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Ice nucleation in mosses and liverworts

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This work demonstrates mosses and liverworts are able to freeze water at elevated temperatures. They are likely to do this as a means of harvesting additional water by the Bergeron–Findeisen process and as a consequence potentially influence atmospheric processes.

Liquid water can be supercooled to well below the melting point of 0°C without freezing. Pure water does not freeze until the temperature drops to –38°C (Franks 1985) or even –42°C for very small samples (Debendetti and Stanley 1996). There are a wide range of ice nuclei (IN) which increase the temperature of freezing. These include inert minerals (Mason 1975), inorganic and organic molecules (Fukuta 1966) and living biological particles (Möhler et al. 2007), including plants (Anderson and Ashworth 1985). However those IN which freeze at the highest temperatures are bacterial. Ice nucleation in bacteria appears to be limited to a small number of plant pathogenic bacteria which use specific proteins to induce freezing in order to damage plants and so gain nutrients (Lindow 1983). Previous work (Kieft 1988) demonstrated the presence of warm temperature IN in a number of lichens. In lichens the ability to freeze water is thought to be for water harvesting but nothing is known about the underlying mechanism. As mosses and liverworts also lack roots, ice-nucleation may be a useful ability as the majority of bryophytes rely on atmospheric water for their basic metabolic processes (Vanderpoorten and Goffinet 2009). Here I show all mosses and all liverworts tested are ice-nucleation active. If these become airborne they could influence atmospheric processes.

Material and methods

Ice nucleation was determined using differential scanning calorimetry (DSC) (DSC822e, Mettler Toledo, Leicester, UK). Approximately 0.1 mg of leaf material immersed in 10 µl of molecular grade water (Sigma, Gillingham, Dorset, UK) was contained within a sealed aluminium crucible. This was then cooled progressively from 0°C to –30°C (1°C min^{-1}). When the sample freezes, the release of latent heat is detected by an array of thermocouples below the crucible and the temperature recorded. The water control never froze above –24°C.

Results

The results for liverworts and mosses are shown in Table 1. The IN temperature for mosses ranged from –5 to –22.4°C with a median of –8.4 with 63% of species tested active above –10°C. For liverworts the range was –6.3 to –10.6°C with a median of –7.5 with 87.5% active above –10°C.

The difference in vapour pressure over ice and water is at its maximum at –15°C and is significant between –5°C and –20°C. Therefore at these temperatures ice grows quickly at the expense of water. This is known as the Bergeron–Findeisien mechanism (Pruppacher and Klett 1997). If a raindrop or dew drop on the surface of a bryophyte is frozen due to the IN described here, then, due to the lower vapour pressure, extra water will be absorbed onto the ice from the atmosphere. Therefore, when the ice melts, in the spring or in the morning, metabolic activity is potentially greater due to the increased availability of water.

Figure 1 shows a moss growing on a dry stone wall in Yorkshire UK. The ice crystals are much larger on the moss than the rock and there is a halo of ice-deficient rock

Table 1. Threshold ice nucleation temperature (°C) for mosses and liverworts determined by DSC.

Liverworts	Threshold freezing temperature
Aneura pinguis	–7.6
Eucalypta streptocarpa	–6.8
Fissenden bryoides	–8.0
Frullania tamarisci	–6.5
Lepidozia reptans	–7.5
Lunularia cruciata	–10.6
Metzgeria temperata	–6.3
Plagiochila porelloides	–8.1
Mosses	
Andrae rothui	–11.5
Anthoceros punctatus	–7.2
Atrichum undulatum	–5.0
Aulacomnium turgidum	–8.4
Dichodontium palustre	–8.3
Dicranella palustris	–8.6
Homalothecium sericeum	–7.8
Hypnum cupressiforme	–10.8
Orthotrichium anomalum	–6.9
Orthotrichum diaphanum	–14.9
Polytrichum commune	–16.3
Polytrichum juniperinum	–6.5
Racomitrium lanuginosum	–9.8
Sphagnum cuspidatum	–7.7
Sphagnum palustre	–12.1
Syntrichia latifolia	–8.2
Tortula muralis	–12.8

around the base of the moss. This dry zone forms because as the temperature drops ice forms earlier on the moss. Surrounding liquid water and water vapour is then absorbed by the ice on the moss. This results in larger crystals of ice on the moss and an ice-free zone on the surrounding rock due to a lack of moisture.

Discussion

Although further investigation is required, ice nucleation appears to be a general feature of mosses and liverworts. Using the DSC only the single most efficient nucleus in the sample is detected. However, I have also carried out

Figure 1. Ice on moss. The ice crystals are larger on the leaves than the surrounding stone. There is also a 'dry' area on the stone around the base of the moss. This is the region from which extra water has been taken up by the moss via the Bergeron–Findeisen process.

drop freezing analysis on a very limited number of moss samples, which have been ground in liquid nitrogen. This enables quantification of the IN per gram of tissue. The mosses I have so far analysed harbour 10^6–10^7 g^{-1} an order of magnitude greater than Kieft (1988) observed in lichens. By culturing on selective cetrimide medium we also have evidence that there are very few ice-nucleating bacteria associated with the surfaces of mosses. Therefore the ice-nucleation activity is unlikely to be due to surface contamination with bacteria. The most likely alternative hypothesis is that the ice nucleation in these bryophytes is due to an indigenous protein or other macromolecule.

In temperate climates ice is required for the initiation of precipitation. When a cloud drop freezes the reduction in vapour pressure means that the ice grows at the expense of available water. As the ice grows it splinters and each splinter continues the process. Once the ice crystals are too large to be supported by updraughts in the parcel of air, they fall. Depending on the conditions, they typically melt as they descend to produce rain. One current issue in the field of cloud microphysics is the relevant contribution of organic and inorganic sources of ice nuclei to the initiation of precipitation (Georgakopoulos 2008). Mosses and liverworts are previously unrecognised potential sources of atmospheric IN .

Estimates for global abundance of mosses and liverworts are difficult to find. However there are estimates for a variety of different terrestrial ecosystem types (Vanderpoorten and Goffinet 2009). Lichen biomass has been estimated to be 10^{14} tonnes (Margulis 1998) and I suggest that globally mosses are at least as abundant as lichens. Using a figure of 150 million km^2 for the global area of peat bogs and an average biomass of 700 g m^{-2} of *Sphag-*

num, the biomass of *Sphagnum* moss in peat bogs can be estimated at 10^{11} tonnes. Only a tiny fraction of this biomass would need to become airborne to have an effect on atmospheric processes. In particular radiation balance, cloud dynamics and precipitation could be influenced by bryophyte ice nuclei. There is some evidence that mosses become airborne although much of this appears to be anecdotal. However, using standard DNA-based methodologies on air samples, Despres and colleagues (2007) found 50% (two out of four) plant gene sequences recovered were attributable to moss (Bryophytes; 95% identity with AY156588 and 98% with AY156592). In addition, on both occasions when bacteria and fungi were being attempted to be cultured from rain collected in London UK, I have inadvertently cultured moss *Funaria hygrometrica.*

Conclusion

It appears that the ice nuclei (IN) phenotype is widespread in mosses and liverworts and was selected for primarily as a water gathering mechanism. Due to the abundance of these IN, both in their apparent ubiquity in mosses and liverworts, the high numbers per gram of tissue, and the temperatures at which they are active, it is possible that they have an influence on atmospheric processes, in particular the initiation of rainfall.

This preliminary work requires refinement and extension but suggests a number of testable hypotheses.

1. Ice nucleation is a ubiquitous feature of bryophytes
2. Ice nucleation is used as a water gathering mechanism
3. Ice nucleation is of greater selective advantages to bryophytes growing in habitats such as rock and tree surfaces
4. Ice nucleation in bryophytes is due to a surface expressed protein
5. Ice nuclei from bryophytes become airborne and influence atmospheric processes.

References

Anderson, J. A. and Ashworth, E. N. 1985. Ice nucleation in tomato plants. – J. Am. Soc. Horticult. Sci. 110: 291–296.

Despres, V. R. et al. 2007. Characterization of primary biogenic aerosol particlesin urban, rural and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. – Biogeosciences 4: 1127–1141.

Debenedetti, P. G. and Stanley, H. E. 2003. Supercooled and glassy water. – Physics Today 56: 40–46.

Franks, F. 1985. Biophysics and biochemistry at low temperatures. – Cambridge Univ. Press.

Fukuta, N. 1966. Experimental studies of organic ice nuclei. – J. Atmos. Sci. 23: 191–196.

Georgakopoulos, D. G. et al. 2008. Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles. – Biogeosciences 5: 1469–1510.

Kieft, T. 1988. Ice nucleation activity in lichens. – Appl. Environ. Microbiol. 54: 1678–1681.

Lindow, S. 1983.The role of bacterial ice nucleation in frost injury to plants. – Annu. Rev. Phytopathol. 21: 363–384.

Margulis, L. 1998. The symbiotic planet: a new look at evolution. – Guernsey Press

Mason, B. J. 1975. Clouds, rain and rainmaking. – Cambridge Univ. Press.

Möhler, O. et al. 2007. Microbiology and atmospheric processes: the role of biological particles in cloud physics. – Biogeosciences 4: 1059–1071.

Pruppacher, H. and Klett, J. 1997. Microphysics of clouds and precipitation, 2nd edn. – Kluwer Academic Publishers.

Vanderpoorten, A. and Goffinet, B. 2009. Introduction to bryophytes. – Cambridge Univ. Press.