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Ultraviolet Radiation and Plant Frost Hardiness in the Subarctic

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Abstract

The effects of ultraviolet radiation on plant frost hardiness were studied at two subarctic experimental sites, namely Abisko (northern Sweden, 68°N) and Sodankylä (northern Finland, 67°N). The Abisko treatment mimicked 15% ozone depletion and had been running for 7 yr. The investigated plant species was bilberry (*Vaccinium myrtillus* L.). The Sodankylä treatment consisted of lingonberry (*V. vitis-idaea*) and seedlings of mountain birch (*Betula pubescens* ssp. *czerepanovii*), which were exposed to both elevated UV-B and UV-A radiation in a modulated system corresponding to about 20% loss of the ozone layer. The frost hardiness tests in the Sodankylä experiment were carried out after UV exposure for one growing season. The frost hardiness of bilberry in Abisko was marginally decreased as a consequence of elevated UV-B exposure similarly to the frost hardiness of lingonberry and mountain birch in the Sodankylä experiment in August. The work provides evidence for rather than against a reducing effect of ultraviolet radiation on plant frost hardiness. The results suggest that elevated UV-A radiation probably reduces plant frost hardiness more efficiently than UV-B radiation.

Introduction

Frost hardiness in the plant kingdom provides for survival over freezing periods, i.e., the time during which the ambient temperature is below 0°C. Frost hardening in autumn starts with shortening days and cooling temperatures. Apart from these two major driving forces for frost hardiness, other environmental factors, such as nutrient or water supply, may modify the state of frost hardiness (Sakai and Larcher, 1987). Moreover, anthropogenic environmental factors, such as pollutants and carbon dioxide, may influence frost hardiness through changes in general health and stress (Barnes et al., 1996).

The increased ultraviolet B (UV-B radiation; 280–320 nm) flux reaching the Earth's surface is a consequence of depletion in the stratospheric ozone layer. The predictions based on recent climatic models already estimate that 20% stratospheric ozone depletion and a consequent 50%–60% increase in the springtime UV-B radiation will occur in the northern areas within the next two decades (Shindell et al., 1998; Taalas et al., 2000). Numerous papers report consequences of UV-B radiation on plant health and survival (e.g., Schnitzler et al., 1996; Sullivan et al., 1996; Björn et al., 1997; Schumaker et al., 1997; Nagel et al., 1998; Liakoura et al., 1999; Laakso et al., 2000; Bilger et al., 2001; Dring et al., 2001), while as yet, the effect of UV radiation on plant frost hardiness has received only scant attention. This is a peculiar gap, since the problem of ozone depletion and the consequent increase in UV-B radiation (i.e., >50%) will be significant in the subarctic, where the changes in air chemistry, reflectance from snow (albedo), and sparse forest structure explain the high intensity of UV-B radiation received by vegetation (e.g., Laakso and Huttunen, 1998).

Dunning et al. (1994) made pioneering work in investigating the relation between UV-B radiation and frost hardiness in a *Rhododendron* species. They concluded that exposure to UV-B radiation increases cold hardiness in this species. However, a more recent study suggests that an increase in UV-B radiation in the subarctic may increase the frost sensitivity of plants, i.e., decrease their frost hardiness (Beerling et al., 2001). The latter effect has also been discussed by the present authors elsewhere (Taulavuori et al., 2003a, 2003b), and it is gaining support by experimental evidence of the present results.

Material and Methods

EXPERIMENT I: ABISKO

The experiment was carried out at Abisko Scientific Research Station in Swedish Lapland (68.4°N). The experimental plots were exposed to daily increasing UV-B radiation from fluorescent lamps mimicking 15% ozone depletion (Björn and Teramura, 1993; Johanson et al., 1995). The experiment had been run for 7 growing seasons (1991–1997) before the sampling for frost hardiness. The samples were collected on 10 August 1997. A detailed description of the experimental system has been published by Taulavuori et al. (1998).

The studied plant material was a natural stand of bilberry (*Vaccinium myrtillus* L.), a dwarf shrub of the field layer of heath forests common from Central Europe to the High Arctic. Frost hardiness was tested with a freezing-induced electrolyte leakage method adopted for bilberry (E. Taulavuori et al., 1997; K. Taulavuori et al., 1997; Taulavuori et al., 2002). Five shoot tips of approximately 5 cm in length were detached from each plot and transported to the University of Oulu in a cool styrofoam box filled with crushed ice ($\pm 0^\circ\text{C}$). The frost hardiness testing procedure was started the next day and continued as described in detail by E. Taulavuori et al. (1997). Frost hardiness is expressed as a LT₅₀ value, i.e., the temperature causing 50% lethality of stem tissue. Antioxidant status (i.e., glutathione, ascorbate, etc.) was also analyzed and has been reported elsewhere (Taulavuori et al. 1998). The statistical analyses were performed with T-tests using a SPSS software package.

EXPERIMENT II: SODANKYLÄ

The experiment was established on a naturally growing forest at the Finnish International Ultraviolet Research Center (FUVIRC) in Sodankylä in Finnish Lapland (67.2°N) in June 2002. FUVIRC is jointly managed by Finnish Meteorological Institute—Arctic Research Center and Sodankylä Geophysical Observatory. The experimental system was organized and built by FUVIRC. A modulated UV-B radiation exposure system maintains the daytime UV-B radiation treatment level at a constant 46% above the ambient level of UV-B_{CIE}

TABLE 1

Monthly means for UV-B_{CIE} and UV-A radiations as percent of the ambient controls (= 100%) in the enhanced UV-A and UV-B treatments during the period June–September 2002. NOTE: (1) No records for UV-A radiation in June, and (2) its low % level in July due to shading frames in the lamp systems (see text for more details).

Radiation	Treatment	Month			
		Jun	Jul	Aug	Sep
UV-A	UV-A	—	103	130	116
	UV-B	—	94	118	109
UV-B _{CIE}	UV-A	95	98	103	102
	UV-B	135	136	133	122

radiation, corresponding to about 20% loss of the ozone layer. Table 1 shows that the daily mean (including the night hours) elevations of UV-B radiation were approximately 35% above the ambient level during the summer months of 2002. The UV-B radiation facility consists of 21 plots (1.2 × 1.2 m) with UV-B radiation treatment, UV-A radiation control, and ambient control, providing altogether seven replicates (n = 7) of each treatment. Each array of lamps consists of four UV-B radiation lamps (UV-B TL 12 RS/40W). The UV-B radiation treatment is achieved by encasing the lamp tubes in a cellulose diacetate film, which cuts off the radiation below 290 nm. Because the above treatment results in an increase of UV-A radiation, a control with enhanced UV-A radiation is included in the experiment. The lamps for this UV-A radiation control are wrapped in a polyester film that excludes all UV-C and UV-B radiation wavelengths. The ambient control treatment with shading equal to that beneath the UV arrays is achieved with lamp frames. Table 1 demonstrates that the enhanced UV-A radiation under the lamp systems was evident, except for the time (i.e., July and before) before the shading frames for ambient controls were assembled.

Erythemally weighted (CIE) Solar Light PMA1102 (UV-B radiation) and PMA1111 (UV-A radiation) sensors measure UV-B and UV-A radiation continuously to maintain the IR radiation at a constant level (10 UV-B sensors: one per each UV-B treatment, one for UV-A control, and one as ambient reference; and 3 UV-A sensors: one for UV-B treatment, UV-A control, and ambient reference). In order to ensure proper functioning of the modulated system, the sensor output was compared to the Brewer spectroradiometer and NILU-UV.

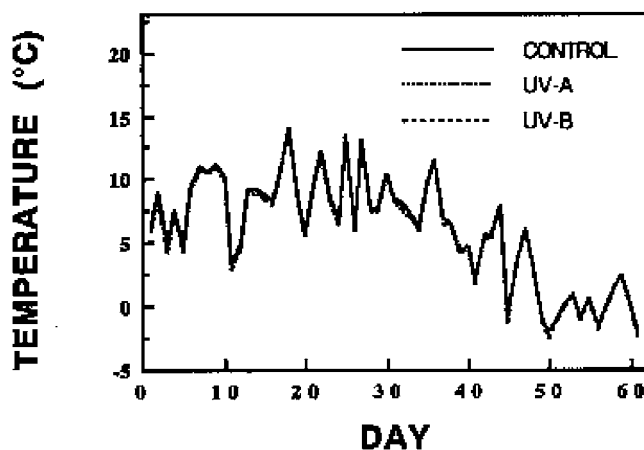


FIGURE 1. Daily minimum temperatures in Sodankylä at the beginning of the plant frost hardening period (August–September) 2002. Day 1 represents 1 August. The line of each treatment illustrates the mean of the seven replicate plots.

TABLE 2

Results on the effect of UV-B radiation on plant frost hardiness ($LT_{50} \pm SE$ (n = 3, Abisko; n = 7, Sodankylä). The different letters (a, b) indicate the significant difference at $P < 0.05$.

Species	Date	Site	Treatment	$LT_{50}(^{\circ}C)$
<i>Vaccinium myrtillus</i>	10 Aug (–97)	Abisko	control	-15 ± 4
			UV-B +	-9 ± 2
<i>Vaccinium vitis-idaea</i>	26 Aug (–02)	Sodankylä	control	-8 ± 1
			UV-A +	-7 ± 1
			UV-B +	-7 ± 1
	19 Sept (–02)	Sodankylä	control	-22 ± 3^a
			UV-A +	-14 ± 1^b
			UV-B +	-17 ± 3^{ab}
<i>Betula pubescens</i> ssp. <i>czerepanovii</i>	26 Aug (–02)	Sodankylä	control	-11 ± 3
			UV-A +	-9 ± 1
			UV-B +	-9 ± 1
	19 Sept (–02)	Sodankylä	control	-44 ± 7
			UV-A +	-47 ± 3
			UV-B +	-44 ± 7

Photosynthetically active radiation (PAR) (400–700 nm) was also continuously monitored with NILU-UV (1-minute average). Electronic controllers (Quictronic HF 2 × 36/230–240V DIM) are used to control the lamp output. UV radiation and PAR are measured continuously at the field site with NILU-UV (1-minute average). The temperature on each experimental plot was recorded with TinyTalk TK-0040 temperature loggers every 15 minutes (Fig. 1).

The studied plant materials were natural stands of lingonberry (*Vaccinium vitis-idaea* L.), and 1-yr-old mountain birch (*Betula pubescens* var. *czerepanovii*) seedlings micropropagated from seeds collected from Abisko. The birch seedlings were in 10 × 10 cm pots. The sampling was performed on 26 August and 19 September, and the samples were transported to the University of Oulu in a cool styrofoam box filled with crushed ice ($\pm 0^{\circ}C$). Frost hardiness was tested with a freezing-induced electrolyte leakage method including some species-specific adaptive modifications. The frost hardiness of lingonberry was determined from its evergreen leaves as described by Taulavuori et al. (2001). The frost hardiness of mountain birch was determined from shoot apices approximately 5 cm in length from the top (Taulavuori et al., 2004). The leaves were removed from the samples before artificial freezing tests and the subsequent electrolyte leakage tests. The data was analyzed with two-way ANOVA and subsequent post hoc comparison with Sheffe's test (SPSS).

Results

ABISKO

A marginal difference ($P < 0.1$) in frost hardiness between the control and UV-B radiation-exposed bilberry plants occurred, showing reduced frost hardiness of bilberry stems as a consequence of the enhanced UV-B radiation exposure (Table 2).

SODANKYLÄ

In lingonberry, the effects of sampling date and treatment on frost hardiness were statistically significant at $P < 0.001$ and $P < 0.05$, respectively. Frost hardiness was most pronounced in the lingonberry plants kept in ambient control plots (Table 2), indicating increased frost sensitivity for plants under elevated ultraviolet radiation. Indeed, the plants grown in enhanced UV-A control plots were less frost hardy. The frost hardiness of mountain birch also increased significantly

UV-radiation

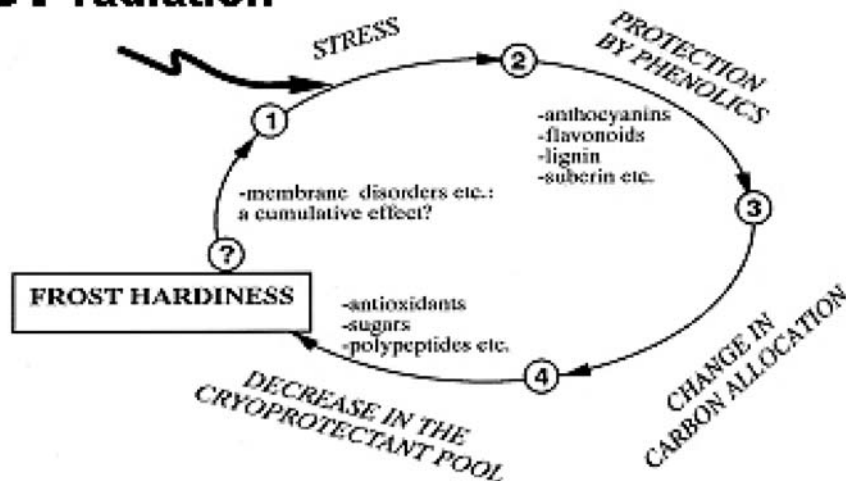


FIGURE 2. Hypothesis. UV radiation (1) is a stressor against which (2) protection mechanisms are activated through (3) altered carbon allocation in cells. (4) Consequently, the cryoprotectant pool is diminished due to carbon preference in protection against stress. The effect may become cumulative since frost-injured tissue is vulnerable to further stress.

during the sampling period ($P < 0.001$). No response to UV-B radiation was seen, although frost hardness during the first round of sampling was slightly higher in the control than in the UV-B radiation-treated seedlings (Table 2).

Discussion

This work shows that UV-B radiation impairs rather than improves frost hardness, and the findings are therefore more consistent with the results of Beerling et al. (2001) than with those reported by Dunning et al. (1994). The overall results on lingonberry demonstrate a statistically significant decrease in frost hardness due to an increase in ultraviolet radiation. In addition, if the results are considered separately according to species and sampling date, we have four cases to support the negative effect of UV-B radiation on plant frost hardness. Only one case (mountain birch in September) is not consistent with this trend, nor does it support the improving effect. The response cannot be attributed to possible heat due to enhanced radiation since no differences between the temperatures of the treatments were found (Fig. 1).

Interestingly, the most significant response of frost hardness to ultraviolet radiation was observed in the UV-A control treatment of lingonberry (Table 2). Actually, UV-B enhancement also produces enhanced UV-A radiation, although not to the same extent. Therefore, the decrease in frost hardness may appear in response to enhancing UV-A radiation. A recent paper (White and Jahnke, 2002) suggests that UV-A radiation, but not UV-B radiation, directly affects photosynthesis, manifested as a loss of photosystem II electron transport efficiency and free radical formation. UV-A radiation elevates the intracellular H_2O_2 concentration and other active oxygen species of the cereal aleurone layer through diminished amounts of enzymes that metabolize them (Fath et al., 2002). UV-A radiation may decrease frost hardness by enhancing oxidative stress, which results in depletion of the antioxidant pool. Indeed, synergistic interactions between UV-B radiation and distinct UV-A radiation pathways may maximize the response (Jenkins et al., 2001) in accordance with the higher UV-A radiation doses in UV-B radiation treatment compared to the control (Table 1).

As antioxidants belong to the plant cryoprotectant pool, they provide reducing power to stabilize the membranes and structures of macromolecules under such conditions as frost, which is favorable for free radical formation. Therefore, it is not surprising that antioxidant activities and concentrations increase toward winter (Levitt, 1972; Esterbauer and Grill, 1978; Guy and Carter, 1982; De Kok and Stulen, 1993; Kunert and Foyer, 1993; Polle et al., 1996). Frost hardness in

bilberry stems from the Abisko treatment was consistently lower in plants grown under enhanced UV-B radiation, and the plants also showed lower ascorbate and glutathione concentrations and lower enzymatic activity of ascorbate peroxidase in September (Taulavuori et al., 1998). It is thus suggested that oxidative stress, originating from either the UV-A or the UV-B region of the light spectrum, is a factor in reducing plant frost hardness.

The role of sugars as cryoprotectants has been recognized for a long time (Levitt, 1956, and references therein; Ericsson, 1979; Sakai and Larcher, 1987; Sauter, 1988; Sauter and van Cleve, 1991; Witt and Sauter, 1994; Vágújfalvi et al., 1999; Greer et al., 2000; Zhang et al., 2003), although some results fail to agree completely with this assumption (e.g., Pomeroy et al. 1970; Obrist et al., 2001). Our earlier findings in bilberry are also in accordance with the significance of soluble sugars in cryoprotection, as the most hardened plants in spring had the highest concentrations of glucose, fructose, and sucrose (K. Taulavuori et al., 1997). The plants exposed to enhanced UV-B radiation in the present Abisko treatment also showed lower glucose contents (Taulavuori, unpublished) attributed to reduced frost hardness. According to Stushnoff et al. (1998), glucose, fructose, and sucrose oscillate during the dormant stages, but oligosaccharides (raffinose, stachyose) are associated with cold hardness, low temperatures, and dormancy in woody species. Irrespective of the biochemical nature of carbohydrate, their role in cryoprotection is to act as compatible solutes, i.e., (1) to stabilize membranes and macromolecules through hydrogen bonds between sugar OH groups and phospholipids (Crowe et al., 1984; Caffrey et al., 1988; Blackman et al., 1992), (2) to compensate for concentrating salts during desiccation (Blackman et al., 1992), and (3) to prevent excess dehydration by favoring the vitrification process to pass ice formation (Bruni and Leopold, 1991; Koster, 1991; Blackman et al., 1992; Stushnoff et al., 1998).

The roles of antioxidant metabolism and carbohydrates provide interesting cues to the relation between ultraviolet radiation and plant frost hardness. In principle, frost hardness is reduced if carbohydrates are allocated more toward protection against ultraviolet radiation: the more phenolics (pigmentation, lignification) (e.g., Laakso et al., 2000; Searles et al., 2001; Tegelberg et al., 2001) are needed, the less carbon there is available for the synthesis of antioxidants in the glutathione-ascorbate cycle (cryoprotection). Consistently, the present paper demonstrates reduced frost hardness in response to UV-A radiation, and it is shown elsewhere (Tegelberg et al., 2002) that only minor increase in UV-A may significantly increase certain UV-absorbing phenolics. While ultraviolet radiation may somewhat activate the antioxidant defense system, the net surplus for cryoprotection is lost in

the activities of glutathione S-transferase and GS-X pumps, which enzymes are responsible for the accumulation of glutathione conjugates, such as GSH-anthocyanin in the vacuole (Marrs, 1996; Alfenito et al., 1998). In addition, the UV-B radiation effect may be cumulative (long-term effects) since plants that have suffered from frost injuries may become more susceptible to further UV-B radiation stress as a consequence of frost injuries: the structure and function of cell membranes is the primary site of frost injuries (e.g., Steponkus, 1990; Yoshida, 1991) as well as other environmental injuries (e.g., Leshem, 1992; Lüttge et al., 1995). The concept for the above hypothesis on the reduced frost hardiness of plants under ultraviolet radiation stress is shown in Figure 2.

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References Cited

- Alfenito, M. R., Souer, E., Goodman, C. D., Buell, R., Mol, J., Koes, R., and Walbot, V., 1998: Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione S-transferases. *Plant Cell*, 10: 1135–1149.
- Barnes, J. D., Hull, M. R., and Davison, A. W., 1996: Impact of air pollutants and elevated carbon dioxide on plants in wintertime. In: Yunus, M., and Iqbal, M. (eds.), *Plant Responses to Air Pollution*. Chichester, U.K., Wiley, 135–166.
- Beerling, D. J., Terry, A. C., Mitchell, P. L., Callaghan, T. V., Gwynn-Jones, D., and Lee, J. A., 2001: Time to chill: effects of simulated global change on leaf ice nucleation temperatures of subarctic vegetation. *American Journal of Botany*, 88: 628–633.
- Bilger, W., Johnsen, T., and Schreiber, U., 2001: UV-excited chlorophyll fluorescence as a tool for assessment of UV-protection by the epidermis of plants. *Journal of Experimental Botany*, 52: 2007–2014.
- Björn, L.O., and Teramura, A. H., 1993: Light sources for UV-B photobiology. In: Young, A. R., Björn, L. O., Moan, J., and Nultsch, W. (eds.), *Environmental UV Photobiology*. New York, Plenum Press, 41–71.
- Björn, L. O., Callaghan, T. V., Johnsen, I., Lee, J. A., Manetas, Y., Paul, N. D., Sonesson, M., Wellburn, A. R., Coop, D., Heide-Jørgensen, H. S., Gehrke, C., Gwynn-Jones, D., Johanson, U., Kyparissis, A., Levizou, E., Nikolopoulos, D., Petropoulou, Y., and Stephanou, M., 1997: The effects of UV-B radiation on European heathland species. *Plant Ecology*, 128: 252–264.
- Blackman, S. A., Obendorf, R. L., and Leopold, A. C., 1992: Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. *Plant Physiology*, 100: 225–230.
- Bruni, F., and Leopold, A. C., 1991: Glass transition in soybean seed. Relevance to anhydrous biology. *Plant Physiology*, 96: 660–663.
- Caffrey, M., Fonseca, V., and Leopold, A. C., 1988: Lipid-sugar interactions. *Plant Physiology*, 86: 754–758.
- Crowe, J. H., Crowe, L. M., and Chapman, D., 1984: Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science*, 223: 701–703.
- De Kok, L. J., and Stulen, I., 1993: Role of glutathione in plants under oxidative stress. In: De Kok, L. J., Stulen, I., Rennenberg, H., Brunold, C., and Rauser, W. (eds.), *Sulfur Nutrition and Assimilation in Higher Plants*. The Hague, Netherlands, SPB Academic Publishing bv, 122–138.
- Dring, M.-J., Wagner, A., and Luening, K., 2001: Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. *Plant Cell Environment*, 24: 1153–1164.
- Dunning, C. A., Chalker-Scott, L., and Scott, J. D., 1994: Exposure to ultraviolet-B radiation increases cold hardiness in *Rhododendron*. *Physiologia Plantarum*, 92: 516–520.
- Ericsson, A., 1979: Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needle on 20-year-old pine trees (*Pinus sylvestris*). *Physiologia Plantarum*, 45: 270–280.
- Esterbauer, H., and Grill, D., 1978: Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*. *Plant Physiology*, 61: 119–121.
- Fath, A., Bethke, P., Beligni, V., and Jones, R., 2002: Active oxygen and cell death in cereal aleurone cells. *Journal of Experimental Botany*, 372: 1273–1282.
- Greer, D. H., Robinson, L. A., Hall, A. J., Klages, K., and Donnison, H., 2000: Frost hardening of *Pinus radiata* seedlings: effects of temperature on relative growth rate, carbon balance and carbohydrate concentration. *Tree Physiology* 20: 107–114.
- Guy, C. L., and Carter, J. V., 1982: Effect of low temperature on the glutathione status of plant cells. In: Li, P. H., and Sakai, A. (eds.), *Plant Cold Hardiness and Freezing Stress. Mechanisms and Crop Implications*, Vol. 2. New York, Academic Press Inc., 169–180.
- Jenkins, G. I., Long, J. C., Wade, H. K., Shenton, M. R., and Bibikova, T. N., 2001: UV and blue light signalling: pathways regulating chalcone synthase gene expression in *Arabidopsis*. *New Phytologist*, 151: 121–131.
- Johanson, U., Gehrke, C., Björn, L. O., Callaghan, T. V., and Sonesson, M., 1995: The effects of enhanced UV-B radiation on the sub-arctic heath ecosystem. *Ambio*, 24: 106–111.
- Koster, K. L., 1991: Glass formation and desiccation tolerance in seeds. *Plant Physiology*, 96: 302–304.
- Kunert, K. J., and Foyer, C., 1993: Thiol/sulfide exchange in plants. In: De Kok, L. J., Stulen, I., Rennenberg, H., Brunold, C., and Rauser, W. (eds.), *Sulfur Nutrition and Assimilation in Higher Plants*. The Hague, Netherlands, SPB Academic Publishing bv, 139–151.
- Laakso, K., and Huttunen, S., 1998: Effects of the UV-B radiation (UV-B) on conifers: a review. *Environmental Pollution*, 99: 319–328.
- Laakso, K., Sullivan, J. H., and Huttunen, S., 2000: The effects of UV-B radiation on epidermal anatomy in loblolly pine (*Pinus taeda* L.) and Scots pine (*Pinus sylvestris* L.). *Plant Cell and Environment*, 23: 461–472.
- Leshem, Y. Y., 1992: *Plant membranes: A Biophysical Approach to Structure, Development and Senescence*. Dordrecht, Kluwer Academic Publishers, 280 pp.
- Levitt, J., 1956: *The Hardiness of Plants*. New York, Academic Press, 278 pp. A Series of Monographs Prepared under the Auspices of The American Society of Agronomy, A. G. Norman (ed.).
- Levitt, J., 1972: *Responses of Plants to Environmental Stresses, second edition*. New York, Academic Press, 697 pp.
- Liakoura, V., Stavrianakou, S., Liakopoulos, G., Karabourniotis, G., and Manetas, Y., 1999: Effects of UV-B radiation on *Olea europaea*: comparisons between a greenhouse and field experiment. *Tree Physiology*, 19: 905–908.
- Lüttge, U., Ratajczak, R., Rausch, T., and Rockel, B., 1995: Stress responses of tonoplast proteins: an example for molecular ecophysiology and the search for eco-enzymes. *Acta Botanica Neerlandica*, 44: 343–362.
- Marrs, K. A., 1996: The functions and regulation of glutathione S-transferases in plants. *Annual Review in Plant Physiology and Molecular Biology*, 47: 127–158.
- Nagel, L. M., Bassman, J. A., Edwards, G. E., Robbrecht, R., and Franceschi, V. R., 1998: Leaf anatomical changes in *Populus trichocarpa*, *Quercus rubra*, *Pseudotsuga menziesii* and *Pinus ponderosa* exposed to enhanced ultraviolet-B radiation. *Physiologia Plantarum*, 104: 385–396.
- Obrist, D., Arnone, J. A., and Körner, Ch., 2001: *In situ* effects of elevated atmospheric CO₂ on leaf freezing resistance and carbo-

- hydrates in a native temperate grassland. *Annals of Botany*, 87: 839–844.
- Polle, A., Kröniger, W., and Rennenberg, H., 1996: Seasonal fluctuations of ascorbate-related enzymes: acute and delayed effects of late frost in spring on antioxidative system in needles of Norway spruce (*Picea abies* L.). *Plant Cell Physiology*, 37: 717–725.
- Pomeroy, M. K., Siminovitch, D., and Wightman, F., 1970: Seasonal biochemical changes in the living bark and needles of red pine (*Pinus resinosa*) in relation to adaptation of freezing. *Canadian Journal of Botany*, 48: 953–967.
- Sakai, A., and Larcher, W., 1987: *Frost Survival of Plants. Responses and Adaptation to Freezing Stress*. In: Billings, W. D., Golley, F., Lange, O. L., Olson, J. S., and Remmert, H. (eds.), Ecological Studies, Volume 62. Berlin, Springer Verlag, 321 pp.
- Sauter, J. J., 1988: Temperature-induced changes in starch and sugars in the stem of *Populus x canadensis* “robusta”. *Journal of Plant Physiology*, 132: 608–612.
- Sauter, J. J., and van Cleve, B., 1991: Biochemical and ultrastructural results during starch-sugar-conversion in ray parenchyma cells of *Populus* during cold hardening. *Journal of Plant Physiology*, 139: 19–26.
- Schnitzler, J.-P., Jungblut, T. P., Heller, W., Köfferlein, M., Hutzler, P., Heinzmann, U., Schmelzer, E., Langebartels, C., and Sander-mann, H., Jr., 1996: Tissue localization of UV-B-screening pigments and of chalcone synthase mRNA in needles of Scots pine seedlings. *New Phytologist*, 132: 247–258.
- Schumaker, M. A., Bassman, J. H., Robbrecht, R., and Radamaker, G. K., 1997: Growth, leaf anatomy, and physiology of *Populus* clones in response to solar ultraviolet-B radiation. *Tree Physiology*, 17: 617–626.
- Searles, P. S., Flint, S. D., and Caldwell, M. M., 2001: A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia*, 127: 1–10.
- Shindell, D. T., Rind, D., and Lonergan, P., 1998: Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentration. *Nature*, 392: 589–592.
- Steponkus, P. L., 1990: Cold acclimation and freezing injury from the perspective of the plasma membrane. In: Katterman, F. (ed.), *Environmental Injury to Plants*. San Diego, California, Academic Press Inc., 1–16.
- Stushnoff, C., Seufferheld, M. J., and Creegan, T., 1998: Oligosaccharides as endogenous cryoprotectants in woody plants. In: Li P. H., and Chen T. H. H. (eds.), *Plant Cold Hardiness. Molecular Biology, Biochemistry, and Physiology*. New York, Plenum Press, 301–310.
- Sullivan, J. H., Howells, B. W., Ruhland, C. T., and Day, T. A., 1996: Changes in leaf expansion and epidermal screening effectiveness in *Liquidambar styraciflua* and *Pinus taeda* in response to UV-B radiation. *Physiologia Plantarum*, 98: 349–357.
- Taalas, P., Kaurola, J., and Kylling, A., 2000: The impact of greenhouse gases and halogenated species on the future solar UV radiation doses. *Geophysical Research Letters*, 27: 1127–1130.
- Taulavuori, E., Taulavuori, K., Laine, K., Pakonen, T., and Saari, E., 1997: Winter hardening and glutathione status in the bilberry (*Vaccinium myrtillus* L.) in response to trace gases (CO₂, O₃) and nitrogen fertilization. *Physiologia Plantarum*, 101: 192–198.
- Taulavuori, E., Bäckman, M., Taulavuori, K., Gwynn-Jones, D., Johansson, U., Laine, K., Callaghan, T., Sonesson, M., and Björn, L. O., 1998: Long-term exposure to enhanced UV-B radiation in the sub-arctic does not cause oxidative stress in *Vaccinium myrtillus*. *New Phytologist*, 140: 691–697.
- Taulavuori, K., Laine, K., Taulavuori, E., Pakonen, T., and Saari, E., 1997: Accelerated dehardening in bilberry (*Vaccinium myrtillus* L.) induced by a small elevation in air temperature. *Environmental Pollution*, 98: 91–95.
- Taulavuori, K., Taulavuori, E., Niinimaa, A., and Laine, K., 2001: Acceleration of frost hardening in *Vaccinium vitis-idaea* by nitrogen fertilization. *Oecologia*, 127: 321–323.
- Taulavuori, K., Laine, K., and Taulavuori, E., 2002: Artificial deacclimation response of *Vaccinium myrtillus* in mid-winter. *Annales Botanici Fennici*, 39: 143–147.
- Taulavuori, K., Taulavuori, E., and Laine, K., 2003a: A review: Frost survival of woody plants at high latitudes with special reference to global change. *Recent Research Development in Biochemistry*, 4: 1017–1028.
- Taulavuori, K., Taulavuori, E., and Laine, K., 2003b: Northern frost survival of plants under global change. In: Strand, K., Taskinen, P., and Kallio, K. (eds.), *Proceedings of the Thule Science Day. Northern Dimensions and Arctic Environments*. Nov 25 2003, University of Oulu, 17–20.
- Taulavuori, K. M. J., Taulavuori, E. B., Skre, O., Nilsen, J., Igeland, B., and Laine, K., 2004: Dehardening of mountain birch (*Betula pubescens* ssp. *czerepanovii*) at elevated winter temperatures. *New Phytologist*, 162: 427–436.
- Tegelberg, R., Julkunen-Tiitto, R., and Aphalo, P. J., 2001: The effects of long-term elevated UV-B on the growth and phenolics of field-grown silver birch (*Petula pendula*). *Global Change Biology*, 7: 839–848.
- Tegelberg, R., Julkunen-Tiitto, R., and Aphalo, P. J., 2002: The effects of long-term elevated ultraviolet radiation on phytochemicals in the bark of silver birch (*Betula pendula*). *Tree Physiology*, 22: 1257–1263.
- Vágújfalvi, A., Kerepesi, I., Galiba, G., Tischner, T., and Sutka, J., 1999: Frost hardiness depending on carbohydrate changes during cold acclimation in wheat. *Plant Science*, 144: 85–92.
- White, A. L., and Jahnke, L. S., 2002: Contrasting effects of UV-A and UV-B on photosynthesis and photoprotection of β -carotene in two *Dunaliella* spp. *Plant and Cell Physiology* 43: 877–884.
- Witt, W., and Sauter, J. J., 1994: Enzymes of starch metabolism in poplar wood during fall and winter. *Journal of Plant Physiology*, 143: 625–631.
- Yoshida, S., 1991: Chilling-induced inactivation and its recovery of tonoplast H⁺-ATPase in mung bean cell suspension cultures. *Plant Physiology*, 95: 456–460.
- Zhang, G., Ryppö, A., Vapaavuori, E., and Repo, T., 2003: Quantification of additive response and stationary of frost hardiness by photoperiod and temperature in Scots pine. *Canadian Journal of Forest Research*, 33: 1772–1784.

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