

CHAPTER 7-6

WATER RELATIONS: REHYDRATION AND REPAIR

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CHAPTER 7-6

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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.



Figure 3. *Syntrichia ruralis* on sand dunes at Harlech, Wales. Photo by Janice Glime.



Figure 4. *Brachythecium rivulare* decurrent leaf base with enlarged hyaline cells at leaf base. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

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*.



Figure 5. *Pleurozium schreberi* with moribund lower shoot tissues exposed. Photo by Janice Glime.



Figure 6. *Bryum argenteum* showing the moribund lower leaves. Photo from Botany Website, UBC, with permission.



Figure 7. *Sphagnum girgensohnii*. Note the change in color in lower branches, indicating senescing conditions. Photo by Bernd Haynold through Wikimedia Commons.

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**.



Figure 8. *Polytrichum piliferum* in hydrated state. Photo by Janice Glime.



Figure 9. *Schistidium apocarpum* in its dry state with leaves wrapped around stem. Photo by Michael Lüth, with permission.

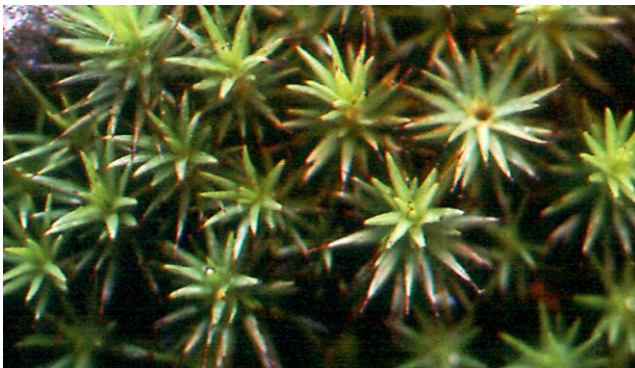


Figure 10. *Polytrichum juniperinum* in hydrated state. Photo by Janice Glime.

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.

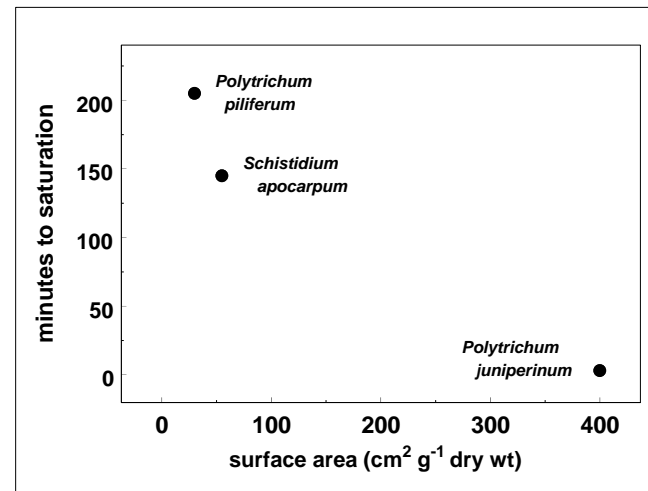


Figure 11. Relationship between surface area and time to saturation upon rewetting of three drought-tolerant mosses. Based on Larson (1981).

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 *Campyllum 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.

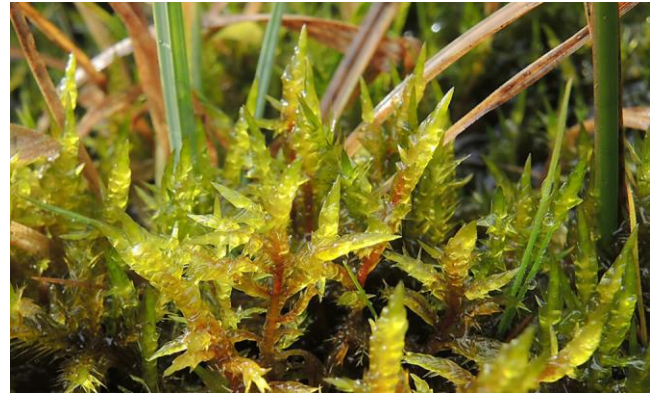


Figure 16. *Calliergonella cuspidata*, a species with moderate resilience to desiccation. Photo by Michael Lüth, with permission.

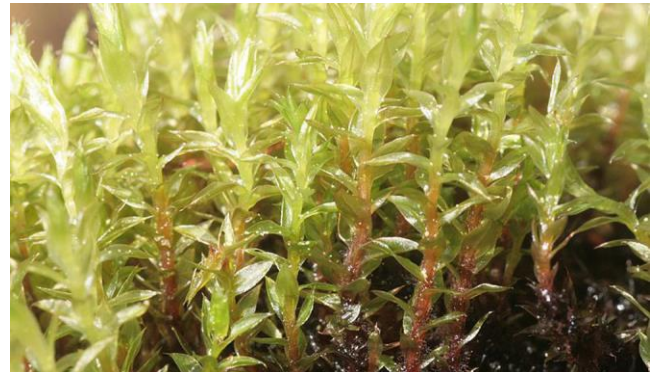


Figure 17. *Bryum pseudotriquetrum*, a species with moderate resilience to desiccation. Photo by Hermann Schachner, through Creative Commons.



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.

Species	Duration Dry	Reference
Mosses		
<i>Andreaea rothii</i>	13 mos	Proctor 1981
<i>Anisothecium staphylinum</i>	45-48 yr (spores, tubers, or rhizoids in dry soil)	Whitehead 1984
<i>Anoetangium compactum</i>	19 yr	Malta 1921
<i>Anomodon longifolius</i>	2 yr	Richardson 1981
<i>Anomodon viticulosus</i>	45 d	Hinshiri & Proctor 1971
<i>Archidium ohioense</i>	20 yr ⁴	Makinde & Fajuke 2009
<i>Barbula torquata</i>	18 mos	Moore <i>et al.</i> , 1982
<i>Bryum argenteum</i>	2 yr	Richardson 1981
<i>Bryum coronatum</i>	20 yr ⁴	Makinde & Fajuke 2009
<i>Dicranella heteromalla</i>	0 d ¹	Streusand & Ikuma 1986
<i>Dicranoweisia cirrata</i>	9 yr	Richardson 1981
<i>Fissidens minutifolius</i>	6 yr ⁴	Makinde 1993
<i>Fissidens subglaucescens</i>	20 yr ⁴	Makinde & Fajuke 2009
<i>Fissidens taxifolius</i>	0 d ¹	Streusand & Ikuma 1986
<i>Fontinalis flaccida</i>	3 mos	Glime 2015
<i>Grimmia apocarpa</i>	8 mos	Alpert & Oechel 1987
<i>Grimmia laevigata</i>	10 mos; 10 yr (shoots), 1 mo (protonema)	Alpert & Oechel 1985; Breuil-Sée 1994; Keever, 1957
<i>Grimmia muehlenbeckii</i>	1.5 yr	Richardson 1981
<i>Grimmia pulvinata</i>	<7 yr	Segreto <i>et al.</i> 2010
<i>Grimmia elatior</i>	5 yr	Richardson 1981
<i>Grimmia torquata</i>	<7 yr	Segreto <i>et al.</i> 2010
<i>Hookeria lucens</i>	~15 d	Dilks & Proctor 1974
<i>Hylocomium splendens</i>	~160 d	Dilks & Proctor 1974
<i>Neckera crispa</i>	~160 d	Dilks & Proctor 1974
<i>Octoblepharum albidum</i>	29 wk (leaves); 20 yr ⁴	Egunyomi 1979; Makinde & Fajuke 2009
<i>Orthotrichum rupestre</i>	9 mos; ~2 yr	Alpert & Oechel 1987; Richardson 1981
<i>Plagiothecium undulatum</i>	100 d	Dilks & Proctor 1974
<i>Racomitrium lanuginosum</i>	>239 d	Dilks & Proctor 1974
<i>Rhytidiadelphus loreus</i>	>100 d	Dilks & Proctor 1974
<i>Scorpiurium circinatum</i>	~120 d	Dilks & Proctor 1974
<i>Sphagnum fallax</i>	14 d	Sagot & Rochefort 1996
<i>Sphagnum fuscum</i>	14 d; 0 d ²	Sagot & Rochefort 1996; Schipperges & Rydin 1998
<i>Sphagnum magellanicum</i>	14 d; 0 d ²	Sagot & Rochefort 1996; Schipperges & Rydin 1998
<i>Sphagnum</i> [3 spp.]	0 d ²	Schipperges & Rydin 1998
<i>Syntrichia caninervis</i>	3 yr; 6 yr	Oliver <i>et al.</i> 1993; Oliver <i>et al.</i> 2005
<i>Syntrichia norvegica</i>	3 yr	Oliver <i>et al.</i> 1993
<i>Syntrichia ruralis</i>	3 yr; 14 yr	Oliver <i>et al.</i> 1993; Maheu 1922; Stark <i>et al.</i> 2016
<i>Tortula muralis</i>	3 yr; 14 yr	Kosnar & Kolar 2009; Glime 2015
<i>Triquetrella papillata</i>	8 wk	Moore <i>et al.</i> 1982
13 Antarctic species	<1 yr	Davey 1997
8 fen spp.	8–20 wk	Manukjanová <i>et al.</i> 2014
protonemal resting cells	49 yr	Bristol 1916
Liverworts		
<i>Bazzania trilobata</i>	0 d	Sollows <i>et al.</i> , 2001
<i>Marchantia berteroana</i>	<1 yr	Davey 1997
<i>Oxymitra paleacea</i>	4 yr	Volk 1984
<i>Plagiochila spinulosa</i>	~30 d	Dilks & Proctor 1974
<i>Reboulia hemisphaerica</i>	4 yr	Volk 1984
<i>Riccia canescens</i>	7 yr	Volk 1984
<i>Riccia macrocarpa</i>	23 yr	Breuil-Sée 1993
<i>Riccia macrospora</i>	2 yr	Volk 1984
<i>Riccia marginata</i>	2 yr	Volk 1984
<i>Saccogyna viticulosa</i>	~200 d	Dilks & Proctor 1974
13 species of hepatics ³	≤20 mos	Volk 1984

¹ shoots allowed to regenerate only 10–14 d² 13 species of *Sphagnum* were shown capable of hardening to DT when partially desiccated at high RHs (Hájek & Vicharová, 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 (Kranter *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.

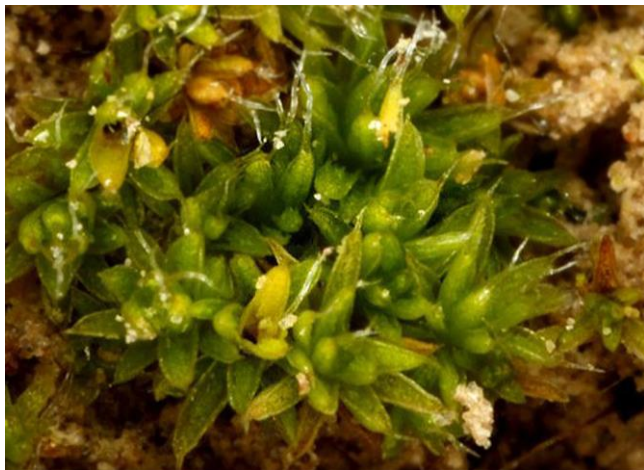


Figure 20. *Crossidium crassinerve*, a species in the Mojave Desert where it requires at least 2 mm of rain to fully rehydrate. Photo by Jan-Peter Frahm, with permission.

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).

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 mRNAs (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).



Figure 21. *Syntrichia ruralis*, a moss that loses hydration proteins upon drying and synthesizes rehydration proteins upon rewetting. Photo by Michael Lüth, with permission.

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!



Figure 22. *Schistidium chrysoneurum* (formerly *Grimmia antarctici*) in Antarctica. Photo by Sharon Robinson, through Creative Commons.

Deltoro *et al.* (1998a) compared recovery in seven desiccation-tolerant bryophytes [Figure 23: *Hedwigia ciliata*, *Hypnum cupressiforme*, *Leucodon sciurioides*, *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), J.]. 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.

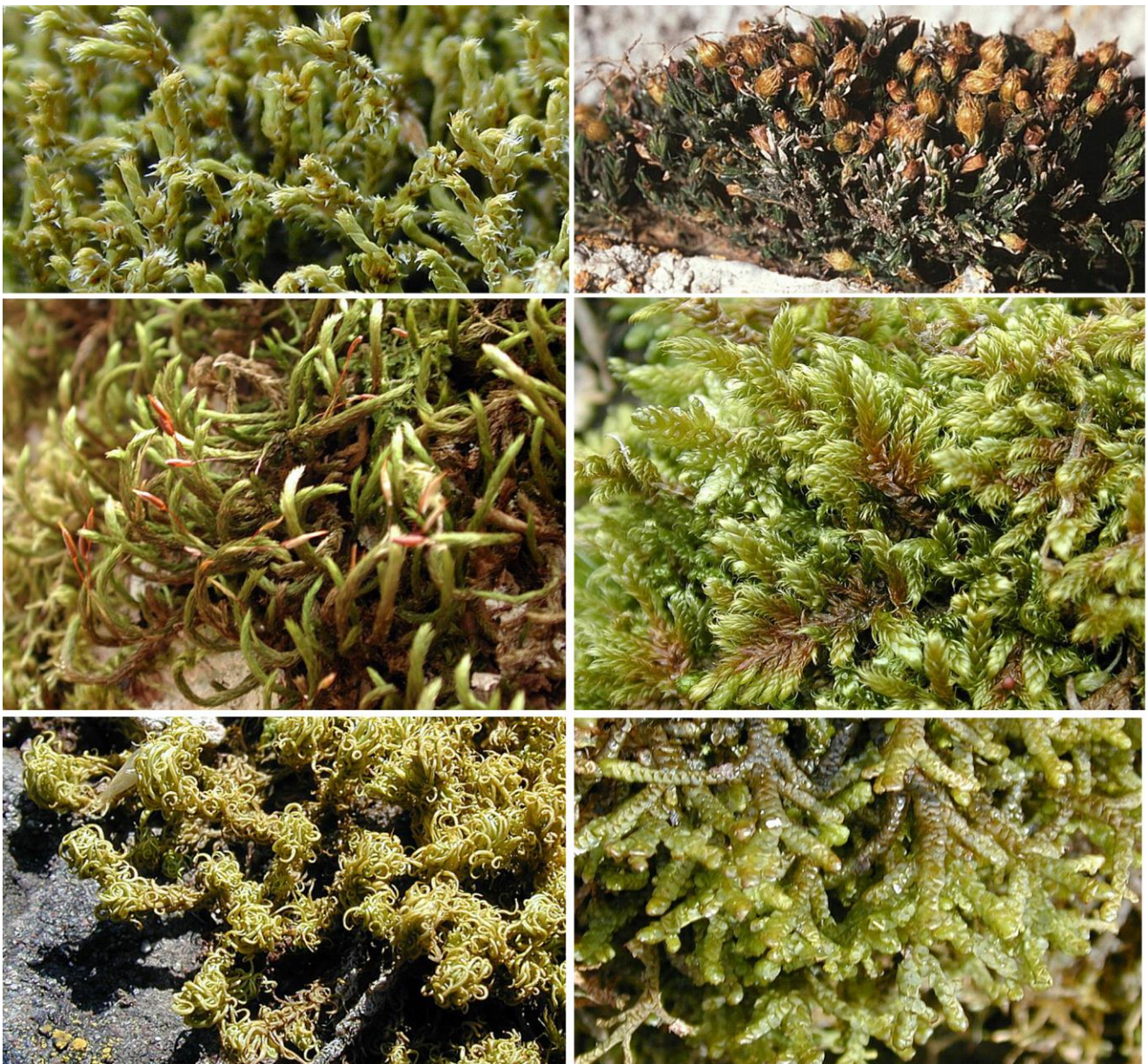


Figure 23. Examples of drought-tolerant bryophytes. **Left, top:** *Hedwigia ciliata*, **Left, Middle:** *Leucodon sciurioides*, **Left, bottom:** *Pleurochaete squarrosa*, **Right, top:** *Orthotrichum cupulatum*, **Right, middle:** *Hypnum cupressiforme*, **Right bottom:** *Porella platyphylla*. Photos by Michael Lüth, with permission.

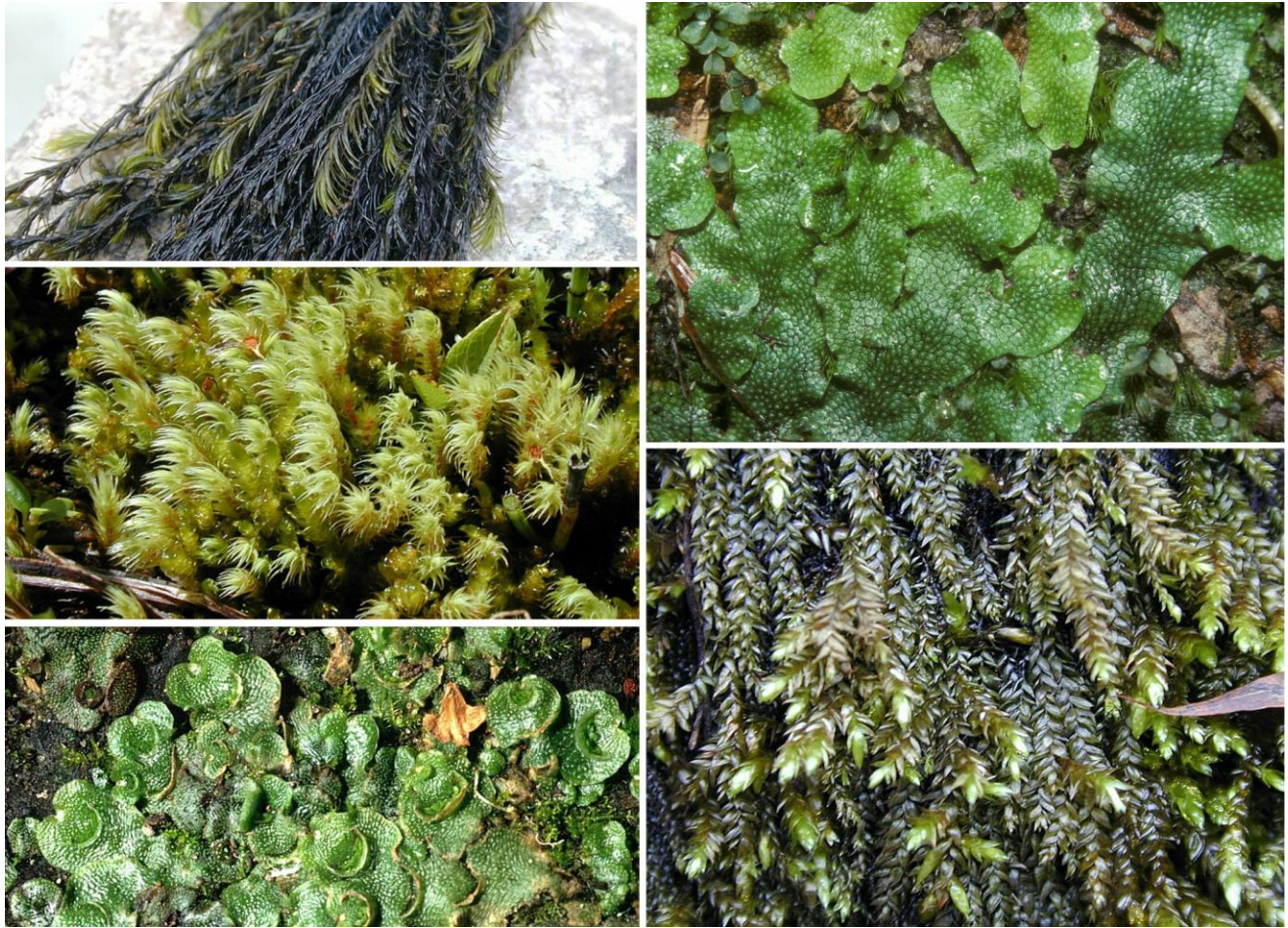


Figure 24. Examples of desiccation-intolerant bryophytes. **Left, top:** *Cinclidotus aquaticus*, **Left, middle:** *Philonotis calcarea*, **Left, bottom:** *Lunularia cruciata*, **Right, top:** *Conocephalum conicum*, **Right, bottom:** *Platyhypnidium riparioides*. Photos by Michael Lüth; *Conocephalum conicum* photo by Janice Glime.

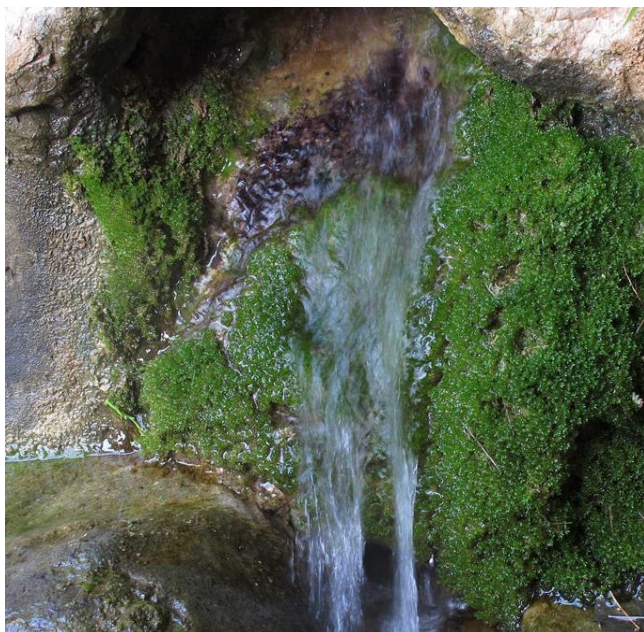


Figure 25. *Barbula bolleana* in a seepage waterfall. Photo by Michael Lüth, with permission.



Figure 26. *Barbula bolleana*, a desiccation-intolerant moss. Photo by Michael Lüth, with permission.



Figure 27. *Palustriella commutata*, a desiccation-intolerant species. Photo by J. C. Schou, 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.



Figure 28. *Polytrichastrum formosum*, a moss that can drop to 40% relative water content before the net CO₂ uptake ceases. Photo by Des Callaghan, with permission.

Even aquatic bryophytes may not die following total desiccation. My experience with boiling *Fontinalis* (Figure 29) and with dead-looking mosses following snow-melt 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.



Figure 29. *Fontinalis antipyretica* in dry stream. This dead-looking moss will recover when water returns to the stream. Photo by Janice Glime.

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 photosynthate 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*.

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).

<i>Anthoceros natalensis</i> (H)	89%
<i>Pellia epiphylla</i> (TL)	83%
<i>Hookeria lucens</i> (M)	77%
<i>Dumortiera hirsuta</i> (TL)	55%
<i>Atrichum androgynum</i> (M)	45%
<i>Sphagnum auriculatum</i> (M)	38%
<i>Plagiochila natalensis</i> (LL)	21%
<i>Rhodobryum roseum</i> (M)	0%



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 rewetting (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 hydrophytic *Cratoneuron filicinum* (Figure 30) slowly loses ATP after rewetting 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 (Noailles 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 Ca⁺² 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 3. Relative cell and vacuole sizes among bryophytes as listed by Oliver & Bewley (1984b).

	cell size	vacuoles
Desiccation tolerant		
<i>Ceratodon purpureus</i>	small	large
<i>Syntrichia ruralis</i>	small	small
<i>Neckera crispa</i>		small
<i>Pleurozium schreberi</i>	long & narrow	small
<i>Barbula torquata</i>	small	large
<i>Triquetrella papillata</i>	small	small
Desiccation sensitive		
<i>Cratoneuron filicinum</i>	long & narrow	small

Melick and Seppelt (1992, 1994) considered that the membrane integrity is restored rapidly and that intracellular carbohydrates likewise are replenished rapidly in the xerophytic *Syntrichia caninervis* (Figure 31). 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

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*.

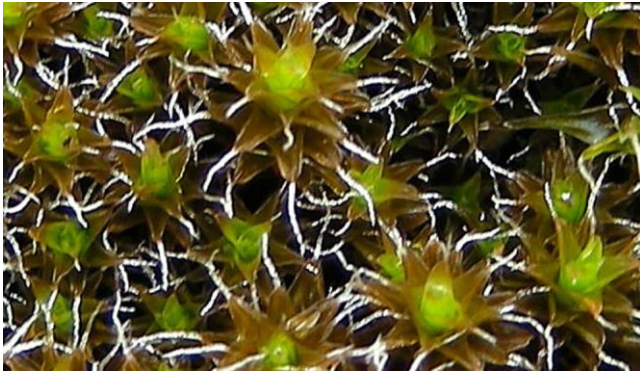


Figure 31. *Syntrichia caninervis*, a desiccation-tolerant desert moss. Photo by Michael Lüth, with permission.

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.



Figure 32. *Hylocomium splendens* on forest floor, a species that grows as well with 6 or 7 days of hydration a week, but not with other hydration regimens. Photo by Amadej Trnkoczy, through Creative Commons.

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/Figure 31) 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.



Figure 33. *Mnium hornum*, a species that doubles its oxygen uptake upon rehydration. Photo by Michael Lüth, with permission.

Reactive Oxygen Species

The greatest damage to cells is caused by reactive oxygen species (Kranner *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.



Figure 34. *Porella platyphylla*, a species that doubles its oxygen uptake upon rehydration. Photo by Janice Glime.



Figure 35. *Scapania undulata*, a species that has 6X as much oxygen uptake when recovering from desiccation. Photo by David Holyoak, with permission.



Figure 36. *Dumortiera hirsuta*, a species that produces extracellular superoxide at a high rate, increasing production following mild desiccation stress. Photo by Paul Davison, with permission.

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** (F_v/F_m) (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 (F_v/F_m) 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 *Rhytidiadelphus loreus* (Figure 38), a mesophytic forest floor species. For desiccation-tolerant bryophytes such as *Anomodon viticulosus*, *Racomitrium lanuginosum*

(Figure 39), and *Rhytidiadelphus loreus*, recovery of photosynthesis upon rehydration is rapid (Proctor & Smirnov 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 CO₂ (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 CO₂ slowly declines as the moss dehydrates. After that, water stress occurs, the moss rapidly proceeds to suspended metabolism, and CO₂ fixation rapidly ceases. Following rehydration, *S. ruralis* immediately begins CO₂ 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).



Figure 37. *Anomodon viticulosus*, a moss that rapidly rehydrates and is ready for photosynthesis. Photo by Michael Lüth, with permission.



Figure 38. *Rhytidiadelphus loreus* on the forest floor, a species that is rapid to regain photosynthetic activity after rehydration, but slower than *Anomodon viticulosus*. Photo by Michael Lüth, with permission.



Figure 39. *Racomitrium lanuginosum* on rock, a species that rapidly regains photosynthetic activity after rehydration. Photo by Michael Lüth, with permission.

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?

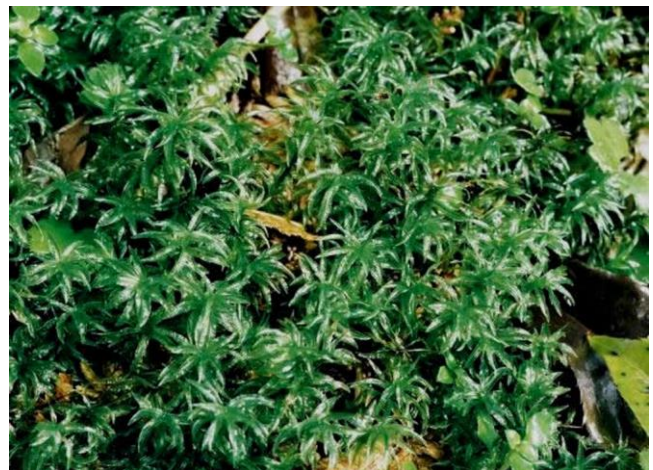


Figure 40. *Atrichum androgynum*, a moss that uses ABA to aid in rapid recovery from desiccation. Photo by Clive Shirley, Hidden Forest <www.hiddenforest.co.nz>, with permission.

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.



Figure 41. *Riccia macrocarpa*, a species that resumed normal metabolism upon rehydration after 25 years in a dry state. Photo by Michael Lüth, with permission.

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.

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.



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* & *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 ELIPa 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 (Deltoro *et al.* 1998a; Marschall & 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. Deltoro 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 Fajuke (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. Dhindsa 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*, *Racomitrium lanuginosum*, *R. aquaticum*, *Leucodon sciurioides*, *Pleurochaete squarrosa*, *Ulota 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 F_v/F_m are less than 0.1, *Physcomitrella patens* fails to regenerate. The F_v/F_m fluorescence is the standard measurement for stress in plants, testing whether or not plant stress affects photosystem II in a dark adapted state. F_v refers to fluorescence in its variable state; F_m 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.

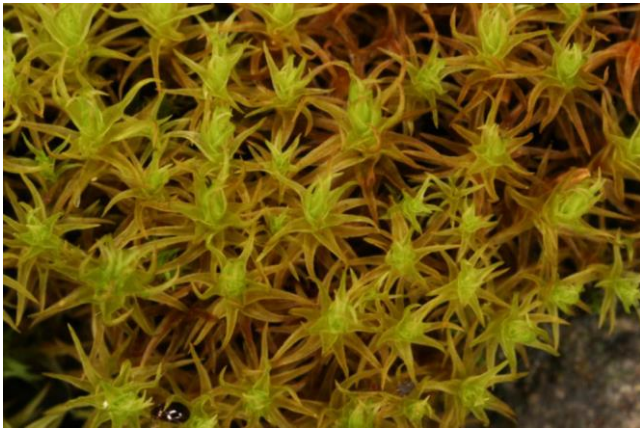


Figure 44. *Didymodon vinealis*, a moss that is able to recover within one hour of hydration after 18 months of desiccation. Photo by Jan-Peter Frahm, with permission.

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 – 7dry 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.



Figure 47. *Syntrichia norvegica*, a species in which fragments and spores germinate better in continuous moisture than in other moisture regimes. Photo by Michael Lüth, with permission.



Figure 48. *Syntrichia laevipila*, a species in which fragments and spores germinate better in continuous moisture than in discontinuous regimes. Photo by Jonathan Sleath, 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 *Rhytidiadelphus 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.



Figure 49. *Dicranum majus*, a moss that seems to do best when the number of wet and dry days are about equal. Photo by Michael Lüth, with permission.

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).

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. caninervis* > *S. ruralis* > *S. norvegica*.



Figure 50. *Pterygoneurum lamellatum*, a desert moss with inducible desiccation tolerance when dried slowly. Photo by Michael Lüth, with permission.

Implications

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 reactivate 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 **rehydrins**, 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 rehydration between frequent drying periods deplete resources and are more harmful than long dry periods, issuing foreboding for moss gardeners.

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