

CHAPTER 7-7

WATER RELATIONS: BIOCHEMICAL ADAPTATIONS TO DRYING

TABLE OF CONTENTS

Membrane Chemistry	7-7-2
ABA Role.....	7-7-3
Protection from Oxidation.....	7-7-6
Oxidative Damage.....	7-7-6
Glutathione.....	7-7-8
Pathogen Danger	7-7-9
Shoot Tips – Variable Tolerance within Plants	7-7-10
The Genes	7-7-10
Summary	7-7-11
Acknowledgments.....	7-7-11
Literature Cited	7-7-11

CHAPTER 7-7

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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 antarctici* (Figure 4; least tolerant). This is another example showing that not all bryophytes have the same adaptations to desiccation.

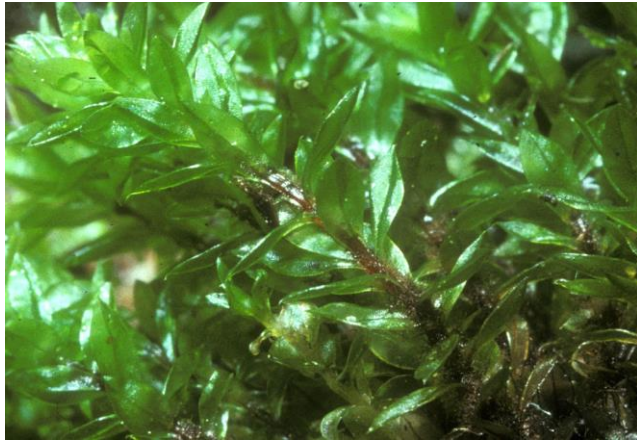


Figure 2. *Bryum pseudotriquetrum*, a moss in which the sugar stachyose aids in desiccation tolerance. Photo by Janice Glime



Figure 3. *Ceratodon purpureus*, a moss that does not use stachyose to aid in desiccation tolerance. Photo by Janice Glime.



Figure 4. Drought-intolerant *Schistidium antarctici* on Macquarie Island. Photo by Rod Seppelt, with permission.

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*.



Figure 5. *Phaeoceros laevis* sporophytes, a hornwort with stomata in the capsule. Photo by Robert Klips, with permission.

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**.



Figure 6. *Physcomitrella patens*, a moss in which ABA increases stress tolerance. Photo by Michael Lüth, with permission.

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.



Figure 7. *Riccia fluitans*, exposed here to air drying. ABA can facilitate conversion to the wider terrestrial form. Photo by Jan-Peter Frahm, with permission.

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.



Figure 8. *Ricciocarpos natans*, stranded here out of water. ABA can facilitate conversion to the terrestrial form. Photo by Janice Glime.



Figure 9. *Lunularia cruciata*, a thallose liverwort that produces the ABA-like lunularic acid as a dormancy hormone. Photo by Michael Lüth, with permission.

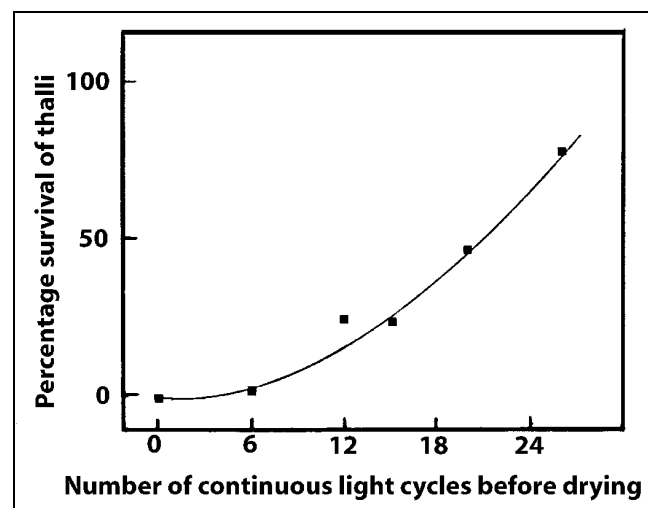


Figure 10. Effect of long-day (continuous) light on induction of drought resistance, resulting in drought survival in the thallose liverwort *Lunularia cruciata*. Based on Schwabe (1990).

Although the presence of lunularic acid seems to be universal in liverworts, and has functions like those of

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 CO₂ 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.



Figure 13. *Atrichum undulatum* showing some plants drying and curling. This moss changes its phosphoglyceride composition during drought stress. Photo by Janice Glime.

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).

Absciscic 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 absciscic 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. *diptera* (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.



Figure 14. *Physcomitrella patens*, a species that exhibits oxidative bursts in response to a fungal presence. Photo by Michael Lüth, with permission.

Minibayeva and Beckett (2001) conclude that patterns of oxide production are correlated with the moisture status of the habitat. Those species with high basal rates of oxide production grow in moist microhabitats, have a moderately high thallus water content, have high K^+ contents, and have well developed oxidative bursts. Species with such oxidative bursts also lose a high proportion of their intracellular K^+ (55-98% in liverworts and hornworts) upon rehydration. Mosses and the one leafy liverwort were all collected from wet habitats and all produced oxides at low rates compared to the thallose liverworts and hornworts.



Figure 15. *Marchantia paleacea* subsp. *diptera* from Japan. Photo by Janice Glime.

The aquatic moss *Fontinalis antipyretica* (Figure 18) exhibits the potential danger of high oxygen levels. De Carvalho *et al.* (2012) demonstrated that under slow dehydration, this species exhibits low production of reactive oxygen species upon rehydration, a phenomenon that reduces the cellular damage and increases cell survival. The slow drying apparently reduces the oxidative burst by limiting production of reactive oxygen species.



Figure 16. *Barbula unguiculata* dry, retaining its green color that permits it to respond quickly to rehydration. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.



Figure 17. *Anthoceros punctatus*, a hornwort having similar isozyme patterns to those of the moss *Barbula unguiculata*. Photo by Jonathan Sleath, with permission.



Figure 18. *Fontinalis antipyretica* in dry stream. Photo by Janice Glime.

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 $1000 \mu\text{mol g}^{-1} \text{ dry mass h}^{-1}$, 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 (H_2O_2), 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 H_2O_2 . Mayaba and coworkers suggest that peroxidases might be responsible for the production of H_2O_2 . They determined that ABA and light influenced the rate of production of peroxide.



Figure 19. *Atrichum androgynum*, a moss with an oxidative burst, especially during the first 15 minutes of rehydration. Photo by Clive Shirley, Hidden Forest <www.hiddenforest.co.nz>, with permission.

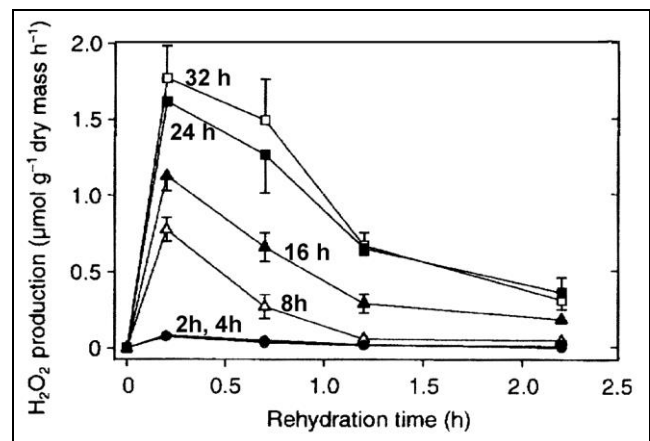


Figure 20. Peroxide (H_2O_2) production during rehydration following various dehydration periods (indicated on each line) in *Atrichum androgynum* from KwaZulu-Natal Province, Republic of South Africa, during summer. Vertical bars indicate standard deviation; $n=5$. Redrawn from Mayaba *et al.* (2002).

This peroxidase system would have several advantages. Peroxidases oxidize phenolics to quinones and generate peroxide (H_2O_2). 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.

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 O₂) activity in *Syntrichia ruralis* var. *arenicola* (= *Tortula ruraliformis*; 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 anti-oxidants 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; Smirnov 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.



Figure 23. *Dicranella palustris* in flush near Swallow Falls, Wales. This moss has limited desiccation tolerance. Photo by Janice Glime.

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 24. *Thuidium tamariscinum*, a species that is able to use glutathione as protection against heavy metals. Photo by Michael Lüth, with permission.

Pathogen Danger

The damaging effects of oxides in the cells leads us to question the advantages that may have kept the oxidative burst in the bryophytes for eons. This may be explained by their role in limiting pathogen invasion and damage.

Cells with damaged membranes resulting from desiccation would be vulnerable to invasion by pathogenic microorganisms. Such oxidative bursts as seen upon rehydration can help to limit the spread of invading pathogens because of oxidation toxicity, as well as inducing expression of defense-related genes. Low and Merida (1996) considered the oxidative bursts in plants to facilitate cross-linking of cell wall proteins, induction of

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).



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 *Dumortiera 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 H_2O_2 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

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 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 29. *Dumortiera 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.

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).

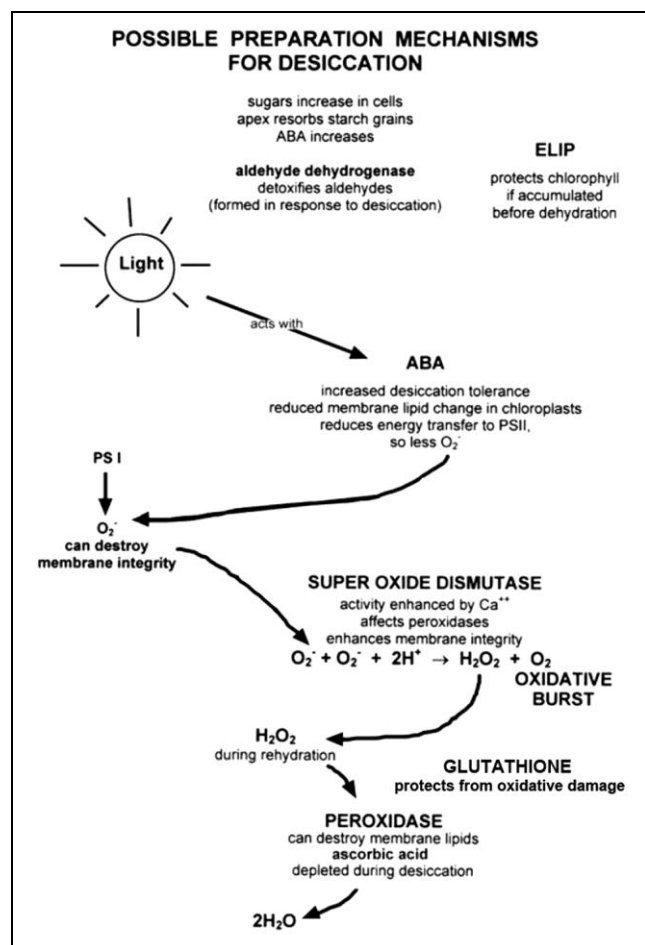


Figure 31. Speculation on possible relationships of the observations that have been made on pre-desiccation events and related rehydration events in desiccation-tolerant bryophytes.

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 H_2O_2 (**peroxide**), which is harmful to plants. We know that H_2O_2 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 tow 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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