Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen *Cladonia stellaris*

Johan ASPLUND[1#]*, Andy SIEGENTHALER[2#] and Yngvar GAUSLAA[1]

[1]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, NO-1432 Ås, Norway.

[2]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.

[#] These authors contributed equally to this work

*Corresponding Author:

johan.asplund@nmbu.no

This manuscript is published in *The Lichenologist* and can be cited as:

Asplund, J., Siegenthaler, A. & Gauslaa, Y. (2017) Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen *Cladonia stellaris*. *The Lichenologist*, **49**, 269–274.

Publisher's version is available at http://dx.doi.org/10.1017/S0024282917000159

Abstract

Lichens as sessile and slow-growing symbiotic associations have evolved various carbon based secondary compounds (CBSCs) to mitigate effects of some stressors in their often extreme environments. The mat-forming lichen *Cladonia stellaris* – an important fodder for reindeer – produces usnic acid in the outermost layer and perlatolic acid in the medulla. Here, we studied effects of simulated global warming on these CBSCs in *C. stellaris* cultivated in climate chambers with: 1) ambient conditions as control or 2) ambient conditions +4°C. The chambers simulated, at an hourly resolution, an averaged 10-years growing season dynamics from a long-term monitored boreal mire in Northern Sweden. After two months of acclimation, +4°C warming in one simulated growing season increased the concentration of usnic acid by 31 % compared with ambient conditions. Whereas the warming decreased the concentration of perlatolic acid by 14 %. Because lichen CBSCs play important roles in ecosystem processes such as lichenivory and decomposition, these changes may profoundly affect lichen-dominated ecosystems.

Key-words: carbon based secondary compounds; climate change; global warming; reindeer lichen; perlatolic acid; usnic acid; boreo-arctic ecosystems

Introduction

As sessile and slow-growing symbiotic associations in extreme environments, lichens face various unpredictable threats. To be successful, lichen mycobionts have evolved carbon based secondary compounds (CBSCs) occurring as extrolites outside fungal hyphae. These compounds protect lichens from lichenivores and excess solar radiation (as reviewed by Solhaug & Gauslaa 2012). CBSCs may also protect lichens from other biotic and abiotic stressors and thus serve multiple functions (Lawrey 2009).

There has been a growing interest in how abiotic factors regulate CBSCs in lichens (Rundel 1969; Bjerke *et al.* 2003; McEvoy *et al.* 2006*b*; Nybakken *et al.* 2007; e.g. Asplund & Wardle 2014). UV-B induces CBSCs located in the upper cortex (e.g. usnic acid, atranorin and parietin) (Rundel 1969; Solhaug *et al.* 2003; McEvoy *et al.* 2006*a*), whereas medullary CBSCs are less responsive to light exposure (McEvoy *et al.* 2007; Nybakken *et al.* 2007). Nevertheless, both cortical and medullary CBSCs concentration peak in summer (Gauslaa & McEvoy 2005; Bjerke *et al.* 2005; Gauslaa *et al.* 2013).

Few have investigated how temperature *per se* affects CBSCs. For instance, the widespread cortical compound, usnic acid has been found to decrease with increasing temperature (Bjerke *et al.* 2004; Nybakken *et al.* 2011). Meanwhile, Bjerke *et al.* (2003) found higher concentrations of the medullary gyrophoric acid and methyl gyrophorate in the cyanolichen *Peltigera extenuata* in open top chambers (OTCs) inducing e.g. warming. Likewise, the medullary salazinic acid had higher concentrations in *Ramalina siliquosa* collected at warmer sites (Hamada 1982). By contrast, CBSCs in many other species did not respond to increased temperature (Nybakken *et al.* 2011). However, because temperature affects water availability (Bjerke *et al.* 2004), we need experimental studies controlling for confounding factors that may interfere with the CBSC metabolism. Earlier studies on lichen CBSCs and temperature did not control important confounding factors, such as light and

humidity. For example, field-based experiments using open-top chambers (OTC) or infra-red lamps artificially decrease the water potential and the soil moisture (e.g. Allison & Treseder 2008; Johnson *et al.* 2013).

The relative humidity and the water potential influence lichen growth (Čabrajić *et al.* 2010; Gauslaa 2014). Many lichens are designed to utilize dew rather than rain as a source of hydration (Gauslaa 2014). Climate models at all latitudes (Allen & Ingram 2002) assume that the relative humidity remains constant in the atmosphere over long time scales because the atmospheric water capacity increases with warming and nearby oceans function as water vapour pumps that can endlessly recharge the atmospheric water pool (Johnson *et al.* 2013). Also recent observations support this and suggest that increasing precipitation and total atmospheric water concur with the rise in temperature over the past two decades (Wentz *et al.* 2007). In northern Sweden, the precipitation is predicted to increase by 11% until 2100 at a warming rate of 4°C (Lind & Kjellström 2008).

It is important to understand the regulation of lichen CBSCs because these compounds influence ecosystem processes such as lichenivory and decomposition, and thus carbon and nutrient cycling (Asplund & Wardle 2013; Asplund *et al.* 2013). The mat-forming terricolous *Cladonia stellaris*, with usnic acid in the outermost layer and perlatolic acid in its medulla. often dominates well-drained inland terrain at high latitudes (Kershaw 1977; Ahti & Oksanen 1990). Such mat-forming lichens contribute to ecosystem processes by e.g. providing the main winter fodder for reindeer/caribou (Scotter 1967; Gaare & Skogland 1975; e.g. Danell *et al.* 1994; Storeheier *et al.* 2002).

Here, we studied the effects of +4°C warming on CBSCs in *C. stellaris* cultivated in two climate chambers without confounding effects of the decreased relative humidity, hydration, and altered levels of UV-B. By compensating for the greater air-water holding capacity of warmed mesocosms and by keeping the relative humidity constant, the capacity of

the mesocosms to form dew was equal across treatments. By taking such precautions, we aimed to quantify the temperature effect *per se* on secondary metabolism.

Material and methods

On November 19, 2011, a homogeneous mat (approximately 4 dm^2) of *C. stellaris* was collected on a *Sphagnum* fen in Lappmyran mire, 2.2 km from the Degerö mire experimental site, Vindeln, Sweden (64º09'54.91"N, 19º35'02.26"E). The mat was cleaned from debris and the partly senesced lowermost layer before it was air-dried for 72 h and stored at –18ºC until the start of experiment. A small portion was dried at 70°C to determine the dry bulk density.

On December 4 2011, we placed 12 *Sphagnum* fen mesocosms (monoliths), taken from a homogenous fen lawn at the same location as the lichen material, and placed in polypropylene boxes (54 cm x 36 cm x 25 cm) evenly distributed in two walk-in climate chambers (Karl Weiss, Giessen, Germany). Thirty 400 W metalhalogen lamps (Powerstar HQI-TS, OSRAM, Munich, Germany) were set 110 cm above the top of the mesocosms. The light spectrum comprised the wavelength range 315 (UV-A) - 800 nm. The Photosynthetic Active Radiation (PAR) was programmed to follow the PAR under natural conditions at an hourly time scale. Lamps could generate a PAR up to 1075 µmol m^{-2} s^{-1} at the surface of the mesocosms. With these light sources, 96.2% of the hourly settings could match the *in-situ* PAR levels of the entire growing season at Lappmyran. UV filters kept the UV radiation below the maximum permitted thresholds to IEC 61167. A linear AccuPAR probe model LP-80 (Decagon, Pullman, USA) recorded PAR. The air temperature and relative humidity were monitored with a QFM3160 sensor (Simens, Munich, Germany), and the chambers were continuously controlled to meet the hourly ambient settings. Two fans ensured mixing of the conditioned or heated air inside the chambers with the spray nozzle humidified air. The mean

surface temperature of the substrate was 18.5 ± 0.3 °C and 20.9 ± 0.2 °C in the ambient and warming treatments, respectively (t = 6.5, P <0.001, *t*-test).

On February 1, we placed one 5 cm × 5 cm *C. stellaris* mat fragment (0.4 g) on top of each *Sphagnum* fen mesocosm. The lamps and filters were pre-burned for almost 2 months before placing the lichens in the chambers. During the experiment, all mesocosms experienced the conditions of a 10-year average growth season-simulation. Six randomly selected mesocosms experienced 4°C warming. This warming corresponds to the land temperature projection in Northern Sweden in summer for the year 2100 using SRES scenario A1FI (Randall *et al.* 2007; Lind & Kjellström 2008). Lichens experienced nearly two months acclimation in 12/12 h night/day cycles at the *in situ* seasonal average daylight PAR (538 µmol m^{-2} s^{-1}), 12° and 80% relative humidity. Then, we set the chambers on March 26 to simulate the 10-year-averaged hourly *in-situ* measurements of air temperature (0.3 to 21.0°C), relative humidity (35 to 99%), PAR (0 to 1384 µmol m^{-2} s^{-1}), precipitation occurrence (0-1) and water level (-3.5 to -19.1 cm) in the mesocosms. We simulated all growing season days >0°C (March 26 - September 26; 148 d in total). We kept the relative humidity equal between the two chambers. Thereby, the absolute humidity was always higher with the 4°C enhanced air treatment, as warmer air holds more water vapour at a given relative air humidity.

To simulate a natural precipitation regime, the mesocosms received variable amounts of water once during a watering day. To determine a watering day, we used the following watering criteria (WC) for each day (i):

$$WC_i = (1+MP_i)^2 * MPO_i \text{ for i= day of the year 122 to 270}$$

Here, MP_i is the mean precipitation for day_i (mm d^{-1}) averaged over 10 years, and MPO_i is the mean precipitation occurrences (1 to 10) for day_i over 10 years. We watered the lichens on the

75 days with the highest WC_i values. During these 75 days, corresponding *in situ* to the average number of daily precipitation occurrences during a mean season, we watered the mesocosms with a watering can fitted with a rose until the water level reached the foreseen 10-year average water level for that day. The mesocosms always remained moist by watering every 1.97 days on average; but they received condensation water (dewfall) when the chamber temperature reached the dew point. The added water was a 9:1 mixture of deionized:tap-water to mimic nutrients, conductivity and pH recorded in the fen's pore water.

To avoid confounding effects of the within-chamber position, we swapped six times the position of the mesocosms within the chambers. To avoid confounding chamber effects, we swapped the mesocosms and the treatment settings from one chamber to the other twice during the experiment. We monitored air temperature and humidity simultaneously in the two chambers using additional device for cross-checking.

After harvest, thalli were air-dried and weighed. The air-dry mass was converted to oven (at 70°C) dry mass by using the ratio between air-dry and oven-dry mass obtained from additional thalli. Growth was recorded as percent biomass change in relation to start weight. The upper 10 mm of each 50 mm tall lichen mat was finely ground with a ball mill. Approximately 35 mg of the powder was extracted for three 45 min periods. The combined extract was evaporated to dryness and dissolved in 1000-2000 µl acetone. The extracted compounds were then quantified on a 1100 Series HPLC (Agilent Technologies, Waldbronn, Germany) including a 1,040-M diode array detector (following Nybakken et al. 2007). Separation was achieved on an ODS Hypersil 50 × 4.6 mm column. The injection volume was 10 µl and the flow rate was 2 ml min^{-1} Solvent A consisted of 0.25% orthophosphoric acid and 1.5% tetrahydrofuran in Millipore (Millipore, Billerica, Massachusetts, USA) water and solvent B was 100% methanol. The run started with 30 % B. Within 15 min, solvent B was increased to 70 % and further to 100 % the next 15 min, and then isocratically in 100 % B for

a further 5 min. At the end of the run, solvent B was reduced to 30 % within 1 min, and the column was flushed with 30 % B for 5 min before the next run. The detection wavelength was 245 nm. Usnic acid was quantified against the response curve of a commercial standard of (+)-usnic acid (Sigma Chemical Co, St. Louis, MO, U.S.A.). Different isomers of usnic acid were not separated. Because no standard was available for perlatolic acid, we reported it in absorbance units mg^{-1}. Perlatolic acid was identified comparing our spectra by the UV-spectra reported by Huneck & Yoshimura (1996). Start concentration of both compounds was quantified from one composite sample of the start material. This value represent the concentration on the collection date.

Numerical analysis

The effect of warming on lichen growth rates, usnic and perlatolic acid concentrations was tested with help of a Welch's *t-test.* One lichen mat fragmented during the experiment was excluded for growth measurements. Thus n = 6 for controls and n = 5 for the warming treatment. In order to test whether concentrations of CBSCs changed during the cultivation we calculated change in CBSCs relative to the bulk start value and performed one sample *t*-tests on these values. All analyses were performed using the R 3.2.5 software (R Core Team 2016).

Results

The lichens had an average mass growth of 6.1 ± 0.9 % (pooled mean ± 1 S.E.), with no significant difference between treatments ($t = 0.86$, $P = 0.423$). Warming increased the concentration of usnic acid by 31 % compared to thalli kept under ambient conditions (Fig. 1; $t = 2.92$, $P = 0.021$). As such, thalli kept in the warming treatment significantly increased their usnic acid concentration compared with the start values ($t = 5.87$, $P = 0.002$) Meanwhile,

thalli kept at ambient temperatures did not change their usnic acid concentration during cultivation (t = 0.48, P = 0.659). The concentration of perlatolic acid fell significantly during cultivation at both temperature regimes (Fig. 1; warming: t = 5.69, P = 0.002; ambient: t = 6.40, P = 0.003). Nevertheless, by the end of the experiment, lichens subjected to warming had 14 % lower concentration of perlatolic acid as compared to those kept under ambient conditions (Fig.1; t = 2.32, P = 0.049).

Discussion

The usnic acid concentration increased during the warming regime, but stayed constant in the ambient regime. Because of the net biomass gain, usnic acid was synthesized in both temperature regimes. Meanwhile, at the end of cultivation, the concentration of perlatolic acid was just 70-80 % of the initial concentration. This could be consistent with dilution due to biomass growth and low or absent perlatolic acid synthesis under the growth chamber conditions. A low synthesis of perlatolic acid could also explain why we only found weak differences between the treatments for this compound.

In contrast to our findings, field studies report constant or lower concentrations of usnic acid at the higher temperature inside OTC (Nybakken *et al.* 2011). For example, *C. arbuscula* inside OTCs (raising air temperature by 1.5 ºC) had lower usnic acid concentration than thalli outside, whereas usnic acid in *Flavocetraria nivalis* was indifferent to this temperature treatment (Nybakken *et al.* 2011). However, OTCs screen UV-B levels and modify relative humidity and dewfall strongly influencing poikilohydric organisms. Such concurring effects may question the ecological relevance of OTC-data for lichens. In studies along natural environmental gradients, *F. nivalis* had higher concentration of usnic acid in the coldest, but also the most humid site (Bjerke *et al.* 2004). Because lichens need hydration for metabolic activity, and because photosynthates boost usnic acid synthesis (McEvoy et al.

2006a), improved water availability could drive usnic acid synthesis (Bjerke *et al.* 2003). Thereby, earlier reported increases in usnic acid with decreased temperatures (Bjerke *et al.* 2004; Nybakken *et al.* 2011) may have been driven by the confounding factor relative humidity. Northern latitudes, where *C. stellaris* is common, are predicted to be warmer and wetter (Kirtman *et al.* 2013). Here, we kept all other factors constant, meaning that the observed increase in usnic acid and decrease in perlatolic acid is attributed to increased temperature only.

Opposite responses in cortical vs medullary compounds, as those in Fig. 1, have also been reported in *Parmotrema hypotropum* showing increased concentration of the cortical atranorin and decreased medullary norstictic acid with increasing temperature-to-water-potential ratio (T/Ψ) driven by sun exposure in the field (Armaleo *et al.* 2008). The authors argued that high versus low T/Ψ would activate cortex-specific polyketide synthases and medulla-specific polyketide synthases, respectively, causing such contrasting responses. Our results could be consistent with such a hypothesis, because the water potential should be similar across our treatments at constant water levels and relative humidity, resulting in higher T/Ψ in the warming treatment.

Mat-forming terricolous lichens with usnic acid dominate continental low alpine soils and forest floor on well drained, nutrient-poor terrain at high latitudes (Kershaw 1977; Ahti & Oksanen 1990). In Canada alone, there is 4.4×10^6 km^2 of lichen woodland (Auclair & Rencz 1982). Given the large biomass of usnic acid lichens at high latitudes, climate-driven changes in the usnic acid concentration will result in large quantitative changes in usnic acid at the ecosystem level. Because lichen CBSCs play important roles in ecosystem processes, e.g. lichenivory and decomposition, such changes may profoundly affect lichen-dominated ecosystems (Asplund & Wardle 2013, 2016).

Acknowledgments

We would like to thank Björn Erhagen, Andrés Peralta Tapia and Rose-Marie Kronberg and Daniel Metcalfe for helping with the chamber maintenance or fieldwork, and Matthias Peichl for resuming the climatic data from the Degerö site. We thank the Kempe Foundation for financial support. We would also like to thank anonymous reviewers for constructive comments on an earlier version of this manuscript.

References

Ahti, T. & Oksanen, J. (1990) Epigeic lichen communities of taiga and tundra regions. *Vegetatio* **86**: 39–70.

Allen, M. R. & Ingram, W. J. (2002) Constraints on future changes in climate and the hydrologic cycle. *Nature* **419**: 224–232.

Allison, S. D. & Treseder, K. K. (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* **14**: 2898–2909.

Armaleo, D., Zhang, Y. & Cheung, S. (2008) Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential. *Mycologia* **100**: 565–576.

Asplund, J. & Wardle, D. A. (2013) The impact of secondary compounds and functional characteristics on lichen palatability and decomposition. *Journal of Ecology* **101**: 689–700.

Asplund, J. & Wardle, D. A. (2014) Within-species variability is the main driver of community-level responses of traits of epiphytes across a long term chronosequence. *Functional Ecology* **28**: 1513–1522.

Asplund, J. & Wardle, D. A. (2016) How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*: doi:10.1111/brv.12305.

Asplund, J., Bokhorst, S. & Wardle, D. A. (2013) Secondary compounds can reduce the soil micro-arthropod effect on lichen decomposition. *Soil Biology and Biochemistry* **66**: 10–16.

Auclair, A. N. D. & Rencz, A. N. (1982) Concentration, mass, and distribution of nutrients in a subarctic *Picea mariana – Cladonia alpestris* ecosystem. *Canadian Journal of Forest Research* **12**: 947–968.

Bjerke, J. W., Zielke, M. & Solheim, B. (2003) Long-term impacts of simulated climatic change on secondary metabolism, thallus structure and nitrogen fixation activity in two cyanolichens from the arctic. *New Phytologist* **159**: 361–367.

Bjerke, J. W., Joly, D., Nilsen, L. & Brossard, T. (2004) Spatial trends in usnic acid concentrations of the lichen *Flavocetraria nivalis* along local climatic gradients in the Arctic (Kongsfjorden, Svalbard). *Polar Biology* **27**: 409–417.

Bjerke, J. W., Elvebakk, A., Domínguez, E. & Dahlback, A. (2005) Seasonal trends in usnic acid concentrations of Arctic, alpine and Patagonian populations of the lichen *Flavocetraria nivalis*. *Phytochemistry* **66**: 337–344.

Čabrajić, A. V. J., Lidén, M., Lundmark, T., Ottosson-Löfvenius, M. & Palmqvist, K. (2010) Modelling hydration and photosystem II activation in relation to *in situ* rain and humidity patterns: a tool to compare performance of rare and generalist epiphytic lichens. *Plant, Cell & Environment* **33**: 840–850.

Danell, K., Utsi, P. M., Palo, R. T. & Eriksson, O. (1994) Food plant selection by reindeer during winter in relation to plant quality. *Ecography* **17**: 153–158.

Gaare, E. & Skogland, T. (1975) Wild reindeer food habits and range use at Hardangervidda. *Fennoscandian Tundra Ecosystems* Ecological Studies. (F. E. Wielgolaski, ed): pp. 195–205. Springer Berlin Heidelberg.

Gauslaa, Y. (2014) Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist* **46**: 1–16.

Gauslaa, Y. & McEvoy, M. (2005) Seasonal changes in solar radiation drive acclimation of the sun-screening compound parietin in the lichen *Xanthoria parietina*. *Basic and Applied Ecology* **6**: 75–82.

Gauslaa, Y., Bidussi, M., Solhaug, K. A., Asplund, J. & Larsson, P. (2013) Seasonal and spatial variation in carbon based secondary compounds in green algal and cyanobacterial members of the epiphytic lichen genus *Lobaria*. *Phytochemistry* **94**: 91–98.

Hamada, N. (1982) The effect of temperature on the content of the medullary depsidone salazinic acid in *Ramalina siliquosa* (Lichens). *Canadian Journal of Botany* **60**: 383–385.

Huneck, S. & Yoshimura, I. (1996) *Identification of Lichen Substances*. Berlin: Springer.

Johnson, C. P., Pypker, T. G., Hribljan, J. A. & Chimner, R. A. (2013) Open top chambers and infrared lamps: a comparison of heating efficacy and CO_2/CH_4 dynamics in a northern michigan peatland. *Ecosystems* **16**: 736–748.

Kershaw, K. A. (1977) Studies on lichen-dominated systems. XX. An examination of some aspects of the northern boreal lichen woodlands in Canada. *Canadian Journal of Botany* **55**: 393–410.

Kirtman, B., Power, S. B., Adedoyin, A. J., Boer, G. J., Bojariu, R., Camilloni, I., Doblas-Reyes, F., Fiore, A. M., Kimoto, M., Meehl, G., Prather, M., Sarr, A., Schar, C., Sutton, R., van Oldenborgh, G. J., Vecchi, G. & Wang, H.-J. (2013) Chapter 11 - Near-term climate change: Projections and predictability. *Climate Change 2013: The Physical Science Basis. IPCC Working Group I Contribution to AR5* (IPCC, ed): p. Cambridge: Cambridge University Press.

Lawrey, J. D. (2009) Chemical defense in lichen symbioses. *Defensive Mutualism in Microbial Symbiosis* Mycology. (J. F. White & (M. S. Torres, eds): pp. 167–181. Boca Raton, FL: CRC Press.

Lind, P. & Kjellström, E. (2008) *Temperature and precipitation changes in Sweden; a wide range of model-based projections for the 21st century.* Norrköping: RMK 113 rapport, SMHI.

McEvoy, M., Nybakken, L., Solhaug, K. A. & Gauslaa, Y. (2006*a*) UV triggers the synthesis of the widely distributed secondary lichen compound usnic acid. *Mycological Progress* **5**: 221–229.

McEvoy, M., Solhaug, K. A. & Gauslaa, Y. (2006*b*) Ambient UV irradiation induces a blue pigment in *Xanthoparmelia stenophylla*. *Lichenologist* **38**: 285–289.

McEvoy, M., Gauslaa, Y. & Solhaug, K. A. (2007) Changes in pools of depsidones and melanins, and their function, during growth and acclimation under contrasting natural light in the lichen *Lobaria pulmonaria*. *New Phytologist* **175**: 271–282.

Nybakken, L., Asplund, J., Solhaug, K. A. & Gauslaa, Y. (2007) Forest successional stage affects the cortical secondary chemistry of three old forest lichens. *Journal of Chemical Ecology* **33**: 1607–1618.

Nybakken, L., Sandvik, S. M. & Klanderud, K. (2011) Experimental warming had little effect on carbon-based secondary compounds, carbon and nitrogen in selected alpine plants and lichens. *Environmental and Experimental Botany* **72**: 368–376.

R Core Team (2016) *R: A language and environment for statistical computing. R Foundation for Statistical Computing*. Vienna. URL: http://www.R-project.org/.

Randall, D. A., Wood, R. A., Bony, S., Colman, R., Fichefet, T., Fyfe, J., Kattsov, V., Pitman, A., Shukla, J., Srinivasan, J., Stouffer, R. J., Sumi, A. & Taylor, K. E. (2007) Climate models and their evaluation. *Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC (FAR)* (S. Solomon, (D. Qin, (M. Manning, (Z. Chen, (M. Marquis, (K. B. Averyt, (M. Tignor & (H. L. Miller, eds): pp. 589–662. Cambridge: Cambridge University Press.

Rundel, P. W. (1969) Clinal variation in the production of usnic acid in *Cladonia subtenuis* along light gradients. *Bryologist* **72**: 40–44.

Scotter, G. W. (1967) The winter diet of barren-ground caribou in northern Canada. *Canadian Field-Naturalist* **81**: 33–39.

Solhaug, K. A. & Gauslaa, Y. (2012) Secondary lichen compounds as protection against excess solar radiation and herbivores. *Progress in Botany Vol 73* (U. Lüttge, (W. Beyschlag, (B. Büdel & (D. Francis, eds): pp. 283–304. Berlin, Heidelberg: Springer.

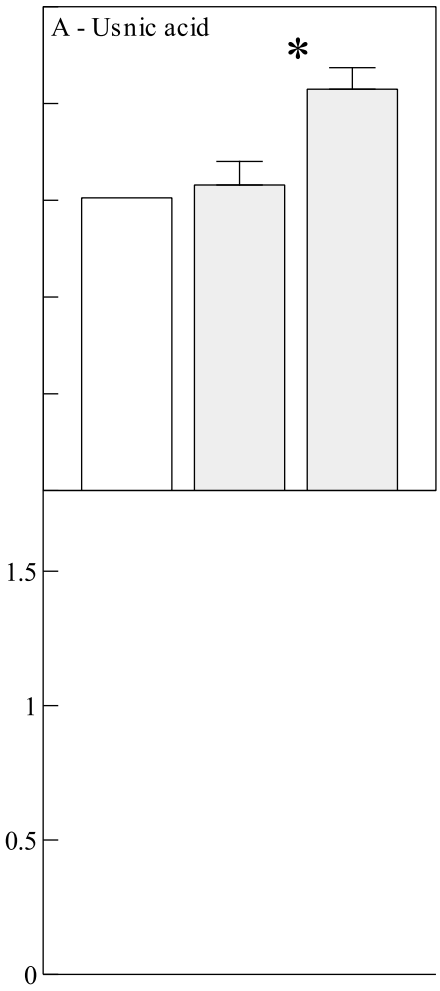
Solhaug, K. A., Gauslaa, Y., Nybakken, L. & Bilger, W. (2003) UV-induction of sun-screening pigments in lichens. *New Phytologist* **158**: 91–100.

Storeheier, P. V., Mathiesen, S. D., Tyler, N. J. C. & Olsen, M. A. (2002) Nutritive value of terricolous lichens for reindeer in winter. *Lichenologist* **34**: 247–257.

Wentz, F. J., Ricciardulli, L., Hilburn, K. & Mears, C. (2007) How Much More Rain Will Global Warming Bring? *Science* **317**: 233–235.

Figure 1. Concentrations (mean + 1 S.E.) of usnic acid and perlatolic acid in *Cladonia stellaris* grown under ambient or increased (+4°C) temperature conditions. The white bars represent start value from a bulk sample. Asterisks denotes signficant difference between the two treatments at $P < 0.05$.

360



361