

CHAPTER 10-2

TEMPERATURE: COLD

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Figure 1. *Racomitrium heterostichum* mostly imbedded in ice while some branches are free and available for photosynthesis. Photo by Michael Lüth, with permission.

Low Temperature Limits

In general, bryophytes seem able to withstand cold in their leafy state much better than their tracheophyte counterparts. Ochi (1952) found that most mosses (18 species tested) were resistant to cold to -20°C . Seven of these species were resistant to -27°C . He was unable to find any trend in relationships to osmotic value, permeability, or seasonal fluctuations. Ochi's results support the later statement of Kallio and Heinonen (1973), that *Racomitrium lanuginosum* (Figure 2), a cosmopolitan moss, is pre-adapted to its abode in the Arctic and Antarctic (see Table 1) and suggest that such pre-adaptation may be a common feature of bryophytes. This contention is supported by the low temperatures that become lethal for bryophytes in the tropics (Table 2).



Figure 2. *Racomitrium lanuginosum*, a species pre-adapted to living in the polar regions with long, white hair tips. Photo by Janice Glime.

Surprisingly, Arctic liverworts do not seem to be so cold resistant. Among the nine species tested by Biebl (1968), seven were mostly dead at -16°C , with only the leafy liverworts *Barbilophozia hatcheri* (Figure 3) and *Chandonanthus setiformis* (Figure 4) surviving well. The moss *Aulacomnium turgidum* (Figure 5-Figure 6) also survived at -16°C . All species survived -6°C . But these were July responses in Greenland; a quite different picture might emerge in winter. On the other hand, all of them survived up to 42°C for half an hour, but twelve-hour exposures killed parts of most of them, the same seven, at 38°C . *Aulacomnium turgidum* survived up to 48°C for half an hour and up to 40°C for twelve hours. This supports the hypothesis that low temperature survival is coupled with high temperature survival.



Figure 3. *Barbilophozia hatcheri*, a leafy liverwort that survives to -16°C . Photo by Michael Lüth, with permission.



Figure 4. *Chandonanthus setiformis*, a leafy liverwort that survives to -16°C . Photo by Michael Lüth, with permission.

Tropical mosses seemed rather similar. After 24 hours of exposure, *Homaliodendron flabellatum* (Figure 7) and *Leucoloma amoene-virens* survived -14°C and *Schistochila commutata* (Figure 8) survived -11°C (Biebl 1967). Tropical *Plagiochila* (Figure 9), *Metzgeria* (Figure 10), and *Bryum* (Figure 11) species each survived to at least -4°C . Try doing that to a tropical *Maranta* (Figure 12).



Figure 5. *Aulacomnium turgidum* in a mountainous habitat. Photo by Michael Lüth, with permission.

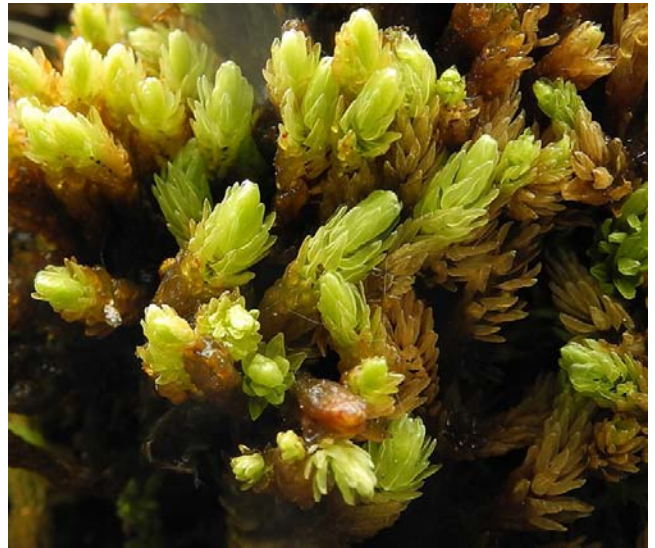


Figure 6. *Aulacomnium turgidum*, a moss that survives to -16°C . Photo by Michael Lüth, with permission.



Figure 7. *Homaliodendron flabellatum*, a tropical species that can survive to -14°C . Photo by Jiang Zhenyu, Mou Shanjie, Xu Zawen, Chen Jianzhi, through Creative Commons.



Figure 8. *Schistochila* sp, a tropical species that survives to -12°C. Photo by Jan-Peter Frahm, with permission.



Figure 9. *Plagiochila* sp. from the Neotropics. Some tropical members of this genus survive to -4°C. Photo by Michael Lüth, with permission.



Figure 10. The tropical thalloid liverwort, *Metzgeria claviflora*. Photo by Michael Lüth, with permission.



Figure 11. *Bryum apiculatum* from the Neotropics. Some tropical members of this genus survive to -4°C. Photo by Michael Lüth, with permission.

Table 1. Temperature limits for net photosynthesis under natural CO₂ and light saturation. From Larcher 1983, compiled from many authors; *Liu *et al.* 2001.

Plant group	Low-temp limit for CO ₂ uptake °C	Temp opt of P _n °C	High-temp limit for CO ₂ uptake (°C)
Herbaceous flowering plants			
C ₄ plants of hot habitats	+5 to 7	35-45	(50) 50-60
Sun plants (temperate zone)	-2 to 0	20-30	40-50
Shade plants (temperate zone)	-2 to 0	10-20	~40
Desert plants	-5 to 5	20-35 (45)	45-50 (56)
CAM plants (CO ₂ fixation at night)	-2 to 0	5-15	25-30
Winter annuals, spring-flowering and alpine plants	-7 to -2	10-20	30-40
Woody plants			
Evergreen trees of the tropics and subtropics	0 to 5	25-30	45-50
Winter-deciduous trees of the temperate zone	-3 to -1	15-25	40-45
Evergreen conifers	-5 to -3	10-25	35-42
Dwarf shrubs of heath and tundra	~-3	15-25	40-45
Cryptogams			
Arctic and subarctic mosses	~-8	~-5	~-30
<i>Racomitrium lanuginosum</i>	-8 to -10	5	25-30
<i>Pleurozium schreberi</i>	-5	10-15	28-33
<i>Plagiomnium acutum</i> *	-10 to -15	20-35	40-45
<i>Plagiomnium maximoviczii</i>	-10 to -15	20-35	40-45
Lichens of cold regions	(-25)-15 to -10	5-15	20-30
Desert lichens	~-10	18-20	38-40
Tropical lichens	-2 to 0	~20	
Snow algae	~-5	0-10	30
Thermophilic algae	20 to 30	45-55	65-70



Figure 12. *Maranta leuconeura*, a tropical plant that dies in cool temperatures well above freezing. Photo by Stickpen, through public domain.

Table 2. Comparison of temperature resistance of leaves of plants from different climatic regions. Limiting temperatures are for 50% injury (TL₅₀) after exposure to cold for 2 or more hours, or after exposure to heat for 0.5 h. Bryophytes appear in **bold**. Tracheophyte data from Larcher 1983, based on data from many authors; cold tracheophytes had been cold-hardened. Data marked by * from Biebl 1967; Data marked by + from Liu *et al.* 2003.

Plants	°C for cold injury	°C for heat injury in growing season
Tropics		
Trees	+5 to -2	45-55
Forest undergrowth	+5 to -2	45-48
Mountain plants	-5 to -10	~45
<i>Schistochila commutata</i> *	-14	44
<i>Plagiochila</i> sp.*	-7	44
<i>Homaliodendron flabellatum</i> *	<-14	52
<i>Leucoloma amoenervis</i> *	<-14	
<i>Bryum</i> sp.*	-11	52
Subtropics		
Sclerophyllous woody plants	-8 to -12	50-60
Subtropical palms	-5 to -14	55-60
Succulents	-5 to -10	58-65
C ₄ grasses	-1 to -3(-8)	60-64
Temperate zone		
Evergreen woody plants of coastal regions with mild winters	-6 to -15 (-25)	50-55
<i>Plagiomnium acutum</i> ⁺		45 (50 dry)
Arcto-tertiary relict trees	-10 to -25 (-15 to -30)	
Dwarf shrubs of Atlantic heaths	-20 to -30	45-50
Winter-deciduous trees and widely distributed shrubs	(-25 to -40)	~50
Herbs		
Sunny habitats	10 to -20 (-30)	48-52
Shady habitats	40-45	
Water plants	~10	38-42
Cold-winter areas		
Evergreen conifers	-40 to -90	44-50
Boreal broad-leaved trees	(-196)	42-45
Arctic and alpine dwarf shrubs	-30 to -70	48-54
Herbs of the high mountains and arctic	(-30 to -196)	44-54

Stress Protection

Bryophytes are well known for their secondary compounds. These defend against competition, microbes, and herbivory, while often protecting against UV radiation, providing drought tolerance, and freezing survival (Xie & Lou 2009). These latter protections can all be associated with cold temperatures. Specifically, bibenzyls and bis(bibenzyls) have provide desiccation tolerance; fatty acid derivatives and phenylpropanoids provide freeze tolerance.

But bryophytes seem to have a large arsenal of protectors against cold stress. They are able to accumulate soluble sugars and abscisic acid (ABA) (Bhyan *et al.* 2012). The latter increases freezing tolerance in plant cells and also is important in desiccation tolerance – a likely consequence of ice crystal formation. During acclimation in *Physcomitrella patens* (Figure 13) that was developed to be insensitive to ABA, the cells accumulated sucrose to levels similar to those found in ABA-normal plants. But the trisaccharide theandrose did not accumulate in the ABA-deficient plants. Furthermore, these deficient plants had very limited accumulation of LEA-like boiling-soluble proteins. On the other hand, Minami *et al.* (2005) found an accumulation of several transcripts for LEA proteins and boiling-soluble proteins during freeze-tolerance acclimation. Bhyan *et al.* (2012) concluded that cold acclimation requires an ABA-dependent signalling system. Cold-induced sugar acclimation, however, may or may not be dependent on the ABA system. This ABA dependence is in contrast to the study by Minami *et al.* (2005), which concluded that ABA had no role in cold hardening in *P. patens*.



Figure 13. *Physcomitrella patens* with young sporophytes. Photo by Michael Lüth, with permission.

Freezing

As the external temperature is depressed, the bryophyte cell cools rapidly, presenting a rather different pattern from that of tracheophytes. In tracheophytes, leaf hairs, thick cuticle, and epidermis all serve to insulate the internal leaf cells from rapidly changing temperatures. Bryophyte leaves have none of these.

Freezing presents a number of problems for cells. Formation of crystals can cause physical damage by poking holes in the cell membrane or distorting the cell so that solutes can leak out more easily. Crystals are hygroscopic, attracting the water molecules from the cells to the cell

surface or intercellular spaces where the crystals may reside. This loss of water from the cells causes them to dehydrate. And cell membranes may be damaged or not function properly as fatty acids with higher solidification points become nonpliable.

Despite being perennial above ground, many, perhaps most, bryophytes survive freezing. Fletcher (1982) provided representative species from New Zealand [*Papillaria crocea* (Figure 14), *Hypopterygium* spp. (Figure 15), *Hymenodontopsis bifaria*, *Cyathophorum bulbosum* (Figure 16), *Calyptrochaeta brownii* (Figure 17)], South Africa [*Hypopterygium* sp. (Figure 15)], Australia [*Gigaspermum repens* (Figure 18), *Goniomitrium acuminatum* subsp. *enerve* (Figure 19)], and from Florida, USA [*Rhizogonium spiniforme* (Figure 20)] that survive freezing. In addition, Fletcher demonstrated that *Takakia lepidozoides* (Figure 21-Figure 22) remained healthy, as did *Sphagnum* spp. (Figure 24) and *Mnium* spp. (Figure 23). That number only provides us proof that some species survive, but gives us no idea of the world picture.



Figure 14. *Papillaria crocea* in a cloud forest at Mt Budawang, Australia, a species that is able to survive freezing. Photo by Peter Woodard, through Public Domain.



Figure 15. *Hypopterygium arbuscula*, in a New Zealand and South African genus in which some species are able to survive freezing. Photo by Scott Zona, with permission.



Figure 16. *Cyathophorum bulbosum*, a New Zealand species that is able to survive freezing. Photo by Peter Woodard, through Creative Commons.



Figure 17. *Calyptrochaeta brownii*, a South African species that is able to survive freezing. Photo by Tom Thekathiyil, with permission.



Figure 18. *Gigaspermum repens*, an Australian species that is able to survive freezing. Photo by David Tng, with permission.



Figure 19. *Goniomitrium acuminatum* subsp. .enerve, a species that survives freezing in Australia. Photo by David Tng, with permission.



Figure 22. *Takakia lepidozoides*, a high elevation species that survives freezing. Photo by Rafael Medina, through Creative Commons.



Figure 20. *Rhizogonium spiniforme*, a Florida species that is able to survive freezing. Photo by Janice Glime.



Figure 23. *Mnium thomsonii* from the Khibiny Mountains, Apatity, Murmansk, member of a genus in which some species survive freezing. Photo by Michael Lüth, with permission.



Figure 21. *Takakia lepidozoides* habitat, Japan. Photo from Digital Museum, University of Hiroshima, with permission.

Sphagnum capillifolium (Figure 24) exhibits a critical freezing temperature threshold for photosystem II that is identical to its ice nucleation temperature (-1.1°C) (Buchner & Neuner 2010). But frost damage (LT_{50}) is not visible until the temperature reaches -16.1°C . The LT_{50} is the condition/level at which the condition is lethal to 50% of the population.



Figure 24. *Sphagnum capillifolium*, a species that does not show frost damage above -16°C . Photo by Michael Lüth, with permission.

Something is going on in nature that does not seem to be mimicked in the lab. *Haplomitrium hookeri* (Figure 25) from New Zealand and *H. mnioides* (Figure 26) from Japan are able to grow in winter in their native habitats, but in cultivation all plants were unhealthy after being subjected to frost (Fletcher 1982). *Moerckia blyttii* (Figure 27), *Symphogyna* sp. (Figure 28), *Corsinia coriandrina* (Figure 29), and *Asterella* sp. (Figure 30-Figure 31) became severely bleached by frost in cultivation, but

Corsinia coriandrina remained healthy on an exposed wall top and in an unheated greenhouse down to a temperature of -5.5°C . Blackening occurred in *Dumortiera hirsuta* (Figure 32), but the plants survived. *Asterella* and *Monoclea forsteri* (Figure 33) likewise were blackened by frost in the greenhouse. *Fossombronia* (Figure 34) and *Anthocerotophyta* (Figure 35-Figure 36) experienced thallus decay, a phenomenon that they exhibited commonly in winter in nature. Plants of the hornworts *Anthoceros punctatus* (Figure 35) and *Phaeoceros laevis* (Figure 36) remained healthy in the greenhouse. Likewise, *Lunularia* (Figure 37), *Pellia* (Figure 38), *Preissia* (Figure 39), *Riccardia* (Figure 40), *Riccia* (Figure 41), and *Marchantia polymorpha* (Figure 42) showed no frost damage in the lab.



Figure 25. *Haplomitrium hookeri*, a species that survives frost in nature, but not in the lab. Photo by Štěpán Koval, with permission.



Figure 26. *Haplomitrium mnioides*, a species that survives frost in nature, but not in the lab. Photo by Yang, Jia-Dong, through Creative Commons.



Figure 27. *Moerckia blyttii*, a species that became severely bleached by frost in the lab. Photo by Michael Lüth, with permission.



Figure 28. *Symphyogyna brasiliensis* female plant. A species in this genus became severely bleached by frost in the lab. Photo by George J. Shepherd through Creative Commons.



Figure 29. *Corsinia coriandrina*, a species that became severely bleached by frost in the lab but remained healthy in nature. Photo by Michael Lüth, with permission.



Figure 30. *Asterella lindenbergiana*, a genus in which some species are blackened by frost in the greenhouse. Photo by Michael Lüth, with permission.



Figure 31. *Asterella lindenbergiana*, a frost-sensitive genus in the lab. Photo by Martin Hutten, with permission.



Figure 32. *Dumortiera hirsuta*, a species that survives frost in the lab, but it is blackened. Photo by Michael Lüth, with permission.



Figure 33. *Monoclea forsteri*, a species that is blackened by frost in the lab. Photo by Jan-Peter Frahm, with permission.



Figure 34. *Fossombronia angustata*. This genus commonly exhibits winter thallus decay. Photo by Michael Lüth, with permission.



Figure 35. *Anthoceros punctatus*, a species that remained healthy at temperatures below freezing in the greenhouse. Photo by Tab Tannery, through Creative Commons.



Figure 36. *Phaeoceros laevis* with capsules, a species that remained healthy at temperatures below freezing in the greenhouse. Photo by Michael Lüth, with permission.



Figure 39. *Preissia quadrata*, member of a genus that remained healthy at temperatures below freezing in the greenhouse. Photo by Janice Glime.



Figure 37. *Lunularia cruciata*, a species that remained healthy at temperatures below freezing in the greenhouse. Photo by Des Callaghan, with permission.



Figure 40. *Riccardia*, a genus that remained healthy at temperatures below freezing in the greenhouse. Photo by Li Zhang, with permission.



Figure 38. *Pellia epiphylla*, a species that remained healthy at temperatures below freezing in the greenhouse. Photo by Li Zhang, with permission.



Figure 41. *Riccia nigrella*, member of a genus that remained healthy at temperatures below freezing in the greenhouse. Photo by Jan-Peter Frahm, with permission.



Figure 42. *Marchantia polymorpha* with red edges, a species that remained healthy at temperatures below freezing in the greenhouse. Photo by Brenda Dobbs, through Creative Commons.

This raises the question of how do these exposed bryophytes survive. Why don't they suffer structural damage from internal ice crystals? How are they protected from severe desiccation as crystals on the outsides of leaves draw water from the cells?

Melick and Seppelt (1992) investigated these questions in *Schistidium antarctici* (Figure 43), *Ceratodon purpureus* (Figure 43, Figure 50), *Bryum pseudotriquetrum* (Figure 44-Figure 45), and *Cephaloziella exiliflora* (Figure 46) that were collected in late summer in the Antarctic Wilkes Land. Following 16 days of immersion, the plant loss of the carbohydrates glucose and fructose was relatively low (ca. 10-29% of the sugar content) in healthy mosses. However, in the senescing tissues of *S. antarctici* 69% of these sugars were lost. Following 16 freeze-thaw cycles the bryophytes experienced a sugar loss 2-3 times as great as in non-frozen controls in all but the dead brown tissue. *Bryum pseudotriquetrum* lost 65% of its total sugar content after a freeze-thaw cycle, whereas the other species lost less than 28%. Freezing points varied from -8.3° to -3.5°C , with dead material having the highest freezing temperatures. Freezing temperatures and sugar loss did not correlate and there was no change in the freezing point temperature of tissues after the sugar loss.



Figure 43. *Schistidium antarctici* hummock with *Ceratodon purpureus* in hollows. Photo courtesy of Rod Seppelt.



Figure 44. *Bryum pseudotriquetrum* in Antarctica, a species that loses considerable sugar after a freeze-thaw cycle. Photo by Rod Seppelt, through Creative Commons.



Figure 45. *Bryum pseudotriquetrum* in Norway, a species that loses little sugar following 16 days of immersion. Photo by Michael Lüth, with permission.



Figure 46. *Cephaloziella exiliflora*, a species that loses little sugar following 16 days of immersion. Photo by Tom Thekathyl, with permission.

On Windmill Islands of continental Antarctica, there was almost no seasonal change in the soluble carbohydrate content of the bryophytes and lichens (Melick & Seppelt 1994). The researchers considered that this lack of change

may be the result of the extreme climate and rapid temperature fluctuations.

Desiccation Tolerance

One of the consequences of freezing is dehydration. Consider the loss of moisture from your meat in the freezer when ice crystals form on the meat surface. Ice crystals are hygroscopic, pulling moisture from adjacent tissues. Furthermore, ice within the cell deprives the cell of the use of that water. The desiccation tolerance of bryophytes, therefore, helps them to survive freezing (Segreto *et al.* 2010). In their study of cryopreservation of bryophytes, Segreto and coworkers found that this natural desiccation tolerance negated the need for pretreatment or use of cryoprotectants before preserving live bryophyte tissues through freezing. They also found that longer or larger shoots of the leafy liverwort *Herbertus* (Figure 47) were able to regenerate more easily than smaller fragments, a phenomenon that suggests they are either able to transport from healthy cells to those that have been harmed, or that the greater amount of tissue helps to protect some of the cells.



Figure 47. *Herbertus hutchinsiae*; longer and larger shoots regenerate more easily in this genus. Photo by Michael Lüth, with permission.

Much like their resistance to hot temperatures, at least some bryophytes (*Syntrichia ruralis* – Figure 48-Figure 49) are more likely to survive freezing if they are dehydrated first (Bewley & Thorpe 1974). Those that were frozen in the hydrated state had lower rates of respiration and showed signs of freeze damage when rehydrated. Nevertheless, the respiration of desiccated mosses and of those desiccated and immersed in liquid nitrogen (frozen) was much higher on recovery than that of the controls that had remained hydrated at room temperature.

Desert species should be particularly adapted to freezing. They are endowed with various adaptations to survive desiccation, and they have a high probability of being desiccated when they experience freezing temperatures. But winter is the active season for the semi-desert grassland mosses in Hungary, with overwintering green shoots that are frequently exposed to temperatures below zero at night (Tuba *et al.* 2008). Daytime temperatures reach 0-5°C, and the dark-colored mosses (*Tortula/Syntrichia* – Figure 48-Figure 49) are even warmer (-2.1° to 6.9°C). The bryophytes were among the

18 out of 20 species that exhibited positive net photosynthesis. The abrupt increase in temperature in March did not affect the productivity rate of the mosses.



Figure 48. *Syntrichia ruralis*, a species that survives freezing better if it is dry first. Photo by David Holyoak, with permission.



Figure 49. *Syntrichia ruralis* dry, showing twisting leaves and awns that help to slow drying and protect at least some leaf cells from UV damage. Photo by Misha Ignatov, with permission.

Lenne *et al.* (2010) found that the ubiquitous moss *Ceratodon purpureus* (Figure 50) did not accumulate ice within the moss tissues during freezing. However, external ice induced desiccation. The water-filled hydroid cells cavitiated at -4°C. Parenchyma cells of the stem's inner cortex lost 20% of their original volume and exhibited **cytorrhysis** (permanent and irreparable damage to cell wall after complete collapse of plant cell due to water loss and consequent loss of internal positive pressure) at the lowest temperature of -20°C. Nevertheless, following freezing at -20°C, chlorophyll fluorescence showed no damage to the chlorophyll. Once again, desiccation played a major role. In hydrated mosses, internal ice nucleation occurred at -12°C, but desiccated mosses showed no evidence of freezing at the lowest temperature of -20°C. There was nothing left to freeze.



Figure 50. *Ceratodon purpureus*, a species that experiences **cytorrhysis** due to desiccation that results from freezing, but chlorophyll remains undamaged. Photo by Janice Glime.

Tolerance to desiccation is one feature that helps bryophytes to survive freezing. Since leaves are generally only one cell thick, and most other parts only a few cells thick, water is easily drawn from the tissues during the slow cooling that occurs in nature. This increases the solute concentration and lowers the freezing point. Hence, intracellular freezing does not occur (Mazur 1969, in Smith 1982). In fact, some mosses are able to photosynthesize at temperatures below 0°C. In **nunataks** (area escaping glaciation) of Queen Maud Land, Antarctica, the air temperature rarely exceeds 0°C, yet moss photosynthesis occurs during the summer as long as there is sufficient water availability (Gjessing & Ovstedal 1989). Narrow clefts and stone blocks shield the mosses from desiccation and maintain less heat loss, but they are also shielded from direct solar radiation most of the time. Nevertheless, short-term periods of warming, even to -2°C, can greatly increase the moss temperature. These microsites permit mosses growing in such severe habitats to have the highest photosynthetic rates.

In the Arctic, *Racomitrium lanuginosum* (Figure 2) has an optimum temperature of 5°C at high light intensities (12,000-15,000 lux), but can sustain photosynthesis down to -10°C (Kallio & Heinonen 1973). Even after exposure to -30°C this moss is able to activate quickly (60% within 3 hours) when warmed. Thus, the bryophytes that exist in such harsh environments as the Antarctic and Arctic must have high freezing resistance, a high resistance to light stress, and a low photosynthetic temperature optimum (Alberdi *et al.* 2002).

In *Marchantia berteroana* (Figure 51), an Antarctic liverwort, freezing greatly reduces photosynthesis, but the author suggested that photosynthesis was also possible at temperatures below freezing (Davey 1997). Rather than temperature, this species is greatly limited by desiccation stress.

Protection of Photosynthetic System from Light

High light intensities at low temperature levels can be extremely damaging to bryophytes that have leaves only one cell thick. Nevertheless, it appears that many, and perhaps most, bryophytes have mechanisms that protect them. In the Antarctic, where such conditions are common, the reversible inhibition present during freezing suggests that mosses such as *Schistidium antarctici* (Figure 43)

have processes that protect them from such photoinhibitory damage (Lovelock *et al.* 1995a) and thus do not require the repair processes that would require temperatures favorable for such repair enzyme activity. Rather, these mosses, when subjected to snow removal, suffered photoinhibition that was reversed when the temperature became warmer (Lovelock *et al.* 1995b). Nevertheless, the greatest recovery occurred in low light. Lovelock and coworkers (1995b) suggest that the photoinhibition during freezing is a protective process that down-regulates photosystem II when photosynthesis cannot keep up with the light-stimulated excitation of electrons.



Figure 51. *Marchantia berteroana*, a species limited by desiccation stress, but freezing only reduces photosynthesis. Photo by Andrew Hodgson, with permission.

Pannewitz *et al.* (2003b) showed similar protection for *Hennediella heimii* (Figure 52) at Canada Flush in Antarctica. Constant meltwater in the summer kept this moss continuously hydrated at near-freezing temperatures while light levels were frequently high. Yet there were no signs of either light saturation or photoinhibition. Rather, the electron transport rate response to light was linear at all temperatures. Pannewitz and coworkers suggested that the moss might be acclimated by building up non-photochemical quenching systems.



Figure 52. *Hennediella heimii*, a very cold-tolerant moss while continuously hydrated. Photo by Barry Stewart, with permission.

For those bryophytes that are epiphytes, it is unlikely that enough mechanisms exist to avoid freezing entirely. But living on a dark tree trunk is likely to mean frequent freeze-thaw cycles. This not only presents problems of desiccation, but also presents potential light damage to the photosynthetic system. Working with the Mediterranean epiphytic moss *Leucodon sciurioides* (Figure 53), Deltoro *et al.* (1999) found that one aspect of bryophyte freeze-thaw survival could be their ability to enhance their non-radiative dissipation of absorbed light energy by freeze-induced decrease in CO₂ fixation, hence protecting their photosynthetic system from excess excitation. This temporary reduction in CO₂ fixation is quickly returned to normal after freezing.



Figure 53. *Leucodon sciurioides* on a tree trunk where it is exposed to atmospheric temperatures all year. Photo by Michael Lüth, with permission.

Rütten and Santarius (1992a) found that photosynthetic apparatus in mature tissues of *Plagiomnium* (Figure 55) species was more frost tolerant than that of either young or old leaves. As freezing stress increased, fluorescence decreased and the photosystem II-mediated electron transport system became inactivated. This resulted in inhibition of electron donations to the photochemical reaction of photosynthesis, differing little from the pattern in tracheophytes. Nevertheless, there was little decrease in transfer of excitation energy through antenna pigments to reaction centers of photosystem II as a result of lethal freezing stress.

Role of Calcium

Calcium seems to play a role in cold tolerance through its role in regulation of membrane transport. In *Physcomitrella patens* (Figure 13), wild type plants respond to cold shock (0-10°C) by increasing cellular content of calcium (Russell *et al.* 1996). It is most likely not calcium itself, but its effect on membrane permeability and other processes in the cell that provide actual protection. In the thallose liverwort *Conocephalum conicum* (Figure 54), Krol *et al.* (2003) likewise found that calcium played a role in climate response. A sudden drop in temperature causes it to generate all-or-none action potentials that appear to be the result of membrane potential changes due to influx of Ca⁺⁺ derived from both internal and external sources.



Figure 54. *Conocephalum conicum*, a species in which membrane potentials change in response to freezing. Photo by Janice Glime.

The activity and thermosensitivity of superoxide dismutase (SOD) is highly sensitive to ions of Ca⁺⁺ and Zn⁺⁺ (Christov & Bakardjieva 1999). In *Plagiomnium affine* (Figure 55), calcium was most important for the one cytosolic and mitochondrial SOD's, whereas zinc was more important for the chloroplastic and two cytosolic SOD's.

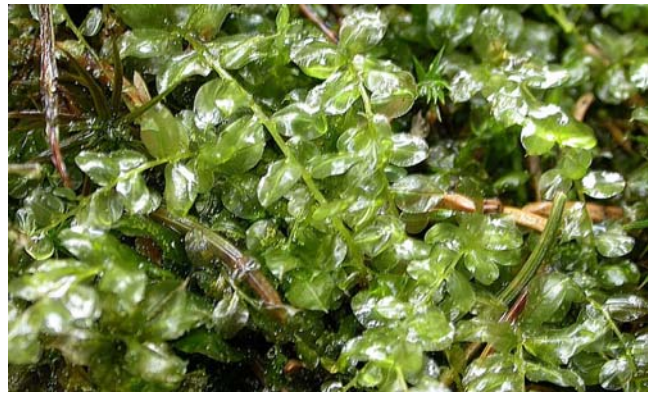


Figure 55. *Plagiomnium affine*, a species that increases its cold tolerance from summer to winter. Photo by Michael Lüth, with permission.

Abscisic Acid

Physcomitrella patens (Figure 13), as in many studies, has contributed to our understanding of freezing protection in bryophytes. When this species was grown on ABA agar, it accumulated up to 22% of its dry weight as sucrose, compared to 3.7% in control (non-ABA) tissues (Davey 1997). Sucrose serves as a protectant during both freezing and drying, but is insufficient as the only agent for freezing protection. When subjected to temperatures down to -80°C, it survived a freeze-cycle only when provided with the cryoprotectant DMSO, a compound that makes membranes more permeable. This species can only survive slow drying, which it does down to 0.02 g H₂O per g DW. Sugar composition and glass transition temperatures differed little between slow and fast drying. Nevertheless, the strength of the hydrogen bonding in the cell's glassy matrix was greater in the slow-drying conditions.

ABA (abscisic acid) is produced in tracheophytes in preparation for cold temperatures and permits plants to survive to lower temperatures, somewhat like antifreeze.

Nagao *et al.* (2005) have shown that media containing ABA does indeed lower the LT₅₀ (temperature at which 50% of cells die) for *Physcomitrella patens* (Figure 13) from -2°C to -10°C and even lower. They observed that there was a "dramatic" alteration in the appearance of the organelles, manifest in slender chloroplasts with reduced starch grains. The vacuoles became segmented rather than the typical single large vacuole. ABA also protected the cells from membrane lesions that occurred in controls at -4°C. One of the mechanisms of protection stimulated by the ABA treatment was an increase in the osmotic concentration of cells of the protonema, most likely due to the increased sugar concentration that accompanied the ABA treatment. But that only tells us what ABA can do. Next we need to determine that mosses do indeed produce it or increase its production at the right time, what stimuli cause this production, and can lunularic acid (ABA analog in liverworts) do the same for liverworts.

But the story does not appear to be straight-forward. Although they reported ABA-induced freezing tolerance in *Physcomitrella patens* (Figure 13) in 2003, Minami *et al.* (2003, 2005) reported that freezing tolerance was not associated with an increase in the level of endogenous abscisic acid in *P. patens*, but that it was associated with increases in the expression of stress-related genes. It seems that the role of ABA is to induce the genes, not to offer protection itself (Nagao *et al.* 2001; Minami *et al.* 2003, 2005). When they subjected protonemata of *P. patens* to -4°C, following normal growth conditions, more than 90% of the cells died, indicating that protonema cells are freezing-sensitive (Minami *et al.* 2003, 2004). ABA treatment resulted in a significant increase in the expression of all PPAR genes within 24 h. These genes are known to participate in the increase of freezing tolerance, and indeed, the death rate decreased significantly.

Minami *et al.* (2005) likewise studied freeze tolerance in *Physcomitrella patens* (Figure 13). They found that in the temperature range of 10°C and 0°C, and especially at 0°C, freeze tolerance increased significantly. But they found that internal tissue levels of ABA did not increase during that acclimation period. Furthermore, removal of ABA by activated charcoal did not affect the developing freeze tolerance. Hence, they concluded that ABA is unimportant in freeze tolerance. I would guess that it is, however, important in surviving the accompanying desiccation.

Transporter Proteins, ABA, and Ca

Further studies on *Physcomitrella patens* (Figure 13) support this conclusion. Two novel transporter-like proteins increase dramatically with low temperature treatment, among other stresses, and increase the cellular tolerance to freezing stress (Takezawa & Minami 2004). It is likely that calmodulin is used by the cell to regulate these novel proteins, and that ABA serves to induce the expression of the necessary genes. However, in *P. patens*, slow freezing to -4°C caused death of more than 90% of the protonema cells (Minami *et al.* 2003). ABA treatment for 24 hours caused a dramatic increase in the freezing

tolerance of this plant, but cold treatment had little effect. This seems to contradict the earlier findings of Nagao *et al.* (2001). They found that both ABA and low temperatures caused an increase in gene expression with concomitant enhancement of freezing tolerance in *Physcomitrella patens*. The LT₅₀ dropped from -2°C to -10°C when the protonemata were grown in a medium with enhanced ABA (Nagao *et al.* 2005). It appears that ABA might be the agent needed to effect expression of the freeze-tolerance genes, but how much advance notice does it require?

Sugars and Plasmolysis

But it appears that ABA also is associated with the increase of soluble sugars in the protonemata of *Physcomitrella patens* (Figure 13) (Nagao *et al.* 2003). Such sugars increase freezing tolerance, most likely by depressing the freezing point.

Rütten and Santarius (1992b) found an increase in cold tolerance from summer to winter in the mosses *Polytrichastrum formosum* (Figure 56), *Atrichum undulatum* (Figure 57), *Plagiomnium undulatum* (Figure 61), *P. affine* (Figure 55), and *Mnium hornum* (Figure 58), and the thallose liverwort *Pellia epiphylla* (Figure 38). The frost resistance between summer and winter differed by more than 25°C in some species, but *Pellia epiphylla* showed little hardening. Concomitant with this increase in frost tolerance, they found a rise in sucrose concentration (except in *Mnium hornum*), and those mosses that were highly frost resistant had a total sugar concentration of 90-140 mM, 80% of which was sucrose. The mosses *Brachythecium rutabulum* (Figure 59) and *Hypnum cupressiforme* (Figure 60) were highly frost tolerant in summer and at that time had high sucrose levels. Furthermore, as sucrose levels declined during artificial exposure to higher temperatures, cold hardiness declined.



Figure 56. *Polytrichastrum formosum*, a species that increases its frost tolerance from summer to winter. Photo by Michael Lüth, with permission.



Figure 57. *Atrichum undulatum*, a species that increases its frost tolerance from summer to winter. Photo by Michael Lüth, with permission.



Figure 58. *Mnium hornum*, a species that increases its cold tolerance from summer to winter. Photo by Michael Lüth, with permission.



Figure 59. *Brachythecium rutabulum*, a species that is highly frost tolerant in summer. Photo by Michael Lüth, with permission.



Figure 60. *Hypnum cupressiforme*, a species that is highly frost tolerant in summer. Photo by Michael Lüth, with permission.



Figure 61. *Plagiomnium undulatum*, a species that increases its cold tolerance from summer to winter. Photo by Michael Lüth, with permission.

However, Rütten and Santarius (1993a) found that different levels of sucrose, glucose, and fructose at the cellular level had no bearing on the frost tolerance of leaves of *Plagiomnium affine* (Figure 55) and *P. undulatum* (Figure 61). Sucrose seemed to contribute in some way to the tolerance, increasing from summer to winter, while temperature limits increased from -10°C in summer to less than -35°C in winter, but there was no correlation between increased sugar content of shoots and frost resistance. They concluded that other factors were also necessary to the increased frost tolerance.

Studies on membrane permeability suggest that sugar uptake and release may be altered as mosses prepare for winter (Rütten & Santarius 1993b). Liu (2000) showed that as the temperature increased above 40°C in these and other species, the membrane permeability increased. At the cold end of the scale, it appears that protection against an increase in membrane permeability may be a necessary step in cold hardiness. Greater retention of sugars could account for the higher concentrations in cold temperatures.

On the other hand, reversible plasmolysis can protect cells by permitting water loss and preventing crystal damage.

This relationship to membrane permeability is supported by studies on *Physcomitrella patens* (Figure 13) (Minami *et al.* 2003). Minami and coworkers subjected protonema cells to hyperosmotic concentrations of NaCl and mannitol, causing an increase in freezing tolerance. They interpreted this increase to indicate that ABA and cold stress trigger the expression of cryoprotectant genes. Oldenhof *et al.* (2006) suggested that sucrose might act as an osmotic spacer in membranes, while at the same time ABA mediates the synthesis of proteins, strengthening the cellular glasses. But we know that ABA can cause membranes to leak. Might there still be a more direct role for ABA than simply a trigger for genes, or is its usual role in membrane leakage one of triggering genes that cause this response?

Aro and Karunen (1988), in studying protonemata of *Ceratodon purpureus* (Figure 50), found that the content and unsaturated level of membrane lipids increased significantly in low growth temperatures, apparently contributing to frost hardiness. Hakala and Sewón (1992) found that both drought and low temperatures (6°C) caused an increased incorporation of ^{14}C into the neutral lipid fraction and decreased its incorporation into the glycolipid fraction in *Dicranum elongatum* (Figure 62), suggesting a preferential accumulation of acetylenic triacylglycerols. Such responses, when adaptive, can permit the moss to prepare for the drought of winter through the signal of low temperature.



Figure 62. *Dicranum elongatum*, a subarctic moss. Photo by Michael Lüth, with permission.

The protonema stage is often ignored in understanding the ecology and physiology of bryophytes. Yet if it is unable to reach a mature state of development and produce gametophores, the species will be greatly limited in its establishment survival. Nagao *et al.* (2006) demonstrated that like the leafy plant, the protonema responds to ABA application, increasing its freezing tolerance. This response includes the accumulation of low-molecular-weight soluble sugars, including theanderose (G6- α -glucosyl sucrose). This accumulation was inhibited by an inhibitor of nuclear-encoded protein synthesis (cycloheximide), resulting in a marked decrease in freezing tolerance. Theanderose is

promoted by cold acclimation and by treatment with hyperosmotic solutes, both of which increase cellular freezing tolerance.

Freezing Longevity

Just how long can a bryophyte remain frozen and survive? In the Antarctic on Signy Island, *Chorisodontium aciphyllum* (Figure 63-Figure 64) and *Polytrichum strictum* (= *P. alpestre*; Figure 65) form a major part of the vegetation. Recently, Roads and Longton (2013) reported *C. aciphyllum* that was extracted from a core at 138 cm depth. This depth remains permanently frozen. There was no great surprise that regrowth occurred from specimens of *C. aciphyllum* retrieved from depths of 0-30 cm, but three new shoots grew from specimens extracted from 110 cm! And in addition the leafy liverwort *Cephaloziella varians* (Figure 66) regenerated new shoots from the muddy base of that core at 123-138 cm. Based on radiocarbon dating, these plants had been there ~1750 years and had been frozen a good portion of that time!



Figure 63. *Chorisodontium aciphyllum* in Antarctica, a species that apparently can remain viable in a frozen state for more than 1700 years! Photo from the Polar Institute through Creative Commons.



Figure 64. *Chorisodontium aciphyllum*, a species that regenerated from a frozen state after more than 1700 years! Photo from the Jan-Peter Frahm, with permission.



Figure 65. *Polytrichum strictum*, a major component of the Antarctic flora. Photo by Michael Lüth, with permission.



Figure 66. *Cephaloziella varians*, a species that regenerated from 1750 year old cores in Antarctica, here nestled among *Polytrichaceae*. Photo by Kristian Peters, with permission.

Internal Cushion Temperatures

The internal temperatures in bryophyte cushions follow the ambient temperature until the cushion temperature drops below 0°C and the water there begins to freeze (Rod Seppelt, Bryonet 27 June 2022). When the cushion begins to freeze, latent heat is released, elevating the cushion temperature ~2°C. This slows freezing and provides more time for physiological processes to acclimate, including that of protein partial denaturing. These physiological modifications reduce tissue damage.

Freezing Effects

Freezing can have many consequences on cells of plants. In bryophytes, it can cause disorganization of the chloroplast lamellae, thus damaging the photosynthetic system (Pihakaski & Pihakaski 1979), damage the cell membranes, and cause desiccation and loss of solutes. In the thallose liverwort *Pellia epiphylla* (Figure 38) that had been chilled and hardened at -22°C, ultrastructural changes occurred. Vacuoles contained a fine granular substance in hardened tissues. Those that had only been chilled had large electron-dense particles embedded in a finer granular substance. The oil bodies changed, with abundant lipid-

like bodies in the cytoplasm. These resembled the oil globules of oil bodies, with oily-looking flecks in the vacuoles. Large starch grains were present in the chloroplasts and the lamellar system lost some of its organization. Interestingly, the net photosynthesis was highest in material that had spent the longest time at -22°C.

Supercooling Intracellular Water

But what is it that permits plants to survive the sub-zero temperatures of winter? One of the first requirements for survival at below freezing temperatures is supercooling of intracellular water (George & Burke 1977). If the water in the cells were to freeze, ice crystals and expansion of water in its frozen state could cause mechanical damage to the cell. We can observe that many trees have as their northern limit the line where -40°C is rarely reached. This is significant since the lower limit for supercooling of water is -41°C (Kuiper 1978), and George and Burke (1977) have observed ice formation in xylem at -30 to -40°C.

Ice Crystals Increase Solutes

Although ice crystals outside the cells can kill plants by desiccation, as in the case of the Florida orange trees, they can also be a means of "winterizing" cells by increasing internal solute concentrations. Molecules have vibrational energy. When an ice crystal forms, the vibrational energy is much reduced, creating an energy gradient between the liquid water molecules in the cell and the crystallized ones outside it (Marchand 1991). The result is that the more active liquid molecules migrate toward the area of less energy on the outside of the cell, adding to the mass of the crystals. Of course the result inside the cell is an increase in concentration of cytoplasmic solutes, thus lowering its freezing point, just as antifreeze does in a car battery. The process of protein denaturation, discussed below, causes the membranes to be leaky, facilitating this emigration of water. In many cells, there seems to be a second change as the temperature continues to decrease, and that change seems to correspond with cell death. One theory suggests that this may be accompanied by failure of water to leave the cell, resulting in internal crystallization and membrane destruction. Even in the absence of internal crystallization, cells still face another problem as the temperature decreases. As additional water is lost, irreversible dehydration may occur and toxic concentrations of solutes may accumulate (Weiser 1970).

Crystal Damage

It is the formation of crystals, not the low temperature itself, that damages cells irreparably, whether it is external crystals that cause dehydration and toxicity, or internal crystals that physically disrupt cell membranes (Schmitt *et al.* 1985). Therefore, another possibility exists for at least some plants to survive the cold, a process called **glass formation** (Marchand 1991). Glass formation results from **vitrification**, in which water solidifies without reorienting into a crystal (Figure 1). This process occurs when we immerse tissue in liquid nitrogen and thus permits us to preserve tissues without ice crystal damage. Balsam poplar trees are known to "form glass" at temperatures below -28°C (Hirsh *et al.* 1985). This means that the contents of

the cell are solid, thus preventing crystal damage, desiccation, and concentration of solutes to toxic levels.

Preventing Ice Crystals

Growers protect oranges by spraying non-nucleating bacteria on them, thus out-competing the bacteria that form the centers for ice crystals on the oranges. Some frogs make tiny proteins that become the centers of small crystals rather than large ones. And it appears that bryophytes and algae may also form special proteins that diminish crystal damage to cells.

One of the means by which plant cells are able to protect themselves from freeze damage is to modify or prevent ice crystals. Crystals form around tiny "nuclei" such as dust particles and bacteria. Being hygroscopic, these crystals grow by taking moisture from their surroundings, including cells. On the outside of the cell, they can desiccate a cell by extracting the water and binding it to the crystal. Inside the cell, they can not only desiccate the cell, but can also cause physical harm by protruding through a cell membrane.

In the Antarctic, Cyanobacteria, algae, and mosses form macromolecular substances that modify growing ice crystals, causing pitting of the crystals, and that cause them to go through an ice phase during freezing (Raymond & Fritsen 2000) – **glass formation** (Figure 67). One Antarctic species of *Bryum* (Figure 68) can modify these crystals by using this macromolecular substance to modify the shape of the growing crystals, and it may be that the mechanism of these macromolecules is to prevent recrystallization of ice (Raymond & Fritsen 2001). These substances are absent in temperate Cyanobacteria and mosses, but do occur in mosses from cold North American habitats. Their actual role is unknown, but their ability to be destroyed by temperatures of 45-65°C suggests that they are protein. It is possible that they may be non-nucleating proteins that reduce crystal formation.



Figure 67. *Hedwigia ciliata* with glass formation (ice) on the surface rather than ice crystals. Photo by Michael Lüth, with permission.



Figure 68. *Bryum cryophilum*, showing the red pigments common in polar regions. One species of *Bryum* can modify ice crystals, somehow reducing damage to the plant cells. Photo by Michael Lüth, with permission.

Rate of Freezing

The effectiveness with which these mechanisms can protect the cell are dependent upon the rate of freezing. White and Weiser (1964) found that leaves on the southwest side of a tree could drop in temperature by 9.5°C per minute across the freezing point of cell water at sunset! The result of this rapid freezing was cell death due to crystallization of water trapped inside the cell. Yet the same species was able to tolerate temperatures as low as -87°C when the temperature decreased slowly. Marchand (1991) contends that slow cooling of 10°C per hour is common in nature and permits time for the removal of water from cells by exterior crystal formation.

But what do all these tracheophyte scenarios mean for bryophytes? In 1912 Irmischer reported that at least some mosses were tolerant to desiccation and cold. Antropova (1974) found that temperatures above optimum for 3 hours did not affect cold resistance of moss cells, nor did temperatures within the optimum range influence either thermal stability or cold resistance. From these experiments he deduced that bryophytes respond similarly to tracheophytes but differently from algae to changes in temperature.

But the cooling process in bryophytes is different from that of tracheophytes (Dilks & Proctor 1975). If a tracheophyte cell is cooled rapidly, the cell contents freeze, and this usually causes fatal damage to the cell. However, the normal condition in nature is slow cooling. Because mosses and liverworts lack protective cells or thick, waxy cuticles, and are mostly one cell thick, this process is much more rapid. As the ambient temperature cools to below freezing, bryophyte cell contents will supercool and lose water to the surroundings, depending on the water-potential gradient. Levitt (1972) found that the injurious freezing rate for cell sections of tracheophytes is 60 times as rapid as for whole plants. Since bryophytes are much like a section of tracheophytes, they could experience a similar rapid freeze, one that could occur during a sudden drop in temperature, making bryophytes more vulnerable than tracheophytes. However, as water freezes outside bryophyte cells, the internal freezing point decreases due to loss of water and increasing concentration of cell sap (Dilks & Proctor 1975). And here tracheophytes have a disadvantage compared to bryophytes. Rather, they are

inhibited from water loss by a hydrophobic cuticle, and even if they accomplished this loss, their cells are more likely than those of bryophytes to be damaged by desiccation. Hence, cells high in water content and having little waxy cuticle for protection, like those of lettuce, turn to mush when frozen.

Among the bryophytes compared in Figure 69, the mosses *Hookeria lucens* (Figure 70) and *Plagiothecium undulatum* (Figure 71) are the most like wet filter paper, with a plateau in cellular cooling as the cell reaches the freezing temperature of water and water leaves the cell. The thallose liverwort (*Conocephalum conicum*, Figure 54), on the other hand, is more similar to the tracheophyte *Arbutus unedo* (Figure 72), with a slow decline in temperature below the freezing point of water.

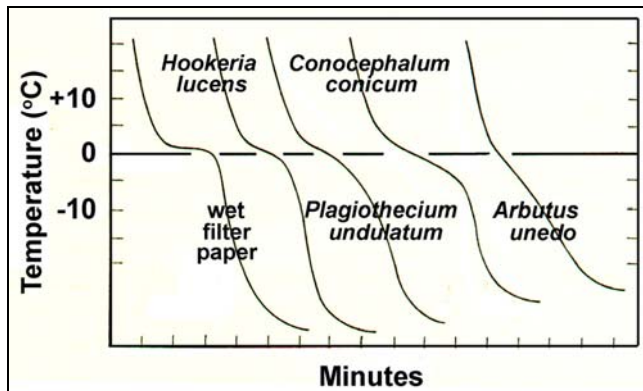


Figure 69. Temperature drop of bryophyte leaf cells compared to that of filter paper and a tree species (*Arbutus unedo* – the strawberry tree). Redrawn from Dilks and Proctor 1975.



Figure 70. *Hookeria lucens*. Photo by Michael Lüth, with permission.



Figure 71. *Plagiothecium undulatum*. Photo by Michael Lüth, with permission.



Figure 72. *Arbutus unedo* (strawberry tree). Photo by Richie Steffen, courtesy Great Plant Picks, with online permission.

Hydration State

The state of hydration is an important consideration in the tolerance of bryophytes to temperature. It is well-known that they tolerate much higher temperatures in the dry state, but they also often tolerate lower temperatures in the dry state as well. This is predictable because of the danger of water forming crystals that can harm membranes.

Dilks and Proctor (1975) subjected nine moss species and one thallose liverwort species to sub-zero temperatures in a desiccator at 32% relative humidity. All survived to -30°C in this dry state except the cushion moss *Leucobryum glaucum* (Figure 73) and leafy liverwort *Plagiochila asplenoides* (Figure 74) var. *major*, both of which died in the desiccator with and without the cold treatment. In the wet state, however, of the 27 mosses tested, 20 had 50% or more death at -10°C and lower. For three of the taxa (*Andreaea* spp., Figure 75), the status could not be determined. *Hylocomium splendens* (Figure 76), *Racomitrium aquaticum* (Figure 77), *R. lanuginosum* (Figure 2), and *Scorpiurium circinatum* (Figure 78) survived to -10°C. *Hookeria lucens* (Figure 71), *Leucobryum glaucum* (Figure 73), *Mnium hornum* (Figure 58), and *Plagiopus oederianus* (Figure 79) were dead or mostly dead at -5°C. Among the liverworts, none of the thallose liverworts survived at -5°C. Among the leafy liverworts, four species survived as well as the mosses, but two had more than 50% mortality at -5°C. Only *Plagiochila spinulosa* (Figure 80) survived to -10°, with 50% survival. It is interesting that such epiphytes as *Porella platyphylla* (Figure 81) had poor survival when moist at -5°C, because that leafy liverwort lives in northern habitats where it is likely to experience such conditions in the winter, but perhaps acclimation and physiological races differ.



Figure 73. *Leucobryum glaucum*, a species that died in the desiccator (32% RH) in a cold treatment to -30°C . Photo by Janice Glime.



Figure 76. *Hylocomium splendens*, a species that survived to -10°C in the lab. Photo by Michael Lüth, with permission.



Figure 74. *Plagiochila asplenioides*, a species that died in the desiccator (32% RH) in a cold treatment to -30°C . Photo by Michael Lüth, with permission.



Figure 77. *Racomitrium aquaticum*, a species that survived to -10°C in the lab. Photo by Michael Lüth, with permission.



Figure 75. *Andreaea nivalis*. In experiments to -30°C and 32% RH, effects on three species in this genus were inconclusive. Photo by Michael Lüth, with permission.



Figure 78. *Scorpiurium circinatum*, a species that survived to -10°C in the lab. Photo by Michael Lüth, with permission.



Figure 79. *Plagiopus oederianus*. Photo by Michael Lüth, with permission.

These data suggest that mosses are more tolerant of wet cold than liverworts and that the thallose liverworts are the most vulnerable.

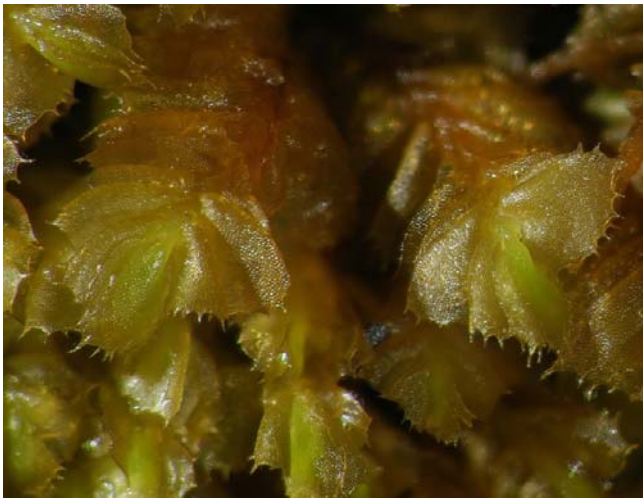


Figure 80. *Plagiochila spinulosa* in Scotland, a species with more than 50% survival at -10°C . Photo by Michael Lüth, with permission.



Figure 81. *Porella platyphylla*, a species that has poor survival if moist at -5°C . Photo by Michael Lüth, with permission.

Lipids in Membranes and Protein Denaturation

We know that bryophytes are able to exist farther north (and south) than woody plants and yet lack the insulating effects of a thick layer of bark. Furthermore, the plasma membrane must remain intact if cellular nutrients and other solutes are to be contained upon thawing. As the temperature drops, the lipid matrix of a plasma membrane can crystallize, and the degree of crystallization depends upon the types of lipids. Saturated lipids crystallize first, with less saturated ones crystallizing at lower temperatures. The crystallization causes membrane proteins to aggregate, setting off a chain reaction. These aggregated proteins make possible the oxidation of sulfhydryl groups of the protein molecules because the close contact permits the formation of disulfide bridges (Levitt 1969). This denaturation of the membrane protein is irreversible and results in membrane destruction, often leading to cell death. It seems then that bryophytes must have some means to prevent this series of events from occurring.

Tracheophytes typically increase their lipid content in response to decreasing temperatures, resulting in winter hardiness. The lipids phosphatidyl choline and phosphatidyl ethanolamine in particular seem to contribute to increased resistance to cold (Kuiper 1970; Yoshida 1974; Siminovitch *et al.* 1975; De La Roche *et al.* 1972, 1975; Willemot 1975). The unsaturated fatty acid linolenic acid likewise seems to play a major role in reducing frost damage (Kuiper 1978).

Unsaturated Lipids

Gellerman and coworkers (1972) reported highly unsaturated lipids in several genera of bryophytes. When Al-Hasan and coworkers (1989) examined *Bryum bicolor* (Figure 82) to determine the effects of temperature on cold hardening, they found that the lipids of this species contained higher proportions of digalactosyldiacyl glycerols and sulfoquinovosyldiacyl glycerols when incubated at 5°C than when plants were incubated at 25°C . An interesting and seemingly non-adaptive aside is the greater production of linolenic acid under continuous illumination at 5°C , since low temperatures generally coincide with short days.



Figure 82. *Bryum bicolor*, a species that has higher concentrations of digalactosyldiacyl glycerols and sulfoquinovosyldiacyl glycerols when incubated at 5°C than when incubated at 25°C . Photo by Michael Lüth, with permission.

Fatty Acid Alterations

One of the means by which organisms prepare for changes in temperature is to alter fatty acid components to those with lower solidification points. Lemmings change the fatty acids in their foot pads by eating bryophytes that contain lots of arachidonic acids, thus providing these tissues with cell membranes that are more pliable at low temperatures (Prins 1981). Meanwhile, the bryophytes are also preparing for winter in a different way.

The protonema of the common moss *Ceratodon purpureus* (Figure 50) prepares for winter by increasing its content and unsaturated level of membrane lipids (Aro & Karunen 1988). The galactolipids typically found in chloroplast membranes increased; phospholipids nearly doubled when plants were acclimated at 4°C vs 20°C. But this seems to have little effect on the frost hardness. Rather, it permits these acclimated protonemata to retain a high phospholipid content. If, as is typical of unhardened protonemata, the phospholipids had been lost, that would have caused irreversible damage to CO₂ fixation following freezing and thawing. Aro and Karunen concluded that while the changes in membrane lipids were themselves not an important component of hardening, they were somehow involved in other factors that contributed to frost hardness.

In *Sphagnum fimbriatum* (Figure 83-Figure 84), when the temperature decreases in the range of 5-15°C, the amounts of linoleic, α linolenic, and arachidonic acids in their glycolipids [both monogalactosyldiacyl glycerols (MGDG) and digalactosyldiacyl glycerols (DGDG)] also decrease (Koskimies-Soininen & Nyberg 1991). These are replaced with increased proportions of palmitic, stearic, and oleic acids, especially in MGDG. However, if light intensity also decreases, as it would as winter approaches, this species exhibits an increase not only of palmitic and stearic acids, but also of linolenic and arachidonic acids, in MGDG, while oleic and α -linolenic acids decrease. But this pattern is certainly not universal. Even the related *S. magellanicum* (Figure 85) responds differently (Koskimies-Soininen & Nyberg 1987). It had its largest changes in fatty acid composition at lower temperatures (0-5°C) and short photoperiods (3-6 hrs daylight). But, unlike *S. fimbriatum*, in decreasing light and temperatures, *S. magellanicum* exhibited a decrease in linolenic acid.



Figure 83. *Sphagnum fimbriatum* frozen in its habitat. Photo by Dick Haaksma, with permission.



Figure 84. *Sphagnum fimbriatum*, a species that decreases its concentrations of various fatty acids, including arachidonic acids, when the temperature decreases in the range of 5-15°C. Photo by Michael Lüth, with permission.



Figure 85. *Sphagnum magellanicum*, a species that has its largest changes in fatty acids at 0-5°C. Photo by Michael Lüth, with permission.

There are indications that the fatty acid composition of bryophyte cells change as the temperatures do (Saruwatari *et al.* 1999). *Marchantia polymorpha* (Figure 42) exhibited changes in the percentages in linolenic acid, arachidonic acid, and eicosapentaenoic acid when the temperature was changed from 25°C to 15°C. Both linolenic acid and eicosapentaenoic acid increased greatly. However, the changes were not equal throughout the cell. Arachidonic acid and eicosapentaenoic acid increased in the chloroplast fraction but not in the rest of the cell, while the level of linolenic acid was increased in both fractions. We need to understand this in the context of the high levels of arachidonic acids known in bryophytes and the suggestion that some animals eat bryophytes to prepare for winter because of these high levels. Prins (1982) has proposed that they provide more fluid fat pads for animals that run around on frozen ground in winter.

One study on lichens might help us predict the way in which bryophytes could respond (Dertien *et al.* 1977). In forested areas, both bryophytes and lichens can be found on tree trunks as well as on the forest floor and in open soil areas. In their study of lichens, Dertien and coworkers (1977) found that lichens of tree trunks contained high levels of the unsaturated linoleic and linolenic acids; however, nearby sand dune species had large quantities of cyclic acids rather than unsaturated acids. This may relate

to the greater likelihood of low temperatures on the tree trunks.

Fatty Acids and N

Using *Ctenidium molluscum* (Figure 86), *Pogonatum urnigerum* (Figure 87), *Dichodontium pellucidum* (Figure 88), and *Tortella tortuosa* (Figure 89), Al-Hasan *et al.* (1991) demonstrated that increasing the nitrogen concentration of the medium causes a decrease in the dominant unsaturated fatty acids arachidonic acid (in *C. molluscum*), eicosatrienic acid (in *P. urnigerum*), and linoleic acid (*D. pellucidum*, *T. tortuosa*). Nitrogen availability generally decreases as the growing season progresses in forests, so it is possible that such a decrease could serve as a signal for mosses to store more unsaturated fatty acids. Arachidonic acid and eicosapentaenoic acid are widespread in mosses (Hansen & Rossi 1990), but arachidonic acid never occurs in angiosperms (Karunen 1990).



Figure 86. *Ctenidium molluscum* in a rock canyon in Europe. This species seems to switch to more unsaturated fatty acids when N concentrations decrease at the end of the growing season. Photo by Michael Lüth, with permission.



Figure 87. *Pogonatum urnigerum*, a species that seems to switch to more unsaturated fatty acids when N concentrations decrease at the end of the growing season. Photo by Janice Glime.



Figure 88. *Dichodontium pellucidum*, a species that seems to switch to more unsaturated fatty acids when N concentrations decrease at the end of the growing season. Photo by Michael Lüth, with permission.



Figure 89. *Tortella tortuosa*, a species that seems to switch to more unsaturated fatty acids when N concentrations decrease at the end of the growing season. Photo by Michael Lüth, with permission.

Triglycerides

The role of triglycerides in low temperature survival seems yet to be explored. Karunen (1981) found that in the subarctic moss *Dicranum elongatum* (Figure 62) triglycerides commonly increased only at low temperatures of 1-6°C. But what might they do for frost hardiness?

Polyribosomes

In the desiccation-tolerant moss *Syntrichia ruralis* (Figure 48-Figure 49), temperatures down to 2°C cause a proliferation of polyribosomes, accompanied by a decrease in single ribosomes (Malek & Bewley 1978). The number of ribosomal subunits does not change. Mosses that have not been desiccated exhibit leucine uptake and were able to synthesize protein at 2° and -2.5°C. However, slowly dried mosses do not contain polyribosomes and instead reform them upon rehydration. There seems to be no change in the

rate of protein synthesis in mosses kept at cold temperatures (2°C) or winter collected. Rather, the moss appears to be pre-acclimated or pre-adapted to freezing year-round. Malek and Bewley concluded that this moss does not have any seasonal cold hardening.

Age Difference to Freezing

Hudson and Brustkern (1965) found that old and young leaves of mosses may differ in their responses to sub-zero temperatures. They found that *Plagiomnium undulatum* (Figure 61) mature leaves experienced extracellular freezing when cooled slowly, thus preventing intracellular freezing. Young shoots, on the other hand, could not tolerate temperatures below 12°C. When subjected to freezing temperatures, young leaves of *P. undulatum* do not experience extracellular ice formation, thus making intracellular freezing more likely. Rütten and Santarius (1992a) found that not only young leaves, but also old leaves of *Plagiomnium*, had much less frost tolerance than mature leaves.

Freezing Effect on Distribution and Niche

Green *et al.* (2007) reported that freeze-thaw cycles are extremely common in parts of Antarctica, with up to 110 occurring in a single year in the northern maritime. Some lichens adapt to this by growing in the pores of rocks, so we should look for bryophytes that have similar safety mechanisms.

The ability to survive freezing will influence both geographic and habitat distribution of bryophytes. Shirasaki (1984) found that *Bryoxiphium norvegicum* (Figure 90) subsp. *japonicum* is distributed in southern Japan at altitudes of 80 m to 2350 m, whereas further north the upper limit declines. Although this species occurs in areas where there is deep snow for a long period of time, it lives mostly on the vertical faces of overhanging rocks in ravines where it is not likely to be covered directly by snow. However, it is positioned where the overhanging soil and snow protect it from the cold wind.

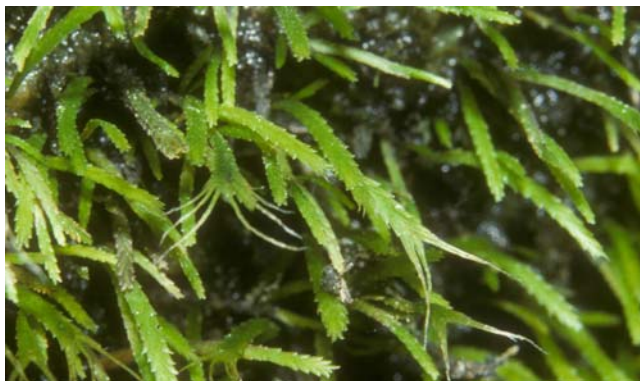


Figure 90. *Bryoxiphium norvegicum* growing on a rock face. Some varieties of this species are able to grow at high elevations. Photo by Janice Glime.

Shirasaki (1987) also found that the distributions of the leafy liverworts *Bazzania trilobata* (Figure 91) and *B. yoshinagana* (Figure 92) in Japan seem to relate to differences in cold and related desiccation tolerance. *Bazzania trilobata* grows on soil that receives sunshine and

good drainage. It is able to survive in areas with little snow where early spring subjects it to severe cold and desiccation. By contrast, *B. yoshinagana* lives primarily on the floor of dense conifer forests where deep snow covers it all winter, thus maintaining moisture and insulating it from the sub-freezing air.



Figure 91. *Bazzania trilobata*, a species that grows in areas that have little snow where early spring subjects it to severe cold and desiccation. Photo by Michael Lüth, with permission.



Figure 92. *Bazzania yoshinagana*, a species is covered by deep snow all winter. Photo by Real thing X 0.3. The copyright of the photograph of this site belongs to the author. Please reprint without permission.

As was seen for *Fontinalis* (Figure 123-Figure 124) species in the previous subchapter on temperature, adaptation to cold can be a contributing difference between species, permitting them to live where they do. It seemed that for centuries we concentrated on morphological differences between species and attempted to see their geographic separations in that perspective. However, physiological differences are much more likely to determine where plants live than are their morphological differences. In some cases, morphology can cause

physiological differences, such as growth forms that alter temperature, but we should not stop there in our quest for niche delineation.

A good demonstration of these physiological differences is seen in the genus *Sphagnum*. In their study of five species, Balagurova *et al.* (1996) found that the photosynthetic leaf cells of *Sphagnum balticum* (Figure 93), *S. subsecundum* (Figure 94), and *S. teres* (Figure 95) were more frost-resistant than were those of *S. magellanicum* (Figure 85) and *S. fuscum* (Figure 96).



Figure 93. *Sphagnum balticum*, a species that is more frost-resistant than the hummock species *S. magellanicum* and *S. fuscum*. Photo by Michael Lüth, with permission.



Figure 94. *Sphagnum subsecundum*, a species that is more frost-resistant than the hummock species *S. magellanicum* and *S. fuscum*. Photo by Michael Lüth, with permission.



Figure 95. *Sphagnum teres*, a species that is more frost-resistant than the hummock species *S. magellanicum* and *S. fuscum*. Photo by Michael Lüth, with permission.



Figure 96. *Sphagnum fuscum*, a hummock species that is somewhat frost-sensitive. Photo courtesy of Andres Baron Lopez.

For the sunny species of *Sphagnum magellanicum* (Figure 85) and *S. papillosum* (Figure 97), short days induce dormancy and long days induce growth (Li & Glime 1990; Gerdol 1995). This corresponds well to their optimum growth temperature of 30-35°C, a high optimum for bryophytes. Nevertheless, *Sphagnum magellanicum* can grow actively whenever it has sufficient moisture and the nighttime temperature exceeds 0°C (Gerdol 1996). It appears that nighttime temperature can be critical to the growth of *Sphagnum* species. *Sphagnum capillifolium* (Figure 24) suffered a five-fold reduction in growth at low nighttime temperatures (Gerdol *et al.* 1998). There seemed to be no alteration in photosynthetic pigments or pigment ratios, but rather enzymatic reactions were limited at low temperatures.



Figure 97. *Sphagnum magellanicum* (red) and *S. papillosum* (olive-green) growing together on a sunny hummock. Those on the right are wet and on the left they are dry. Photo by Janice Glime.

Regulation of Mammal Reproduction?

There is interesting evidence that some plants stimulate reproductive activity in small mammals that eat them by providing to them their own growth substances. Gibberellic acid, common in germinating seeds, and 6-methoxybenzoxazolinone (6-MBOA, a glycoside derivative) have such an effect. Is it possible that bryophytes, developing under the snow, provide a source of

green food to small mammals, such as voles and lemmings, under the snow pack and help to regulate their reproductive cycle?

Overwintering under Snow

Snow affords great protection from the ravages of winter, and we might have a very different polar and boreal flora without it. Flock (1978) found that it was the areas with deep, late-season snow where bryophytes reached their highest species indices on the Niwot Ridge of Colorado, USA, an alpine area. An interesting separation of acrocarpous and pleurocarpous mosses occurred, with acrocarpous mosses being the most abundant ones in the dry areas that had only a light snow cover. Pleurocarpous mosses were nearly restricted to the wet sites with deep snow, where they outnumbered the acrocarpous taxa. Only *Hypnum vaucheri* (Figure 98-Figure 99), *H. revolutum* (Figure 100), and *Abietinella abietinum* (Figure 101) among the pleurocarpous mosses ventured into the dry areas with little snow. Liverworts were rare. This distribution may be more one of moisture needs than of temperature, but at least the possibility exists for some mosses to enjoy the greater protection from extreme cold when most of the area may be free of snow.



Figure 98. *Hypnum vaucheri* habitat in Aversal Graubünden, a species that survives winter in areas with little snow. Photo by Michael Lüth, with permission.



Figure 99. *Hypnum vaucheri* in Norway. Photo by Michael Lüth, with permission.



Figure 100. *Hypnum revolutum*, a species that is able to survive in cold but dry areas with little snow. Photo by Michael Lüth, with permission.

On the other hand, snow cover can be a detriment when the growing season is short, preventing sufficient productivity to complete a life cycle. In the Antarctic, Pannewitz *et al.* (2003a) found that indeed the snow cover was a good insulator, but late-lying snow retained the winter cold that kept the bryophytes inactive long after the ambient air temperature was warm enough for activity. Unlike some north temperate areas where the sub-surface soil may be 10°C in the winter (Jiquan Chen, University of Toledo, unpublished data), temperatures under the Antarctic snow were typically less than -10°C while snowmelt was complete in surrounding areas.



Figure 101. *Abietinella abietina*. Photo by Michael Lüth, with permission.

Snow Temperatures

In the temperate and boreal zones, winter cold and snow can play a major role in ecosystem behavior. Snow cover can be an essential factor in protecting plants from severe cold and wind, while in many cases providing a steady stream of water and nutrients to the soil. Soil temperatures at 5 cm beneath the surface under deep snow in Houghton, Michigan, USA, can remain above freezing for an entire winter while air temperatures plummet to -10°C or lower (Jiquan Chen, University of Toledo, unpublished data). Longton (1979) reported temperatures at the moss level (*Polytrichum strictum*; Figure 65) at Pinawa, Manitoba, Canada, to remain between 0° and -10°C under the winter snow, but in summer sun the diurnal temperatures fluctuated widely. The mosses clearly ameliorate the temperature in spring and autumn, with

fewer freeze-thaw cycles and higher minimum temperatures at moss levels than at 200 cm above the ground.

We have mostly ignored winter ecology in bryophytes. Do they photosynthesize under the snow? How will they respond to shorter winters as the climate warms? Are any or all of them dormant in cold winters? What is the effect of snow depth on their physiological behavior?

Pannewitz *et al.* (2003a) found that it was possible to measure photosynthetic activity of lichens under undisturbed snow. The snow cover provides a good insulator for both lichens and mosses. But this insulation works at both warming and cooling temperatures. These lichens were inactive at subzero temperatures, even when the air temperatures had warmed enough to allow metabolic activity. For lichens, the maximum activity occurred during the 10-14 days following the final disappearance of the snow. But some species were activated at temperatures below -10°C when water became available from the high air humidity. Similar accounts of bryophyte responses are largely lacking in most winter ecosystems.

Nutrients from Snow

Inputs and losses of soil nutrients change as temperatures slow processes and snow melt leaches nutrients from collected dust. During January to March, nitrate export can increase from 0 to 1 kg ha^{-1} as the temperature increases from -10 to -3°C (Park *et al.* 2004 in Campbell *et al.* 2005).

These processes will certainly affect the mosses, positioned at the interface between snow and soil. In her studies on *Sphagnum russowii* (Figure 102) in a Jack pine forest (*Pinus banksiana*), Scafione (unpubl) found that the mosses were frozen in a block of ice under the snow as the melt season began in April. But is this the case all winter? Do the mosses receive nutrients that trickle through the snow, trapping them and sequestering them for an early spring surge of growth? Or do they remain frozen until after the snow is gone, facilitating the movement of nutrients past them to breaks in the ice-covered moss carpet? Figure 103 suggests that they don't. How little we know of their winter ecology!



Figure 102. *Sphagnum russowii*, a species that can freeze in a block of ice and survive. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.



Figure 103. *Racomitrium lanuginosum* emerges from the snow unfrozen and in good health. Photo by Michael Lüth, with permission.

Epiphytes

Mosses in the North Temperate Zone seem to appear in the spring in a much fresher condition than they were in the previous fall, and some of them seem to be further developed. Our data on epiphytes in Keweenaw County, Michigan, USA, suggest that perhaps winter affords them an opportunity to grow in a moist, light environment, protected from winter winds (Trynoski & Glime 1982). We suggested this possibility because, contrary to the popular misconception, the mosses were more abundant on the south side of the trees at 1 m above the ground. In Keweenaw County, the winds come predominately from the north and northwest, bringing desiccation to mosses on that side of the tree. Of course, the south side of the tree is subject to the drying heat of the sun in the summer, but only if the canopy allows it to pass. Our conjecture is that in winter the deep snow (1 m or more) provides a haven. Snow cover does not hug a tree all the way to the surface of the snow. Instead, it forms **tree wells**, where snow is separated from the tree trunk by a small funnel of air, caused at least in part by the reradiation of heat from the dark trunk of the tree (Figure 104). Within this funnel, there is little air movement, and if our theory about the reradiation is correct, the temperature must be near melting, *i.e.* 0°C . Under such conditions, we would assume that the funnel must be moist in winter, at least on sunny days. On the south side of the tree, the temperature would be higher, causing more hours of moist air and above freezing temperatures. Furthermore, sun penetration through the snow should provide ample light at this low temperature. Under such circumstances, we conjecture that mosses could achieve a slow but steady growth during 4-5 months of winter.



Figure 104. Tree well at the base of *Acer platanoides*. Although the snow has melted considerably, this shows the funnel that can form. Photo by Janice Glime.

As we pondered the tree funnels, we also considered that mosses on rocks and soil under the snow probably receive a relatively steady moisture supply, ample light, and a 0°C temperature, permitting the cold-adapted ones to achieve photosynthesis, little respiratory loss, and some level of growth during at least part of the winter. This raises the interesting question as to what role the snow on the side of a tree trunk might play in the distribution of mosses, providing moisture and light for growth in winter and probably occurring on the side that receives the most direct rain in summer, assuming the prevailing wind direction does not change seasonally. But how much, if any, light penetrates several feet of snow?

Light through Snow

Fortunately, Marchand (1993) has provided proof that many of our theories about snow are possible. He was trying to explain how voles managed to be reproductively active just 10 weeks before the snow melted, and when the snow pack was deeper, they delayed their reproductive activity, again being active just 10 weeks before the snow melt, which occurred a full month later. Assuming they had no more ability to see into the future than do we, he began taking measurements under the snow. Some startling facts were discovered (although, I suspect some physicists would not be surprised).

As expected, the more dense the snow at a given depth, the less light penetrated. However, what Marchand did not predict was that as the snow melted and filled in the spaces between the snow crystals, the light penetration increased. (See transparency in Figure 1). Hence, the voles could use light intensity as an indicator of the coming of clear ground, and our bryophytes could carry out photosynthesis and grow or develop well before the snow was gone in the spring.

He found that any combination of depth:density that was greater than 200 gave maximum thermal protection, resulting in a near 0°C temperature under the snow. Thus, 20 cm of snow with a density of 0.1 g cm⁻³ (very fresh snowfall) would completely buffer most temperature fluctuations. When the density increases to 0.2 g cm⁻³, twice as much snow is required for the same thermal

protection. This means that additional snowfall can ameliorate the lowered temperature effects of increasing density of compacted older snow.

But what of light? Marchand knew that only a small amount of light, principally in the blue and blue-green range (Figure 105), could penetrate the deep snow pack. Under only 3-4 mm of older, crystalline snow, no infra-red radiation penetrates (Gates 1962).

Photosynthesis is greatest in the red range, with a smaller second peak in the blue range. When the snow density reaches 0.3 - 0.4 g cm⁻³, typical of the upper part of the snow pack in late winter, only 2 - 3% of the surface light reaches a depth of 15 cm. When Marchand's group compacted the snow as much as they could, attaining a density of 0.5 g cm⁻³, the light penetration was nearly zero. That seemed to be the critical density – the density possible by compaction alone. It was following that experiment when they discovered that melting snow actually increased in transmission of light. Instead of refracted, scattered light passing through tiny ice grains, the light was now passing through larger, fused grains that caused much less scattering and absorption. Although less than 0.1% of incident light seems to reach the ground from late December to early April when the snow depth is greater than 40 cm and density > 0.25 g cm⁻³, the late season snow provides an insulating source of water as it melts, increasing the transmission of light.



Figure 105. Ice cave at Athabasca Glacier, Jasper, British Columbia, Canada, demonstrating the blue-green color of light penetrating ice. Photo by Janice Glime.

Late Snowbeds

Unique communities of bryophytes occur adjacent to summer snowfields, taking advantage of the cooler temperatures and most likely greater moisture. In such cool habitats, one might find red mosses that increase their leaf temperatures by absorbing the light rays and reradiating them as heat. These mosses might have their lower parts in meltwater at 0°C while their growing tips are much warmer in the rays of the sun with this "red body" heating. Such mosses include *Andreaea nivalis* (Figure 106), *Bryum muehlenbeckii* (Figure 107), and *Racomitrium sudeticum* (Figure 108) (Bailey 1933; Belland 1983). Others are white, perhaps being protected from the bright light reflecting from the nearby snow, while

being subjected to temperatures that do not allow rapid use of excited electrons among the chlorophyll antenna pigments (see Figure 2 of *Racomitrium lanuginosum* for an example). The genus that once was *Webera*, and now most likely is *Pohlia* (Figure 109-Figure 110), seems to have several species that thrive in this unique habitat (Bailey 1933; Woolgrove & Woodin 1994). Bailey comments that in the Cascade Range, Washington, USA, all of these taxa are acrocarpous. Only *Isopterygiopsis pulchella* (Figure 111) among these is a pleurocarpous moss.



Figure 106. *Andreaea nivalis*, illustrating the red color of this arctic/alpine species. Photo by Michael Lüth, with permission.



Figure 107. *Bryum muehlenbeckii*, a species that uses "red body" heating in the sun. Photo by Michael Lüth, with permission.



Figure 108. *Racomitrium sudeticum*, a species that uses "red body" heating in the sun. Photo by Michael Lüth, with permission.

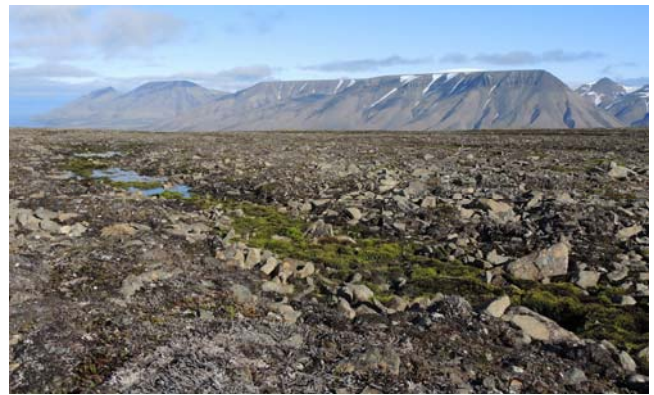


Figure 109. *Pohlia ludwigii*, a late snowbed bryophyte. Photo by Michael Lüth, with permission.



Figure 110. *Pohlia ludwigii*, a late snowbed bryophyte. Photo by Michael Lüth, with permission.

Lösch *et al.* (1983) reported that only the top 4 mm of the late snowbed liverwort *Anthelia juratzkana* (Figure 112-Figure 113) has enough chlorophyll to be capable of net gain in photosynthesis. This species reaches its low temperature compensation point at -4°C . It easily sustains life in 9 months of darkness, cold, and wetness. However, its respiration rate increases, causing the net photosynthetic rate to decrease following snow melt. In *Polytrichum sexangulare* (Figure 114-Figure 115), also a snowbed moss, the low temperature compensation point is -5°C . However, this species did not tolerate being wet and cold in

the dark for so long. Both species survive in these snowbed communities because of their ability to use low light intensities at low temperatures (optimum of 6-11°C). *Anthelia juratzkana* is able to grow at the edge of snowbanks at very cold temperatures. *Polytrichum sexangulare* succeeds because of its more rapid growth rate, permitting it to outcompete the seed plants. But this evades the question, how do these bryophytes survive the alternating warm and freezing temperatures at the edge of the snowbeds, or do they?



Figure 111. *Isopterygiopsis pulchella*, a late snowbed pleurocarpous moss. Photo by Michael Lüth, with permission.



Figure 112. *Anthelia juratzkana* in a recently melted late snowbed. Photo by Michael Lüth, with permission.

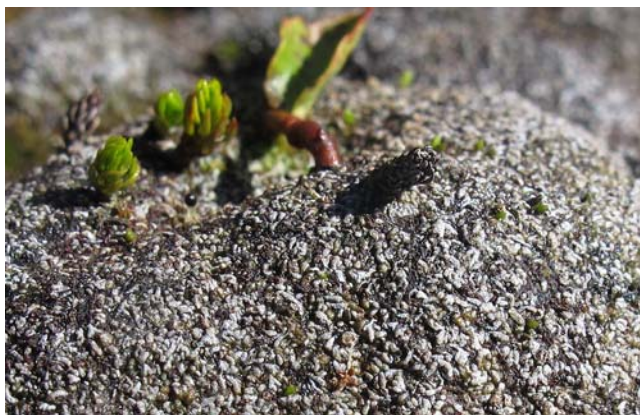


Figure 113. *Anthelia juratzkana*, a late snowbed leafy liverwort that reaches its low temperature compensation point at -4°C and is able to exhibit growth at the edge of a snowbed. Photo by Michael Lüth, with permission.



Figure 114. *Polytrichum sexangulare* with capsules, a late snowbed species. Photo by Michael Lüth, with permission.



Figure 115. *Polytrichum sexangulare*, a species that grows rapidly, permitting it to outcompete other species. Photo by Michael Lüth, with permission.

Acclimation and Adaptation

Could the Antarctic climate be so severe that the bryophytes are always ready? Melick and Seppelt (1994) found little or no change in soluble carbohydrate levels. However, as already noted, both chlorophyll and carotenoids did respond to seasons. But are bryophytes elsewhere ready both to remain dormant when conditions are too cold and to grow during periods that are warm enough?

Winter Growth

I have long suspected that a number of bryophyte species are able to grow in cold winter months, perhaps even under the snow. For example, mosses like *Brachythecium rutabulum* (Figure 59) have better growth at temperatures below 18°C in winter collections than those from summer collections (Furness & Grime 1982).

In a study of 40 bryophyte species in Europe, Furness and Grime (1982) found that most species had an optimum growth temperature of 15°-25°C. Nevertheless, many species continued to grow at temperatures less than 10°C.

Winter Warming Events

What happens to a frozen moss when those sunny days take its temperature above freezing? We know that tracheophytes can be severely damaged when "spring

comes early" and then winter returns. Buds may begin to open, then the tender young leaves killed when frost returns. This expensive energy loss uses stored resources and cannot be tolerated frequently. But what happens to bryophytes under these same circumstances?

Bjerke *et al.* (2011) simulated such events in a sub-Arctic heath using infrared heat lamps and soil warming cables. Among the dominant cryptogamic flora, they subjected the boreal moss *Hylocomium splendens* (Figure 76) to such warming events for three consecutive winters. Unlike the lichen *Peltigera aphthosa* (Figure 116), *H. splendens* exhibited a significant decrease in summertime net photosynthesis (up to 48%) and growth rate (up to 52%). The lichen does not have seasonal life cycle stages, but *H. splendens* has seasonal stages when it produces new branches and leaves. The most critical of these responding to winter warm periods is the initiation of growth. These young shoots are vulnerable if the cold period returns shortly thereafter. Such winter warm periods have been experienced in areas such as my home in the Keweenaw Peninsula of Michigan and are likely to increase in frequency as the global climate changes.



Figure 116. *Peltigera aphthosa*, a lichen that lacks a seasonal life cycle. Photo by Steven K. Sullivan, through Creative Commons.

Snowbed bryophytes are not likely to experience winter melt, but in the spring the bryophytes at the edge of the snowbed may experience alternating warming and freezing periods. We have seen above that late snowbed bryophytes like *Anthelia juratzkana* (Figure 112-Figure 113) and *Polytrichum sexangulare* (Figure 114-Figure 115) survive the short growing season and long period of snow cover because of their ability to have a net photosynthetic gain in low light at low temperatures (Lösch *et al.* 1983). But how do these bryophytes, especially *A. juratzkana*, fare at the edge of the snowbed if it melts, then freezes again? Our knowledge of bryophytes in winter, and especially when experiencing intermittent warming, is extremely meager.

Pigments and Color Changes

One protection against high light intensity is development of red pigments (Quinn 2008). Just as high elevation mosses may be red, like those discussed as living in late snowbeds, and snow algae such as *Chlamydomonas nivalis* (Figure 117), are red, some bryophytes produce red pigments to provide protection against UV radiation and

may even receive an added bonus of warmer daytime temperatures due to color. Anthocyanins, known in both bryophytes and tracheophytes, convert light to heat; this is especially important in the cooler days at the beginning and end of the growing season (Quinn 2008).

Several species of *Sphagnum* (Figure 102) have this color response, wherein cold temperatures induce production of the red cell wall pigment **sphagnorubin**, a flavonoid (Tutschek 1982).



Figure 117. Pink snow caused by the alga *Chlamydomonas nivalis* in the Arctic. Photo through Creative Commons.

Bendz *et al.* (1962) pointed out that the color of a bryophyte can vary widely, depending on solar radiation and nutrient availability. These red pigments appear to be anthocyanins. *Bryum cryophilum* (Figure 68) exhibits deep red color in the Arctic along stream borders. These proved to be anthocyanins in the cytoplasm. Red cell wall pigments occur in *Sphagnum magellanicum* (Figure 85) and *S. capillifolium* (*S. nemoreum*; Figure 24). Likewise, *Warnstorfia pseudosarmentosa* has red anthocyanin cell wall pigments. One of its pigments chemically resembles those of *B. cryophilum* and the other resembles those of the two aforementioned *Sphagnum* species.

In the Antarctic, Post and Vesik (1992) found that the leafy liverwort *Cephaloziella exiliflora* (Figure 46) was green in shaded sites and dark purple in sunny locations. This red color was due to an anthocyanin-like pigment bound in the thick cell walls of the sun plants. These plants grew in dense turfs and their leaves were larger and more closely spaced, most likely increasing moisture-holding capacity and reducing sun damage. It is interesting that the chlorophyll *a/b* ratio did not vary, but the green shade plants had more chlorophyll per unit weight.

Charlie Campbell (Bryonet 12 December 2013) found that the red *Sphagnum magellanicum* (Figure 85) was more photosynthetically active after freezing than the yellow-brown *S. papillosum* (Figure 97). Others (Quinn 2008) have reported that more highly colored species live in colder mountainous regions, compared to those close to the sea. Other color changes are noted in response to sun. *Hypnum imponens* (Figure 118-Figure 120) and *Thuidium delicatulum* (Figure 121-Figure 122) definitely change from medium green (Figure 119, Figure 121) to yellow-green or vivid yellow tones (Figure 120, Figure 122) when exposed to more sunlight (Annie Martin, Bryonet 12 December 2013).



Figure 118. *Hypnum imponens* in forest showing green color. Photo by Janice Glime.



Figure 121. *Thuidium delicatulum* in shaded location at Hocking Hills Ohio, USA. Photo by Janice Glime.



Figure 119. *Hypnum imponens* from forest showing light green color. Photo by Janice Glime.



Figure 122. *Thuidium delicatulum* showing golden color indicative of a sunny location. Photo by David Holyoak, with permission.



Figure 120. *Hypnum imponens* showing typical golden sun colors. Photo by Michael Lüth, with permission.

In *Sphagnum capillifolium* (Figure 24), Gerdol *et al.* (1998) found no trigger for the formation of red wall pigments when nighttime temperatures were 5°C and above

One principle to keep in mind in this discussion is that being cold and in bright light at the same time is a problem for plants, especially bryophytes. The light excites the chlorophyll electrons, but the cold temperature slows down the physiological processes. Hence, pigments that absorb some of that light energy can help to protect the chlorophyll from damage. These should not be part of the chlorophyll antenna system because that would transfer even more energy to the chlorophyll. Rather, they can be cytoplasmic or cell wall pigments. In the chapter on light, I have already discussed the reaction of *Fontinalis antipyretica* (Figure 123-Figure 124) in cold water exiting an underground stream into full sunlight. The moss was crimson!

Exposure to UV-B radiation is often the trigger for higher levels of pigmentation (Robinson *et al.* 2005). However, the Antarctic species *Schistidium antarctici* (Figure 43) did not increase UV-B absorbing pigmentation under higher UV-B radiation, unlike many other species in the Antarctic.

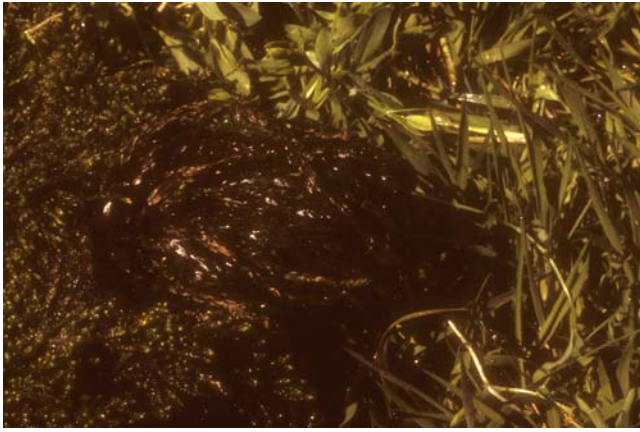


Figure 123. *Fontinalis antipyretica* exhibiting red color from the stress of high light levels and cold temperatures. Photo by Janice Glime.

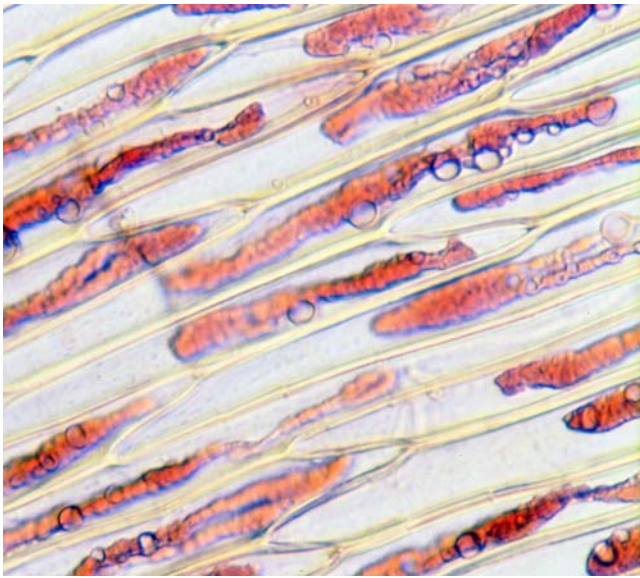


Figure 124. *Fontinalis antipyretica* red cells that result from stress. Photo by Janice Glime.

Dunn and Robinson (2006) suggest that *Bryum pseudotriquetrum* (Figure 44-Figure 45) will have an advantage over other species under conditions of high UV-B radiation that occurs with low temperatures. This will be mediated in *B. pseudotriquetrum* by the presence of UV-B absorbing and anthocyanin pigments that limit physiological activity during periods of low temperatures and desiccation, but also limiting photoprotective and repair mechanisms.

In the same study (Dunn & Robinson 2006), *Ceratodon purpureus* (Figure 50) is intermediate among the three species studied. Rather than responding to high levels of UV-B, it has a stable, constitutive concentration of UV-B- absorbing pigments. However, the anthocyanin pigments in this species were more responsive than those of *Bryum pseudotriquetrum* (Figure 44-Figure 45), most likely providing antioxidant protection during periods of high UV-B radiation (Turnbull & Robinson 2009). *Bryum pseudotriquetrum* did decrease the accumulation of photosynthetic product as the temperature rose. Of the three species, *Schistidium antarctici* (Figure 43) presents the least protection and seems to have no UV-B protective response (Dunn & Robinson 2006).

In a different Antarctic study, Melick and Seppelt (1994) found that pigment levels varied seasonally. Total chlorophyll and the chlorophyll *a/b* ratio dropped during winter. Carotenoids increased in the summer, presumably responding to the higher light intensity.

Summary

The optimum growth temperature for most bryophytes lies between 15 and 25°C, but it can go much lower in habitats that remain cold for most of the year. The lowest extreme for photosynthesis appears to be about -15°C and the uppermost around 40-45°C. However, it is unlikely that there would be a sustained photosynthetic gain at these higher temperatures.

Snow provides insulation and may serve as a source of nutrients and moisture during the winter. Acrocarpous mosses seem more able to tolerate dry areas with only light snow cover, whereas pleurocarpous mosses are more common on wet sites with deep, long-lasting snow. Some epiphytes may benefit from the moist, protected funnels of air between the snow and tree trunk. Light quality is altered through the snow to principally blue and blue-green and diminishes rapidly from the surface.

Bryophytes near late snowbeds remain cold from melt water while experiencing high light intensities and, like bryophytes from regions of extreme cold, are often red, deriving protection from UV and possibly benefitting from warming. White tips also seem to help in reflecting the bright light. Like the exposed bryophytes, these typically are acrocarpous, with *Isoeterygiopsis pulchella* being a notable exception.

Freezing of cells can result in damage from crystals that poke holes in membranes, loss of solutes, and desiccation. Hence, desiccated cells are more likely to survive freezing than hydrated cells. Some bryophytes have net photosynthetic gain on nunataks and other areas where the temperature rarely exceeds 0°C. Net gain at -10°C is not uncommon.

But low temperature and high light intensity can cause photoinhibition. Bryophytes gain protection through colored pigments and down-regulation of photosystem II to prevent over-excitation of electrons. Mature tissues seem to exceed both young and senescing tissues in their frost tolerance.

Calcium and ABA seem to have a role in cold tolerance, although the mechanism is incompletely understood. ABA stimulates the activity of genes that code for stress proteins. These, in turn, increase freezing tolerance and decrease the death rate. Presence of ABA protects cells from membrane lesions and causes an increase in the sugar concentration of cells, but this may be an indirect effect through activation of genes that code for the production of stress proteins. Calcium alters membrane permeability, thus affecting membrane transport. Cold temperatures seem to increase the cellular content of Ca^{++} , which comes from both internal and external sources. An increase in soluble sugars could lower the freezing point or provide energy for rapid repair. Depressed temperatures stimulate the

bryophytes to prepare for winter by activating these mechanisms.

Membrane integrity may be maintained by alteration of fatty acids and lipids, with those having high freezing points being replaced with ones having lower freezing points. There seems to be a change to more unsaturated fatty acids as weather cools. Decreasing N levels may signal this change to occur. Some experiments suggest that arachidonic acids diminish as the temperature cools, but if light intensity decreases, as it would as winter approaches, at least some mosses exhibit an increase not only of palmitic and stearic acids, but also of linolenic and arachidonic acids. Such fatty acids as arachidonic acid may even be important in protecting the footpads of lemmings that eat the mosses prior to the onset of winter.

Bryophytes respond differently from tracheophytes to freezing. Because they are only one cell thick and lack internal air spaces, their external surfaces are able to form ice rather than crystals. This helps to insulate the cell. Furthermore, cellular loss of water in preparation for winter deprives the external surfaces from drawing water from the cells to grow crystals. Presence of macromolecular substances, most likely proteins, help polar and cold region bryophytes to form ice rather than crystals. The rapid cooling achieved by the one-cell-thick leaves also causes water loss from the cell, increasing solute concentration and lowering the freezing point inside the cells. This also contributes to the prevention of internal crystal formation. Thallose liverworts with multiple cell layers are more likely to suffer freezing damage.

The ability to accomplish the various means of surviving freezing plays an important role in the niche width and distribution of closely related species.

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