft 1988; Kieft & Ahmadjian 1989; Kieft & Ruscetti 1990) and that the *Fusarium* proteins are more similar to those of lichens than to those of bacteria (Pouleur *et al*. 1992).

Despres *et al*. (2007) determined aerosol particles in the air by using DNA sequencing. They found that most of the bacteria were **Proteobacteria**, with some **Actinobacteria** and **Firmicutes**. Fungal DNA came from **Ascomycota** and **Basidiomycota**, most likely from spores. Two different DNA sequences came from moss spores.

Christner *et al*. (2008) reported that ice nucleators are widespread in snowfall and the most active ones are biological. Most of these are bacteria. Many of these nucleators, therefore, are likely to be added to the mosses during snowfall and may contribute their survival of low temperatures and winter desiccation.

Fukuta (1966) found that more than 20 organic compounds out of 329 were able to nucleate ice at temperatures >-5°C. Hence, it is possible that even pollutants may contribute to nucleation of water on bryophytes.
Nucleating Proteins in Lichens

Our knowledge of lichens may help us to understand the nucleation relationship in bryophytes. Like bryophytes, lichens are able to survive year round and renew their photosynthetic activity when suitable temperatures and hydration resume. Perhaps the response of lichens can give us some insight into moss behavior relative to nucleation. Most of the epilithic (rock-dwelling) lichens (Rhizoplaca, Xanthoparmelia, and Xanthoria) tested by Kieft (1988) had ice nucleation at temperatures above -8°C, whereas their substrates showed negligible nucleation above that temperature. The nucleation activity in the lichen appears to be non-biological. No nucleation-active bacteria could be isolated, and the activity did not cease when the lichen was heated to 70°C or subjected to sonication. An axenic culture of the fungal part of the lichen Rhizoplaca chrysoleuca showed nucleation activity at -1.9°C. Kieft hypothesized that these frost-tolerant lichens benefit from increased moisture deposition that results from ice nucleation.

Henderson-Begg et al. (2009) remind us that for water to freeze above -36.5°C requires the activity of an ice nucleator. Bacteria are the best known of these, inducing freezing at temperatures up to -1.8°C, but seem to be of little importance in the lichens. The nucleators are common in lichens and can become airborne. Many of these are non-bacterial, but are biological, probably fungal and lichen.

There are several studies that support the presence of lichen fragments in the atmosphere (Tormo et al. 2001; Ahern et al. 2007). Marshall (1996) demonstrated that lichen soredia (asexual reproductive structures) were the most abundant of the airborne propagules of lichens, with peaks occurring after the winter snowmelt while subzero temperatures continued.

Kieft and Ahmadjian (1989) found that of 14 species of mycobionts (fungal partners) in lichens, five have nuclei active at -5°C. However none of the 13 photobionts (algae & Cyanobacteria) have ice-nucleating activity at -5°C or warmer. Hence, the ice-nucleating nuclei are produced by the fungal partner of the lichen. Kieft and Ahmadjian suggested that these ice-nucleating proteins are involved in moisture uptake and frost protection.

Kieft and Ruscetti (1990) found that biological ice nuclei in the lichen Rhizoplaca chrysoleuca were active at ~-4°C. Their sensitivity to various substances indicated that they were proteinaceous, and they were relatively heat stable and active without lipids, demonstrating that they were significantly different from bacterial ice nuclei.

Nucleating Proteins as a Source of Water

Lindow (1983) found that ice-nucleation activity occurs primarily in the outer membrane of the cells of Pseudomonas syringae and Escherichia coli into which it has been inserted. It does not occur in soluble components of these cells. The ability of the ice-nucleating bacteria to operate depends on incubation temperature, growth medium composition, culture age, and genotype (Lindow et al. 1982). Their optimum conditions for nucleation in culture occur on nutrient agar containing glycerol at 20-24°C. Their ability to mitigate ice injury on corn seedlings depends on the bacterial population size and the number of ice nuclei active at that temperature.

Compounds for Winter?

Bryophytes produce record numbers of secondary compounds. These are best known for their antibiotic effects, but they can also play a role in both drought tolerance and freezing survival (Xie & Lou 2009). Among these, bibenzyls and bis(bibenzyls) have desiccation tolerance activity; phenylpropanoids have freeze tolerance activity. But the nature of these activities is unknown.

We know from several studies that the proportions of various fatty acids change with temperature (Saruwatari et al. 1999). Among these, linolenic acid and eicosapentaenoic acid might increase freezing-tolerance, as suggested by Marchantia polymorpha (Figure 27). Xie and Lou (2009) likewise reported the freeze tolerance activity of fatty acid derivatives in bryophytes.

Sugars

Sugar concentrations have a role in frost tolerance. Sucrose can increase the ability of bryophytes to tolerate rapid drying (Stark & Brinda 2015). Among the bryophytes tested by Rütten and Santarius (1992), only Mnium hornum (Figure 73-Figure 74) among seven Bryidae and one of Marchantididae lack an increase in sucrose concentration concomitant with an increase in frost hardiness. Insignificant changes in glucose and fructose contents accompany these frost hardiness events.

Figure 73. Mnium hornum forest floor habitat. Photo by Michael Lüth, with permission.

Brachythecium rutabulum (Figure 75-Figure 76) and Hypnum cupressiforme (Figure 77-Figure 78) have high sucrose concentrations in summer, similar to those of other species in winter, and thus are frost tolerant even in summer (Stark & Brinda 2015). Those mosses that are highly frost-resistant have a total sugar concentration of ~90-140 mM. Of this sugar, 80-90% is sucrose. Artificial degradation of the sucrose during higher temperatures causes a decline in cold hardiness, supporting the hypothesis that it is important in frost hardiness in these species.
Some species [*Polytrichastrum formosum* (Figure 79-Figure 81), *Atrichum undulatum* (Figure 82), *Plagiomnium affine* (Figure 83-Figure 84), *Mnium hornum* (Figure 73-Figure 74), *Pellia epiphylla* (Figure 85-Figure 86)] exhibit a distinct increase in cold tolerance from summer to winter (Rütten & Santarius 1992). Mosses have significant differences in frost resistance between summer and winter (15->25°C), but the thallose liverwort *Pellia epiphylla* experiences relatively little winter hardening capacity.
Figure 80. *Polytrichastrum formosum* with frost, a species that has a distinct increase in cold tolerance from summer to winter. Photo by Aimon Niklasson, with permission.

Figure 81. *Polytrichastrum formosum* leaf lamellae. The role of lamellae in frost protection is unknown. Photo by Michael Lüth, with permission.

Figure 82. *Atrichum undulatum*, a species that has a distinct increase in cold tolerance from summer to winter. Photo by David T. Holyoak, with permission.

Figure 83. *Plagiomnium affine* forest floor habitat. Photo by Michael Lüth, with permission.

Figure 84. *Plagiomnium affine*, a species that has a distinct increase in cold tolerance from summer to winter. Photo by Janice Glime.

Figure 85. *Pellia epiphylla* protected habitat under grass bank of flush in Wales. Photo by Janice Glime.
Melick and Seppelt (1994) suggest that the lack of significant changes in soluble carbohydrates in Antarctic bryophytes may result from the extreme climate and the rapid temperature fluctuations during the growing season. On the other hand, maximum water content is present in the summer. Chlorophyll levels decrease in winter in both total chlorophyll and the chlorophyll $a:b$ ratio, as do the total carotenoids. This decrease may be a response to low light levels that are insufficient for making more pigment.

Using the Physcomitrella patens (Figure 87) protonema as a model organism, Nagao et al. (2003, 2005) concluded that ABA-induced soluble sugars play a role in freezing tolerance. The accumulation of the sugars, at the expense of starches, is associated with morphological changes in the organelles and reduce freezing-induced structural damage to the plasma membrane, while the freezing tolerance of the protonemal cells increases. Nagao et al. (2006) identified the sugar as theanderose, a sucrose that occurs in close association with ABA treatment that enhances freezing tolerance. Cycloheximide inhibits the accumulation of theanderose, resulting in a marked decrease in freezing tolerance. The accumulation of theanderose is promoted during cold acclimation and treatment with hyperosmotic solutes, both of which increase cellular freezing tolerance.

Using the bryological lab rat Physcomitrella patens (Figure 87), Oldenhof et al. (2006) demonstrated that sucrose helps to protect cells during freezing and drying, but accumulation of sucrose alone is not sufficient for survival. ABA serves to cause this sucrose accumulation, up to 22% of dry weight, but only 3.7% occurs in non-ABA-treated tissues. A combination of ABA treatment and the cryoprotectant DMSO permit the tissues to survive a freeze-thaw cycle down to -80°C. DMSO-mediated changes involved in the membranes are important and may be relevant to the essential desiccation tolerance.

Polyols may contribute to cold hardiness as well. Tearle (1987) found that Antarctic lichens contained up to three times the amount of polyols when compared to temperate lichens, endowing them with extra freezing protection. The soluble sugars and polyols from mosses and lichens leach into the fellfield soils in the spring.

ABA

ABA is the stress hormone, and it plays a role in freezing tolerance of plants as well (Minami et al. 2003; Takezawa et al. 2011). Nevertheless, slow freezing of the protonemata of Physcomitrella patens to -4°C under normal growth conditions kills more than 90% of the cells. Application of ABA for 24 hours causes a marked increase in the freezing tolerance (see also Nagao et al. 2001, 2005, 2006). Cold treatment only slightly increases the freezing tolerance within the same period. Treatment with ABA causes a marked increase in expression of all the PPAR genes within 24 hours. Several of these genes also respond to cold, but much more slowly than they respond to ABA. Treatment with hyper-osmotic concentrations of NaCl and mannitol also increases the expression levels of eleven PPAR genes and the freezing tolerance of the protonemata. Minami and coworkers (2003) suggest that these relationships indicate that stresses increase the expression of genes that result in protection of the protonemata, but the nature of that relationship is unclear.

Nevertheless, in Physcomitrella patens (Figure 87) protonemata, as in tracheophytes, freezing tolerance increases following incubation at low temperatures in the range of 0-10°C, indicating the importance of acclimation (Minami et al. 2005). This tolerance is accompanied by an accumulation of several transcripts for late-embryogenesis-abundant (LEA) proteins and boiling-soluble proteins. De-acclimation causes reduction in expression of these proteins and loss of freezing tolerance. But surprisingly, unlike events in tracheophytes, in P. patens low-temperature-induced freezing tolerance does not coincide with an increase in endogenous ABA, despite increases in expression of stress-related genes. In short, the acclimation is somewhat different from that of tracheophytes.

These observations are further confounded by the experiments of Minami et al. (2003) on Physcomitrella patens (Figure 87). They found that treatment with ABA for 24 hours greatly increases the freezing tolerance of the protonemata; cold treatment alone has only a slight effect on freezing tolerance. Even slow freezing to -4°C kills more than 90% of the cells. On the other hand, hyperosmotic concentrations of NaCl and mannitol increase freezing tolerance of protonemata.
At the same time, research by Takezawa and Minami (2004) identified genes coding for membrane transporter-like proteins. These newly identified proteins increase considerably following treatment with low temperatures, hyperosmotic solutes, or ABA. These genes are regulated by calmodulin.

**Arachidonic Acid**

Prins (1982) suggested that one reason small mammals eat mosses in winter is the content of arachidonic acids. These fatty acids make membranes more pliable and may make it easier for these rodents to run around on frozen ground and snow. But what do these do for bryophytes in winter? Does this extra flexibility also make it easier for them to survive? One protection against freezing is the ability to lose water, avoiding crystal formation that could damage membranes and organelles. With flexible membranes and withdrawal of water, the cells could shrink within the walls during the cold (and dry) period.

In *Physcomitrella patens* (Figure 87), production of arachidonic acid increases with higher concentrations of sugar (Chodok *et al.* 2010). Al-Hasan (1989) found that in *Bryum bicolor* (Figure 88) more arachidonic acid is produced at 5°C than at 25°C. Both of these studies support the production of arachidonic acid as winter approaches.

**Polyribosomes**

Polyribosomes (cluster of ribosomes connected by a strand of messenger RNA and active in protein synthesis) respond to cooling temperatures. In the xerophytic moss *Syntrichia ruralis* (Figure 61-Figure 62), when temperatures descend to 2°C an accumulation of polyribosomes occurs while the single ribosomes decrease (Malek & Bewley 1978). This change in numbers reflects rearrangement, but does not involve a change in the number of ribosomal units. Slowly dried *S. ruralis* does not contain any polyribosomes when rehydrated, but these reform at 2, 8, and 20°C. Leucine rapidly incorporates into the protein when the plants are rehydrated at 20°C, but its incorporation is less dramatic at 2°C. Cold-hardened *S. ruralis* has no changes in the rate of protein synthesis at low temperatures (2°C). In fact, even in summer this species can carry out protein synthesis at low temperatures.

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**Summary**

One of the dangers of frost damage is desiccation. Ice crystals on the inside of cells damage membranes and those on the outside pull water from the cells. Some bryophytes are protected by being encased in ice, preventing the formation of crystals and insulating against severe cold. Absence of vacuoles or having only small vacuoles can help to protect the interior of cells.

Many species survive winter and are ready for photosynthesis when the snow disappears, using the snowmelt water to rehydrate their tissues. Slow cooling, like slow drying may be important in survival. Extracellular freezing can protect against intracellular freezing. Some macromolecular substances can modify the shape of ice crystals in ways that do not damage the cells. Some ice-nucleating structures, made by the plants or available from the atmosphere, including proteins, create a small crystalline structure likewise protecting against damage from larger crystals. On the other hand, some ice crystals on the outsides of the cells can sequester water that is available at suitable temperatures. Desiccation can protect the cells by preventing crystal formation. Cell shrinkage helps to prevent crystal formation. Frequent freeze-thaw cycles, like dehydration-rehydration cycles, can damage the cells if the hydration and photosynthetic period is insufficient to repair membranes and accomplish a carbon gain.

Polyribosomes are active immediately following the freeze-thaw cycle. Lipids, starch bodies, sucrose, ABA, bibenzyls, bis(bibenzyls), and phenylpropanoids help to increase freezing and desiccation tolerance. Arachidonic acid helps to make membranes more pliable. These compounds permit some bryophytes to have seasonal tolerance. In desert habitats winter is often the best growing season because mosses remain hydrated for several days following rainfall events.

Some species become bleached from frost damage, but shoot tips and other parts may remain healthy and provide new growth in spring.

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# CHAPTER 7-10
## WATER RELATIONS: SNOW ECOLOGY

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CHAPTER 7-10
WATER RELATIONS: SNOW ECOLOGY

Figure 1. Late snowbeds in an alpine habitat in the Khibiny Mountains, Russia. Bucklandiella microcarpum is in the foreground. Photo by Michael Lüth, with permission.

Snow Effects

Snow can contribute in multiple ways to enhance the productivity. We know little about productivity of bryophytes under snow. What we do know is that light can penetrate snow, but that the light quality is altered. We know that snowmelt provides moisture, and that this melt can occur while the snow pack is still present, even in the middle of winter. We know that the snow can buffer the temperature, maintaining it close to 0°C. Dorrepall et al. (2004) demonstrated that Sphagnum fuscum (Figure 2) experienced an enhancement of 33% in productivity as a result of winter snow addition, while, nevertheless, not increasing growth in length.

Even in areas with considerable snow, bare areas exist, often as a result of winds that clear the snow. Some of these surfaces are rock surfaces that protrude, making them vulnerable to those wind movements of the snow (John 1990). One moss species capable of living in such exposed areas is Grimmia longirostris (Figure 3) in the Canadian Rocky Mountains.

Figure 2. Sphagnum fuscum, a moss that experiences greater productivity as a result of winter snow. Photo by Julita Kluša <daba.dziedava.lv>, with online permission.
I have long suspected that at least some bryophytes are able to carry out photosynthesis under snow as long as it is not too deep for sufficient light penetration. Pannewitz et al. (2003) demonstrate that photosynthesis under the snow occurs in lichens. The snow cover provides effective insulation against the bitter cold of the Antarctic atmosphere, protecting both the mosses and the lichens. But in spring, this insulation proves to be detrimental. It retains the severe cold of winter and prevents the bryophytes from benefiting from the early warming of the air. This delay can last 10-14 days. Furthermore, the hydration provided to the lichens by the snow lasts only briefly once the snow disappears, providing only a brief period for photosynthetic activity. Is this same shortening of the photosynthetic period in effect for bryophytes, or are they able to retain the water longer?

In our study of the bryophytes on trees in the Keweenaw Peninsula of Michigan, USA, we found that those about 1 m above the ground were most common on the south sides of the trees (Trynoski & Glime 1982). We attributed this to a combination of winds from the north and sufficient light and moisture for these bryophytes to have photosynthesis in winter. Trees always have a narrow funnel of space between them and the snow (Figure 4). In this area where snow on the ground reaches a meter or move depth, the snow is an insulator. Dark-colored bark is able to absorb heat and the funnel remains somewhat humid. Light is able to penetrate. I have no measurements of growth or photosynthetic activity for these epiphytes – that needs to be done.

Snow can affect the distribution of species. For example, in Japan Bazzania trilobata (Figure 5-Figure 7) grows on ground that is well drained all year and is typically sunny (Shirasaki 1987). Bazzania yoshinagana (Figure 8), on the other hand, grows on the forest floor in densely shaded coniferous forests. It spends its winter covered with deep snow that insulates it from freezing and provides it with moisture.

Figure 3. Grimmia longirostris, a species of exposed areas. Photo by Michael Lüth, with permission.

Figure 5. Bazzania trilobata habitat where it lives in well-drained locations. Photo by Dick Haaksma, with permission.

Figure 6. Bazzania trilobata, a species of well-drained locations. Photo by Michael Lüth, with permission.

Figure 7. Bazzania trilobata leaf cells showing spherical oil bodies that may help in surviving desiccation. Photo by Walter Obermayer, with permission.
**Snowbed Communities**

"Areas which experience prolonged snowlie and possess a distinctive bryophyte-dominated vegetation are termed snowbeds" (Woolgrave & Woodin 1996). Snowbeds (Figure 9) create their own unique characters. They shorten the growing season but can extend the period of hydration. Some bryophytes are dependent on these sources of hydration. On the Antarctic peninsulas the snow cover and site exposure seem to define the plant distribution (Melick et al. 1994).

Many of the alpine bryophytes display adaptations that make their snowbed habitats tenable. *Andreaea nivalis* (Figure 11-Figure 12) is a reddish moss that hangs where snow water glides over the rocks and cliffs (Bailey 1933). Even its name (*nivalis*) means snow-covered. *Brachymenium erectum* (Figure 13) grows in snow water in alpine areas and disintegrates so rapidly after maturity that it is easy to miss it altogether. *Pohlia ludwigi* (Figure 14-Figure 15) lives in or near snow water. *Pohlia filum* (Figure 16) grows in snow water on the south side of Mount Rainier, Washington, USA, and produces brood bodies (Figure 17) in the snow water. *Polytrichastrum sexangulare* (Figure 18-Figure 20) likewise lives near the snow. Its setae begin to elongate before the snow is completely gone and as a result they become trailing and twisted (Figure 20). *Bryum muehlenbeckii* (Figure 21) has deep red leaves and stems, probably protecting it from UV radiation, and possibly increasing its temperature in its cold habitat near the snowbeds. *Pohlia wahlenbergii* var. *glacialis* (Figure 22-Figure 23), by contrast, has whitish leaves. On Mt. Rainier it covers large expanses that are wet with snow water. *Meiotrichum lyallii* (Figure 24) becomes visible at high elevations as soon as the snow disappears and is common on the higher slopes of Mt. Rainier. All of these bryophytes are acrocarpous mosses. Only *Isopterygiopsis pulchella* (Figure 25) is a pleurocarpous snow lover associated with these snowbeds. In all cases, it is likely that these bryophytes are non-competitors with tracheophytes and that take advantage of their C3 photosynthesis to grow in the cold temperatures when adequate moisture is available.
Figure 12. *Andreaea nivalis* in a location where it receives water that glides over rocks and cliffs. Photo by Michael Lüth, with permission.

Figure 13. *Brachymenium* in India. *Brachymenium erectum* is short-lived in alpine snowbed runoff. Photo by Michael Lüth, with permission.

Figure 14. *Pohlia ludwigii* in its late snowmelt water habitat. Photo by Michael Lüth, with permission.

Figure 15. *Pohlia ludwigii*, a moss that thrives in snowmelt water. Photo by Michael Lüth, with permission.

Figure 16. *Pohlia filum* growing in wet soil from snowmelt. Photo by Michael Lüth, with permission.

Figure 17. *Pohlia filum* showing the bulbils that are produced while it grows in snowmelt water. Photo by Michael Lüth, with permission.
Figure 18. *Polytrichum sexangulare* at alpine lake in Europe. Photo by Michael Lüth, with permission.

Figure 19. *Polytrichastrum sexangulare*, a late snowbed bryophyte. Photo by Martin Hutten, with permission.

Figure 20. *Polytrichastrum sexangulare* showing crooked and twisted setae from developing under snow. Photo by Michael Lüth, with permission.

Figure 21. *Bryum muehlenbeckii* in snowmelt water on rock. Note the red color, a common character of alpine bryophytes. Photo by Michael Lüth, with permission.

Figure 22. *Pohlia wahlenbergii* var. *glacialis* in its snowmelt habitat. Photo by Michael Lüth, with permission.

Figure 23. *Pohlia wahlenbergii* var. *glacialis*, a species that thrives in snow water. Photo by Michael Lüth, with permission.
In Scotland, some of these same species are dependent on the snowbeds (Rothero 2007). These include *Andreaea nivalis* (Figure 11-Figure 12), *Polytrichum sexangulare* (Figure 18-Figure 20), *Kiaeria falcata* (Figure 26-Figure 27), *Anthelia juratzkana* (Figure 10), and *Pohlia ludwigii* (Figure 14-Figure 15). Additionally, snowbeds in the UK are habitats for *Racomitrium heterostichum* (Figure 28), *Marsupella brevissima* (Figure 29-Figure 30), *Kiaeria starkei* (Figure 31), *Moerckia blyttii* (Figure 32), *Pleurocladula albescens* (Figure 33-Figure 34), *Marsupella arctica*, and *Marsupella condensata* (Figure 35). The flushes and mires resulting from melting snowbeds also support growths of *Pohlia wahlenbergii* var. *glacialis* (Figure 22-Figure 23), *Scapania paludosa* (Figure 36), and occasionally *Sphagnum riparium* (Figure 37) and *S. lindbergii* (Figure 38-Figure 39).
Figure 29. *Marsupella brevissima* habitat. Photo by Michael Lüth, with permission.

Figure 30. *Marsupella brevissima*, a snowbed liverwort. Photo by Jan-Peter Frahm, with permission.

Figure 31. *Kiaeria starkei* with capsules, a late snowbed moss. Photo by Rosemary Taylor, with permission.

Figure 32. *Moerckia blyttii*, a snowbed bryophyte. Photo by Michael Lüth, with permission.

Figure 33. *Pleuroclada albescens* in a snowmelt bed in Norway. Photo by Michael Lüth, with permission.

Figure 34. *Pleuroclada albescens*, a snowbed liverwort. Photo by Michael Lüth, with permission.

Figure 35. *Marsupella condensata*, a species that lives in snowbeds in the UK. Photo by Andrew Hodgson, with permission.
In snowbeds, more than 40% of the cover is often comprised of bryophytes (Jägerbrand 2011). This may be as little as 3% of the exposed area during early melt to 80% in the late-melting areas. Björk (2007) found 26 species that grow mostly in snowbeds, 13 of which are found only in those areas. In a late snowbed in western Newfoundland, Canada, Belland (1983) found some of the same species associations as named above for the UK. In particular, *Andreaea nivalis* (Figure 11-Figure 12) was common in late snowbeds. Belland found 49 bryophyte species in the eight late snowbeds he investigated. Other dominant species included *Kiaeria falcata* (Figure 26-Figure 27), *Moerckia blytti* (Figure 32), and a species of *Trematodon* (Figure 40). The uniqueness of this habitat is demonstrated by the disjunct distribution for 13 of these species between western and eastern North America. Eleven of the species are characteristic of snowbed habitats throughout most of the world.

The unique combination of temperature regime and moisture support some of the rare species of the world. On Mt. Washington, New Hampshire, USA, Slack et al. (2013) found *Haplomitrium hookeri* (Figure 41), *Aulacomnium turgidum* (Figure 42-Figure 44), *Dicranum elongatum* (Figure 43), and *Pseudocalicium trifarium* (Figure 45) – all rare species in the northeastern USA.
Growth Form Variability

Snowbeds can create unusual growth forms. For example, in the high Arctic of Spitsbergen, the fellfield type of *Sanionia uncinata* (Figure 46-Figure 48) had few branchless shoots and formed dense colonies, leading to better desiccation avoidance (Ueno et al. 2001). The snowbed type had many branchless shoots and more sparse colonies. Furthermore, there were fewer branches in the upper part of the shoot than in the lower part in the snowbeds. Was this a response to the snow, possibly damaging branch buds, or was the more dense branching in the fellfield an adaptation selected to permit survival in the drier climate there?
or possibly due to growth in the reduced light under snow. *Ceratodon purpureus* (Figure 55-Figure 60) lives in shallow lakes in the Antarctic and was originally known as *Ceratodon minutifolius* there, differing in leaf shape and leaf apex (Horikawa & Ando 1963; Seppelt & Selkirk 1984).

The multiple growth forms of some species in the Antarctic seem to be a response to submersion (Seppelt & Selkirk 1984). For example, *Bryum pseudotriquetrum* (Figure 49-Figure 50) was originally named as *Bryum algens* there due to its different growth form. *Calliergon sarmentosum* (Figure 51-Figure 52) assumes a different morphology when shoots develop under water or in damp conditions (Priddle 1979). *Bryum argenteum* (Figure 53-Figure 54) in the Antarctic assumes longer and narrower leaves with increased cell size in etiolated shoots (Longton 1981; Seppelt & Selkirk 1984), perhaps due to submersion,
Figure 52. *Calliergon sarmentosum* aquatic growth form. Photo by Michael Lüth, with permission.

Figure 53. *Bryum argenteum* from the Neotropics, exhibiting the broader leaves typical there. Photo by Michael Lüth, with permission.

Figure 54. *Bryum argenteum* from alpine area in Europe where it exhibits longer, narrower leaves than plants from the tropics. Photo by Michael Lüth, with permission.

Figure 55. *Ceratodon purpureus* in Antarctica. Photo by Rod Seppelt, with permission.

Figure 56. *Ceratodon purpureus* in Antarctica. (Blackish mosses at right are *Bryum pseudotriquetrum*.) Photo by Rod Seppelt, with permission.

Figure 57. Submerged *Ceratodon purpureus* in the Antarctic. Bubbles from photosynthesis here create a condition known as *pearling*. Photo courtesy of Rod Seppelt.
Chapt. 7-10. Water Relations: Snow Ecology

Duration of Snowbeds

The duration of the snowbeds separates communities in Scotland (Woolgrove & Woodin 1994). Prolonged snowlie has negative effects on the Marsupella-Anthelia community (Figure 29-Figure 30, Figure 35; Figure 10). On the other hand, the Polytrichum-Kiaeria (Figure 18-Figure 20; Figure 26-Figure 27) community is positively affected by its prolongation. Pohlia (Figure 14-Figure 16, Figure 22-Figure 23) seems less affected by the duration, but the substrate moisture content is important for it.

Snowmelt

Kaiser (1921) describes his "journey into mossland" during a February thaw in Pennsylvania, USA. So many mosses appeared, bright green, and ready to grow. These winter survivors, especially along streambanks, included Plagiomnium cuspidatum (Figure 61-Figure 62), Leucobryum glaucum (Figure 63-Figure 67), Bryophyllum illecebra (Figure 68-Figure 69), Dicranum scoparium (Figure 70-Figure 71), Plagiomnium ciliare (Figure 72), Rhizomnium punctatum (Figure 73), Conocephalum conicum (Figure 74), Marchantia polymorpha (Figure 75), Pellia epiphylla (Figure 76), Atrichum (Figure 77), Dicranella (Figure 78), Pohlia nutans (Figure 79-Figure 80), and Bartramia pomiformis (Figure 81-Figure 82), among others, all benefitting from the snowmelt moisture.
Figure 63. *Leucobryum glaucum* on edge of crevice where it escapes the leaf litter. This site benefits from runoff, but can also suffer exposure. Photo by Janice Glime.

Figure 64. *Leucobryum glaucum*, one of the mosses that is ready to photosynthesize when the snow melts. Photo courtesy of Eileen Dumire.

Figure 65. *Leucobryum glaucum* showing the whitish color due to hyaline cells that help to keep the photosynthetic cells hydrated. Photo by Janice Glime.

Figure 66. *Leucobryum glaucum* showing photosynthetic and hyaline leaf cells. Photo by David Wagner, with permission.

Figure 67. *Leucobryum glaucum* leaf cross section showing photosynthetic and hyaline cells. Photo by Walter Obermayer, with permission.

Figure 68. *Bryoandersonia illecebra*, a moss that one can find when the snow melts in Ohio, USA. Photo by Bob Klips, with permission.
Figure 69. *Bryandersonia illecebra* on tree, a species of vertical surfaces. Photo by Bob Klips, with permission.

Figure 70. *Dicranum scoparium* in early autumn, a moss that overwinters and looks bright when the snow melts. Photo by Janice Glime.

Figure 71. *Dicranum scoparium* with capsules. Photo by Michael Lüth, with permission.

Figure 72. *Plagiomnium ciliare* with antheridia, a moss that overwinters and is ready to grow when the snow leaves. Photo by Robert Klips, with permission.

Figure 73. *Rhizomnium punctatum* looking etiolated after its winter snow cover. Photo by Michael Lüth, with permission.

Figure 74. *Conocephalum conicum*, a liverwort that is active in early spring. Photo by Janice Glime.
Figure 75. Marchantia polymorpha with gemmae cups, a liverwort that is active when the snow melts. Photo by David T. Holyoak, with permission.

Figure 76. Pellia epiphylla in the mountains of Wales. Photo by Janice Glime.

Figure 77. Atrichum undulatum in snow, a species that has a distinct increase in cold tolerance from summer to winter and is ready for photosynthesis when the snow melts. Photo by Michael Lüth, with permission.

Figure 78. Dicranella heteromalla, a soil bank moss that is ready to grow when the snow melts. Photo by Michael Lüth, with permission.

Figure 79. Pohlia nutans at snowmelt time in the Khibiny Mountains, Russia. Photo by Michael Lüth, with permission.

Figure 80. Pohlia nutans, one of the first plants to be seen in spring. Photo by Michael Lüth, with permission.
Gaberščik and Martinčič (1987) demonstrated seasonal changes in growth of *Sphagnum papillosum* (Figure 83-Figure 85) in a raised bog in Slovenia, Yugoslavia. They found the greatest growth at the beginning of the growing season, a time when water is usually plentiful. During winter months, the photosynthetic activity declines and ceases completely in February.

One restoration technique in peat-mined bogs in Canada has been an attempt to enhance the moisture content (Rochefort *et al*. 2002). To do this, *Sphagnum* (Figure 83-Figure 85) is reintroduced in the restoration areas and may be covered by extended periods of flooding, especially following snowmelt or heavy rainfall. These flooding events can cause production of innovations in which the buds and shoots grow. Some species grow capitula (compact apical branches of *Sphagnum*) from fragments under a variety of conditions. The most species in their study grew from whole plants under long-term conditions of shallow flooding. However, many of the species under long-term flooding suffered from etiolation (condition of plants grown in partial or complete absence of light, characterized by long, weak stems and smaller, sparser leaves).

In the Niigata Prefecture of Japan, the floating liverwort *Ricciocarpos natans* (Figure 86) is common in cultivated rice fields (Shirasaki 1996). It grows best where there are warmer temperatures and a snow depth of 0.5-3.0 m.
In western Norway, *Andreaea rupestris* (Figure 87-Figure 89) occurs along the flushing gradients created by snowmelt or is associated with snow cover (Hedger 2001). Species in the alpine areas of Norway are sensitive to the timing of snowmelt. In 43 sampled transects, 22 of the 41 taxa show a significant relationship to the time of snowmelt as the altitude increases. But these relationships are not necessarily direct responses to the temperature or water. Rather, at least some of them avoid locations of earlier snowmelt because of competition from other plants, especially tracheophytes.

Snowbed bryophytes must utilize low light and short growing seasons with low temperatures to attain sufficient photosynthesis for carbon gain. *Anthelia* (Figure 10) does especially well in the border zone of snowbeds because of its resistance to long-lasting cold, wet, and dark conditions (Lösch *et al.* 1983). *Polytrichastrum sexangulare* (Figure 18-Figure 20), on the other hand, is more productive and is therefore able to compete with the tracheophytes at less extreme sites.

**Mechanical Effects**

Snow has its down side for plants. The sheer weight can crush or break the plants. So how do tiny plants like bryophytes fare under this weight? Kennedy (1993) commented on how few studies have included the biomechanics of bryophytes, reminding us of their need for snow cover resistance.

Among the mosses receiving the greatest mechanical stress due to height is *Dendroligotrichum dendroides* s.l. This moss stands alone, supporting a height up to 40 cm where it lives in the forests of Chile and New Zealand. For this species, the dense hypodermal sterome provides considerable stiffness comparable to that of woody stems of tracheophytes. But for many smaller mosses, such support is usually not needed. Rather, the mosses of various habitats have a wide range of mechanical conformations. By contrast, size, development, and phylogenetic position seem to be less important than the habitat in determining growth form and mechanical adaptations.

**Freeze-thaw Cycles**

Free-thaw cycles can have some of the same damaging effects as dehydration-rehydration. And like many other epiphytes that tolerate the wet-dry cycles, the rock face and tree-trunk-dwelling *Leucodon sciuroides* (Figure 90) in the Mediterranean tolerates freezing and thawing with its photosynthetic apparatus fully operational after freezing (Deltoro *et al.* 1999). Both CO$_2$ fixation and chlorophyll fluorescence return to pre-freezing values during thawing. And like many desert mosses, it recovers its photosynthesis rapidly during thawing. Deltoro and coworkers suggest that this rapid recovery is possible through dissipative pathways that absorb excess light energy in frozen plants.
Figure 90. *Leucodon sciuroides* on tree bark, a species that tolerates freezing and is ready for photosynthesis as soon as it thaws. Photo by Michael Lüth, with permission.

Melick and Seppelt (1992) experimented with up to 16 freeze-thaw cycles in Antarctic bryophytes. After 16 days of immersion in water, there is a relatively low loss of glucose and fructose [10-29% of the total sugar pool in healthy mosses, but 69% from the dead *Schistidium chrysoneurum* (≈*Grimmia antarctici*; Figure 91-Figure 92)]. Freeze-thaw cycles increase this leakage up to 2-3 times except in the dead mosses. *Bryum pseudotriquetrum* (Figure 49-Figure 50) lost 65% of its total sugar when subjected to 16 freeze-thaw cycles. The other species [*Ceratodon purpureus* (Figure 55-Figure 60, Figure 92), *Schistidium chrysoneurum*, *Cephaloziella exiliflora* (Figure 93)] lost less than 28%. This loss does not seem to be related to the freezing temperature.

Schlensog et al. (2004) compared the recovery of lichens and mosses after winter in the continental Antarctic. Whereas the lichens recover photosystem II (PS II, first protein complex in light-dependent reactions of oxygenic photosynthesis; it captures photons of light to energize electrons) almost fully within a few minutes of hydration, the mosses take much longer to recover. The moss *Bryum subrotundifolium* (Figure 94-Figure 96) maintains highly elevated respiration rates for several days following activation. Like the response to desiccation, it appears that this moss must repair damages before it can make a positive photosynthetic gain.

Figure 91. *Schistidium chrysoneurum* in the Antarctic. Photo by Rod Seppelt, with permission.

Figure 92. *Schistidium chrysoneurum* hummock with *Ceratodon purpureus* in the hollows. Photo by Rod Seppelt, with permission.

Figure 93. *Cephaloziella* sp. *Cephaloziella exiliflora* loses some of its stored sugar during freeze-thaw cycles. Photo by Kristian Peters, with permission.

Figure 94. Bed of *Bryum subrotundifolium* in meltwater on Antarctica. Photo by Rod Seppelt, with permission.

Figure 95. *Bryum subrotundifolium*, a moss that maintains high respiration rates for several days following rehydration. Photo by Rod Seppelt, with permission.
Freeze-thaw protection can be conferred on bryophytes by various compounds present prior to freezing (Rütten & Santarius 1993). In Plagiomnium affine (Figure 97) the uptake and release of sucrose does little to change the permeability of the leaf cell plasma membranes to sugars, proline (amino acid), or polyethylene glycols. However, pretreatment with these compounds sufficient to induce plasmolysis does protect the moss cell membranes from freeze-thaw damage. On the other hand, pretreatment with glycerol (compound that is soluble in water and is hygroscopic) causes plasmolysis (shrinking of cell membrane away from cell wall) without endowing the cells with protection against freeze-thaw damage.

Figure 96. Bryum subrotundifolium in the Antarctic infected with a fungus that is taking advantage of suitable moisture conditions. Photo by Catherine Beard, with permission.

Figure 97. Plagiomnium affine, a moss protected from freeze-thaw damage by various compounds and plasmolysis. Photo by Michael Lüth, with permission.

**Winter Short-term Warming Events**

Noting that climate change in northern high latitudes is likely to be greater in winter than in summer, Bjerke et al. (2011) examined the potential effects of more frequent short-term warming events. Whereas these warming event effects are known to be damaging to tracheophytes, their effect on bryophytes could be quite different. And the bryophytes and lichens are of major importance in these high-latitude ecosystems. By simulating winter warming events with infrared lamps and soil warming cables in a sub-Arctic heath, Bjerke et al. were able to monitor the responses of the feather moss Hylocomium splendens (Figure 142). In the three winters of simulated warming events, this moss experienced significant reductions in net photosynthetic rates and growth rates (of up to 48% and 52%, respectively), starting in the first summer after these events began. In this species, growth begins early in the spring season, exposing young, vulnerable shoots to the effects of cold. The researchers suggest that the damage under winter warming events may be due to breaking dormancy and experiencing premature growth during the winter warming events that causes damage to those sensitive young shoot tissues. Subsequent drying following these events may cause desiccation damage to the tender shoots.

These winter warming events could change the distribution of acrocarpous vs pleurocarpous mosses in areas with winter snow cover. In the Front Range of the Rocky Mountains, USA, Flock (1978) found that acrocarpous mosses are more abundant in areas that are dry and maintain light snow cover. Pleurocarpous mosses, on the other hand, are more abundant in wet sites with deep snow cover.

As Longton (1988) has pointed out, bryophytes in general have phenotypic plasticity, opportunistic responses in CO₂ exchange, and a poikilohydrous water relationship that endows them with considerable tolerance for desiccation and frost. These make it possible for them to occupy snowbeds where few tracheophytes can succeed.

**Protection from Light Damage**

A potentially serious problem for desiccated mosses at low temperatures is that they are still able to absorb light energy. This can be a special problem for forest epiphyte species that experience more light exposure in winter, compared to summer, when the tree canopy has lost its leaves. Particularly on those cold days that lack snow cover, over excitation of chlorophyll electrons can be damaging. However, Heber et al. (2006) report that some mosses have seasonal differences in their ability to dissipate that excess light energy into heat.

Freezing and thawing can result in photoinhibition, as demonstrated by the endemic moss Schistidium chrysoneurum (Figure 91) from the Antarctic (Lovelock et al. 1995a). Jägerbrand (2011) considered the time immediately following snowmelt to be the most dangerous time for UV damage to bryophytes. Rehydration, lingering low temperatures, and rising UV levels coincide with a time when bryophytes must repair the damage due to absence of light and desiccation from winter. This is especially problematic in the Antarctic where the ozone layer is thinning. Fortunately, this highly variable photoinhibition is reversible during periods of warmer temperatures (Lovelock et al. 1995a). The inhibition that occurs between freezing and thawing events recovers best under low light conditions. After four cycles, recovery of hydrated mosses occurred within 12 hours of transfer to 5°C at 15 μmol quanta m⁻² s⁻¹.
During the dry summers, some desiccation-tolerant mosses are more protected against photo-oxidative damage when they are dry than they are in the humid winters (Heber et al. 2006). In mosses such as the poikilohydric (having no mechanism to prevent desiccation) Rhytidiadelphus squarrosus (Figure 98), desiccation reduction of chlorophyll fluorescence does not occur under even strong illumination in the desiccated state once the moss has achieved phototolerance. One protectant is zeaxanthin (one of the most common carotenoid alcohols and a powerful antioxidant), which requires drying in light. If the water is lost slowly, fluorescence is quenched. Quenchers accumulate during desiccation and remain stable until hydration occurs. Hydration results in their reversion to non-quenching molecules.

Figure 98. Rhytidiadelphus squarrosus, a moss that requires drying to induce protection (zeaxanthin) against photoquenching. Photo by Michael Lüth, with permission.

Lovelock and Robinson (2002) found that surface reflective properties of leaves also plays a role in dissipating the light, hence protecting the plants from light damage. They suggested that the water content, but not pigments, of the mosses are important in altering the red-edge and photochemical reflectance index. The water content may account for the differences in reflectance among the species. All the mosses maintain high levels of xanthophyll pigments that serve as photoprotectants. Interestingly, their abilities to reflect UV light differs little. Bryum pseudotriquetrum (Figure 49-Figure 50) has greater reflective values than the other mosses studied and also has higher levels of UV-absorbing pigments, but its carotenoid levels are lower than the other species tested. Ceratodon purpureus (Figure 55-Figure 60) has higher levels of anthocyanins but lower total chlorophyll concentrations. Bryum pseudotriquetrum has higher levels of the specific UV-screening pigments; Ceratodon purpureus and Schistidium chrysoneuron (Figure 91) have higher levels of pigments that protect against excess visible light.

For Schistidium chrysoneuron (Figure 91), freezing in darkness reduced the Fv/Fm ratio (ratio of variable:maximum fluorescence) and the initial fluorescence (Lovelock et al. 1995b). These were reversible when the mosses thawed. The reduction of Fv/Fm may be the result of conformational changes in the pigment-protein complexes due to the desiccation that occurs during freezing. The photoinhibition during freezing is reversible and indicates that processes that protect the moss from photoinhibitory damage during freezing temperatures occur in consort with high solar radiation levels. These protections therefore limit the repair needed when favorable temperatures return.

**Winter Growth**

Proctor (2000) points out that bryophytes have a desiccation tolerance strategy that differs from that of tracheophytes. Bryophytes are able to survive because they can photosynthesize and grow when water is freely available, then suspend their metabolism when it is not. By being ectohydric (conducting water externally), many species can have wide variability in their external capillary water without affecting the water content of the cells. This external source permits the cells to function most of the time with full turgor. When they do desiccate, the period of water stress is brief. They have a carbohydrate content that is similar to that of the maturing embryos of desiccation-tolerant seeds. It is likely that these carbohydrates contribute to their rapid recovery upon rehydration. In short, they mimic temperate winter annuals or mesic desert ephemerals. For example, in the maritime climate of Britain, the wall top moss Grimmia pulvinata (Figure 99) takes advantage of the mild climate of autumn and early winter for most of its growth (Proctor 2004). During that period the moss is able to maintain hydration for long periods of time to carry out photosynthesis. Like many mosses, it is adapted to frequent and often short wet-dry cycles.

Figure 99. Grimmia pulvinata on rock where it grows mostly in autumn an dearly linter when it is well hydrated frequently. Photo by Michael Lüth, with permission.

Even growth rates seem to adjust to differences in temperature, perhaps because of differences in available moisture, perhaps just to acclimation (see Fornwall & Glime 1982). For example, Brachythecium rutabulum (Figure 100) has superior growth in winter compared to summer when grown at temperatures below 18°C (Furness & Grime 1982). Most species of temperate regions seem to have their optimum growth temperature at 15-25°C, but growth can be extensive at temperatures even below 10°C. Gabersčik and Martinečí (1987) demonstrated seasonal changes in growth of Sphagnum papillosum (Figure 83–Figure 85) in a raised bog in Slovenia, Yugoslavia. They found the greatest growth at the beginning of the growing
season, a time when water is usually plentiful. During winter months, the photosynthetic activity declined and ceased completely in February.

Asada et al. (2003) found that winter growth of a number of bryophyte species [Racomitrium lanuginosum (Figure 101-Figure 103), Pleurozium schreberi (Figure 104), Sphagnum austinii (Figure 105-Figure 106), S. fuscum (Figure 2), S. rubellum (Figure 107-Figure 108), S. papillosum (Figure 83-Figure 85), S. lindbergii (Figure 38-Figure 39), S. tenellum (Figure 109-Figure 110), S. pacificum (Figure 111)] in a coastal peatland in British Columbia, Canada, is an important contribution to the productivity of the system. But for this productivity to occur, water must be available during those times when the temperature permits photosynthesis to occur. Asada further supported the importance of water by demonstrating that productivity in these species correlated more strongly with precipitation than with temperature.
Growth can actually occur at sub-zero temperatures. *Brachythecium geheebii* (Figure 112) and *Homalothecium philippeanum* (Figure 113-Figure 114) in Romania montane areas are able to assimilate CO$_2$ down to -9°C (Atanasiu 1971). *Isothecium alopecuroides* (Figure 115-Figure 116) had net gain down to about -8°C. Both of these temperatures are lower than those for evergreen trees tested in winter. But not all bryophytes are created equal. Davey and Rothery (1996) found that in *Brachythecium austrosalebrosum* from the Antarctic, respiration rates were highest in summer and lowest in winter regardless of temperature within the natural range, but that in *Chorisodontium aciphyllum* (Figure 117-Figure 118) and *Andreaea depressinervis* (Figure 119), there was little change with season.
Figure 113. *Homalothecium philippeanum* in a habitat where it can be exposed to sub-zero temperatures. Photo by Michael Lüth, with permission.

Figure 114. *Homalothecium philippeanum* on a boulder where it can photosynthesize when the air temperature is as low as -9°C. Photo by Michael Lüth, with permission.

Figure 115. *Isothecium alopecuroides* growing epiphytically where it is exposed to sub-zero temperatures. Photo by Michael Lüth, with permission.

Figure 116. *Isothecium alopecuroides*, a species that has photosynthesis down to -8°C. Photo by David Holyoak, with permission.

Figure 117. *Chorisodontium aciphyllum* in Antarctica where its respiration differs little with seasons. Photo from Polar Institute, through Creative Commons.

Figure 118. *Chorisodontium aciphyllum*, a moss whose respiration differs little with season. Photo by Juan Larrain, with permission.
A recent addition to the known flora of the British Isles, the thallose liverwort *Athalamia hyalina* (Figure 120) is a Northern Hemisphere montane species (Long et al. 2003). In Scotland it has its active growth in the winter and produces its spores in spring. This permits it to live on the thin soil of eroding limestone ledges where it can take advantage of the moisture in fog of winter and intermittent thaws.

Winter warming (and possibly summer drought?) in the UK seems to account for the increases in *Campyliadelphus chrysophyllus* (Figure 121) and *Fissidens dubius* (Figure 122) in a limestone grassland, as demonstrated with experiments in winter warming and increased supplemental rainfall in summer (Bates 2006). Spread of the epiphytes *Cololejeunea minutissima* (Figure 123) and *Colura calyptrifolia* (Figure 124) seems likewise to be the result of rising temperatures in winter, and possibly a change in the summer moisture. On the other hand, winter warming coincides with decreases in *Rhytidiadelphus squarrosus* (Figure 98) and *Lophocolea bidentata* (Figure 125).
Figure 124. *Colura calyptrifolia* on bark, a species that seems to be spreading in the UK concomitant with rising mean winter temperatures. Photo by Michael Lüth, with permission.

Figure 125. *Lophocolea bidentata*, a species that seems to be disappearing from the UK due to winter warming. Photo by Michael Lüth, with permission.

**Winter and Reproduction**

Since winter is often the season with moisture in some habitats like deserts, we can expect this to be the season of gamete transfer and fertilization. In the Negev Desert of southern Israel, Herrnstadt and Kidron (2005) found that *Bryum dunense* initiates its reproductive organs prior to the first winter rain, using atmospheric humidity from dew and fog as the source of water. The most sporophytes form in the partially shaded microhabitats following winter rains, interestingly exceeding those in the shaded microhabitats. When the first rain arrives, *B. dunense* is ready to disperse its bulbils and to complete fertilization. The shrubs seem to be essential to provide the partial shade in which this species is most successful at reproducing.

Too little rainfall in winter can be detrimental to desert mosses. *Syntrichia inermis* (Figure 126) in the Mojave Desert, Nevada, USA, failed to initiate sporophytes in 1996 and 1997 when the winter-spring rainfall was reduced (Stark 2001). In *Crossidium crassinerve* (Figure 127), the appropriate hydration periods occurred in the cooler months of October to April (Stark 2005). Hydration in the summer was detrimental because the patches dried too quickly (as few as 3 hours) following the rainfall, prohibiting sufficient repair and carbon gain. During the four years of the study, the five patches monitored initiated 248 sporophytes; only 9 survived. Embryonic abortion (69%) and capsule herbivory (30%) accounted for most of the deaths.

*Acaulon triquetrum* (Figure 128) in southwest Germany initiates most of its gametangia in October to December (Ahrens 2003). These develop rapidly, permitting fertilization to occur during the same time period. Sporophytes grow in October-November to January-February, with dispersal in April or May. The *chloronemal* (branches of protonemata that give rise to gametophore buds) filaments are persistent through summer but die off during winter (December – February). The rhizoid system, however, persists throughout the winter, once again giving rise to new chloronemata and gametophores in the spring. Having rhizoids that persist through the winter permits this moss to rapidly occupy bare surfaces, especially the loess created by small, burrowing mammals.

Figure 126. *Syntrichia inermis* dry, a species that frequently fails to produce sporophytes due to insufficient rainfall in winter and spring in the Mojave Desert, USA. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 127. *Crossidium crassinerve*, a species that is hydrated mostly in winter and early spring in the Mojave Desert. Photo by Michael Lüth, with permission.
Laaka-Lindberg and Heino (2001) found that the leafy liverwort *Lophozia ventricosa* (var. *silvicola*; Figure 129) in southern Finland has two types of gemmae. One of these becomes dormant and the other is non-dormant. Only the dormant gemmae are able to survive the winter. She provided the evolutionary argument that if the winter mortality (of non-dormant gemmae) increases compared to the mortality during the growing season, then evolution would favor an increase in the percentage of dormant gemmae, especially among those produced at the end of the growing season.

Spore dormancy is also subject to temperature. Spores require water to germinate, but dormancy loss also occurs in response to temperature. For *Sphaerocarpos texanus* (Figure 130) at 35/20°C, loss of spore dormancy increases faster than that in even modestly lower temperatures of 30/15°C or 25/15°C (McLetchie 1999). The best spore germination occurs at 16/10°C and spores fail to germinate at 35/20 or 30/15°C. But low temperatures induce the spores to return to dormancy. McLetchie considered this behavior to be similar to that of seeds of obligate winter annuals.

Longton and Greene (1969) demonstrated that in Britain the boreal forest moss *Pleurozium schreberi* (Figure 104) sustains survival of its antheridia through winter. The antheridia begin development in August but remain immature through winter. The archegonia likewise overwinter in an immature stage. When spring arrives, both undergo rapid development, preparing them for fertilization in April and May. The sporophyte matures in autumn and spores are dispersed between January and April.

It is likely that the moss *Dichelyma japonicum* is excluded from high altitudes and latitudes because its sporophytes have a late sporophyte maturation (Shirasaki 1997). This species grows on the woody plants beside ponds and streams and is covered by deep snow in winter. In the aquatic family *Fontinalaceae*, this species requires deep snow in winter and high precipitation in summer.

*Riccia cavernosa* (Figure 131) avoids most of the problems of cold, dark, dry winters by having an extremely short life cycle on the banks and sandy flats of the Kobuk River in Alaska (Seppelt & Laursen 1999). Its spore to spore cycle is only three to four weeks of late summer and autumn! Hence, it is able to overwinter as spores and avoid all the problems. On the other hand, this same species (perhaps a different race?) has a life cycle of two - three months of winter and early spring in Australia.
**Hennediella heimii** (Figure 132) holds the record for the most polar sporophytes. Seppelt *et al.* (1992) reported this species with young sporophytes from the Lower Taylor Valley, Victoria Land, Antarctica (77°55'S).

![Figure 132. Hennediella heimii with capsules; this species has the record for capsules at the highest latitude in the Antarctic. Photo by Michael Lüth, with permission.](image)

**Asexual Survival**

We have learned much about preparation for winter conditions through studies in cryopreservation. The pioneer moss *Ditrichum plumbicola* (Figure 133) survives winters and desiccation in the field, but has poor survival of cryopreservation, even with pretreatment (Rowntree *et al.* 2007). Using a series of treatments and observations, Rowntree and co-workers attempted to determine the effects of ABA, sucrose, and desiccation on various stages of the protonemata. What they found was that most of the protonemal cells pretreated with ABA and sucrose died, but the ones that survived had thick cell walls with deep pigmentation, numerous small vacuoles, and cytoplasmic lipid droplets. Those with only desiccation and cryopreservation exhibited little cytological change. Removal of the ABA-sucrose pretreatment permitted normal development and activity of the protonemata, whereas the pretreatment induced propagules from the protonemata, and these propagules were highly desiccation tolerant and easily survived the cryopreservation. In nature, this species forms highly desiccation-tolerant rhizoids that serve the same perennating function.

![Figure 133. Ditrichum plumbicola, a species that survives winter and desiccation in the field, but it has little ability to survive cryopreservation except as propagules. Photo by Des Callaghan, with permission.](image)

**Sympatric Differences**

Seasonal differences in reproduction often separate *sympatric* (two species that exist in same geographic area) species and keep them separated reproductively. Such is the case for three species of *Dicranoloma* in Australia (Milne 2001). In *D. menziesii* (Figure 134) and *D. platycaulon* (Figure 135), the antheridia are initiated during winter and archegonia in the following spring, whereas in *D. billardierei* (Figure 136) the antheridia initiate during late spring to summer whereas archegonia originate in autumn. Differences in development time place the times of fertilization in three different time periods, late summer for *D. menziesii*, mid autumn for *D. platycaulon*, and early winter for *D. billardierei*. For all three species, the winter season is an important period for this process, providing sufficient moisture and avoiding these activities during the high summer temperatures that can cause excessive respiration.

![Figure 134. Dicranoloma menziesii, a species separated from its congeners by its reproductive times. Photo by Andrew Hodgson, with permission.](image)

![Figure 135. Dicranoloma platycaulon, a species separated from its congeners by its reproductive time. Photo by David Tng, with permission.](image)

Figure 136. *Dicranoloma billardierei*, a species separated from its congeners by its reproductive times. Photo by Michael Lüth, with permission.

In Japan, *Trachycystis flagellaris* (Figure 137) has developing sporophytes that do well in the lower temperatures and deep snow at higher altitudes (Shirasaki 1998). *Trachycystis microphylla* (Figure 138), on the other hand, has its sporophyte maturation in early spring; it seems to be restricted to coastal areas with only thin snow cover in winter.

Effects of Bryophytes on their Communities in Winter

We cannot ignore the importance of the perennial nature of most bryophytes. Many remain active in winter, but even more importantly they are present and active within hours of snowmelt on their leaves.

The ability of the mosses to remain green and moist throughout winter in some areas is important for their invertebrate inhabitants. For example, in the Black Forest of Germany, *Rhytidiadelphus squarrosus* (Figure 98) is home to several species of tardigrades (water bears) (Schuster & Greven 2007). These tiny animals are well attuned to the seasonal changes in moss habitats. Their diversity is greatest in winter, although numbers decline then. (See Volume 2, Chapter 5).

Mosses are able to modify the effects of frost on the soil community. In a feather moss community in northwestern Alberta, Canada, dominated by *Pleurozium schreberi* (Figure 104), removal of the moss layer increased soil temperatures in summer and lengthened the frost-free period (Startsev *et al.* 2007). But rather than causing the soil to be warmer, the bare soil had temperatures as low as -13°C during the frost-free times.

In China, mosses are a critical part of the gall nut industry, a valuable source of medicines and chemicals. The gall aphid, *Kaburagia rhusicola* spends its winters on mosses, including *Brachythecium* spp., *Entodon*, and *Oxyrrhynchium* (Lai & Zhang 1994). The mosses are able to provide both cover and a moist location. The gall aphid *Kaburagia ovogallis* uses eight species of the *Brachytheciaceae* for its winter hosts (Li 1990). In fact, as of 1990, 24 species of mosses were identified as winter hosts of various species of gall aphids. By providing more of these mosses, those in the gall nut industry were able to sustain higher yields.

Lichens (especially reindeer "moss") are well known as food for caribou and other large herbivores in winter. But bryophytes are less well known for this role. Olofsson *et al.* (2002) demonstrated the importance of bryophytes and other food sources by using exclosures around parts of snowbeds in Arctic-alpine tundra communities. Following eleven years of exclosure, the snowbed developed significant increase in both tracheophytes and bryophytes. The same response did not occur in the tall herb meadow. The primary herbivores in the study were rodents – grey-sided voles (*Clethrionomys rufocanus*), red voles (*C. rutilus*), field voles (*Microtus agrestis*), root voles (*M. oeconomus*), and lemmings (*Lemmus lemmus*). Bryophytes accounted for most of the cryptogamic changes in biomass. Predominant among those increasing in biomass were *Sanionia uncinata* (Figure 46-Figure 48) (584% increase) and *Polytrichum juniperinum* (Figure 139) (113,584% increase). *Pleurozium schreberi* (Figure 104) was absent in the controls and increased by 2.7 g m⁻² in the exclosures. The researchers concluded that the low competition of the unproductive snowbeds was caused by these mammalian herbivores that depressed the plant biomass. The presence of food under the snow, including the bryophytes, permits these animals to remain hidden from aerial predators.
Prins (1982) asked why mosses are eaten in cold climates only. Could it be that they like the sweet taste of sucrose that is stored in some species in preparation for winter?

Winter Dispersal

Winter can be a time for dispersal. Dry, brittle mosses easily break, presenting fragments that can travel long distances across the smooth snow or caught up in winds unimpeded by canopy leaves. McDaniel and Miller (2000) demonstrated this by collecting bryophyte fragments from late-spring snowbeds in the Adirondack Mountains, New York, USA. The diversity of fragments from the higher elevations of alpine and krummholz vegetation was much greater than that in the forested site. (The diversity also surpassed that of the tracheophytes.)

Miller and Howe Ambrose (1976) were able to collect bryophyte fragments from late snowbeds on Bathurst Island in the Canadian high Arctic. These collections contained large numbers of both mosses and liverworts. Most of the species represented those on the nearby ridges and slopes and Miller and Howe Ambrose presumed that these had been dispersed during the previous winter, resulting from surface winds. In lab cultures, 12% of these fragments exhibited viability, producing protonemata, new shoots, rhizoids, or renewed growth. But parts were not equally viable. Detached moss leaves did not grow and only one leafy liverwort fragment was viable. Rather, the leafy gametophore tips were the most successful. Nevertheless, the researchers estimated that a cubic meter of granular snow contained more than 4000 viable propagules!

Pollution Effects – Vital Water or Deadly Poisons?

Kennedy (1993) reminded us that traditional wisdom tells us that life in the Antarctic is restricted by the arrival of new species and the extreme cold. But recently biogeographical evidence indicates that water may be the primary limiting factor. But it can also bring danger.

Winter can be a particularly dangerous time for bryophytes that are subject to air pollution. The snow collects the pollutants over the extended period of snow cover (Thomas 1981). When melting occurs, the bryophytes are subjected to that long-term collection of pollutants, i.e., concentrated pollutants, in what is known as acid flush (Woolgrove & Woodin 1996). Woolgrove and Woodin documented that these concentrated pollutants in the snowbed moss *Kiaeria starkei* are causing damage to the underlying bryophytes. This damage is greatest when the snow cover is gone and meltwater is delivered to the active plants. This exposure can last for a sufficient period of time that no recovery is measured after 4 weeks. This, combined with the short growing season of these mosses in snowbeds, can have serious impacts on their survival.

Markert and Weckert (1993) found that plants of *Polytrichastrum formosum* (Figure 141) had the maximum concentrations of pollutants in winter and the lowest in summer, attributing this to the higher biomass productivity of this species in the spring. But I suspect that part of this effect was due to the accumulation effect by the snow. Hynninen (1986) attributed the greater winter accumulation of heavy metals by *Sphagnum* (Figure 83-Figure 85) in moss bags in Finland to the summer holiday breaks. Could these winter highs be due to the absence of rain to wash the pollutants away and the long time for continuous collection?

In the boreal forest *Hylocomium splendens* (Figure 142) uses both organic and inorganic nitrogen deposited in the snow (Forsum et al. 2008). Snowmelt N is dominated by nitrates (86%), followed by ammonia (11%) and amino acids (3%). The *H. splendens* is able to take up 24% of the nitrogen from the snow nitrogen. On the other hand, Björk (2007) showed that 1.0 g m\(^{-2}\) yr\(^{-1}\) N added to the snow water had little effect on the bryophyte community over a three-year period. Other nutrients become available in the snowmelt water as well (Björk & Molau 2007; Jägerbrand 2011).
On the other hand, some bryophytes grow best in winter when more moisture is available. Others survive winter through asexual propagules. The life cycle adaptations to winter microclimate are effective means for maintaining species differences among sympatric members of the same genus.

Bryophytes occupy habitats where tracheophytes cannot complete their life cycles in the short growing seasons. These bryophytes provide a refuge and food for invertebrates, rodents, and even some large free-range mammals. They modulate the ground temperature, preventing extremes, hold water longer than bare ground, and prevent destructive runoff during spring flushes.

The dry air of winter facilitates breakage of bryophyte fragments. These easily blow across the snow, taking advantage of the absence of leaves on the trees in deciduous forests, making winter dispersal significant.

Pollutants accumulate in the snow and rapid melt may expose the bryophytes to heavy concentrations in a short time frame. In some cases, the bryophytes gain important nutrients from the collected pollutants, but some are detrimental.

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**Literature Cited**


