Changes of the photosystem 2 activity and thylakoid proteins in spruce seedlings during water stress

P.A.K. EASTMAN*,+, A. RASHID** and E.L. CAMM***,+

*Forest Biotechnology Centre, British Columbia Research Inc., Vancouver B.C. V6S 2L2, Canada**
*Pest Management Branch, Alberta Environmental Centre, Vegreville, Alberta T9C 1T4, Canada***
*Department of Biology, University College of Fraser Valley, Abbotsford, B.C. V2S 7M9, Canada****
Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada+

Abstract

We subjected spruce [*Picea glauca* (Moench) Voss × *P. engelmanni* Parry hybrid complex] seedlings to a severe water stress (shoot water potential ≤-3.5 MPa) to permit assessment of stress effects on photosystem 2 (PS2) in isolated photosynthetic membranes. The thylakoids and *Triton*-treated membranes isolated from stressed seedlings showed declines in O_2-evolving capacity (H_2O → 2,6-dichloro-*p*-benzoquinone, DCBQ) and electron transport activity (H_2O → 2,6-dichlorophenol indophenol, DCIP). A partial restoration of O_2-evolution by adding $CaCl_2$ suggested an effect of water stress on the oxygen-enhancing extrinsic (OEE) polypeptides. Water stress had an additional impact on the reaction centre, shown by the inability of 1,5-diphenylcarbazide (DPC) to restore the electron transport (DPC → DCIP) to the levels seen in control membranes. Quantification of specific photosynthetic membrane proteins by immunoblots strengthened the above suggestions: after drought stress, concentrations of OEE1 and OEE2 declined by 40 %, and amount of the reaction centre protein D1, ATP synthetase, and cytochrome *f* also declined. The specific effect of stress on these proteins was confirmed by the fact that the amount of chlorophyll-protein complex CP2 was unchanged in membranes isolated from drought-stressed seedlings.

Received 14 August 1996, *accepted* 13 November 1996.
*Phone: 603 224 4331, fax: 604 224 0540, e-mail: acastman@bcr.bc.ca.
Abbreviations: BSA, bovine serum albumin; Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCIP, 2,6-dichlorophenol indophenol; DPC, 1,5-diphenylcarbazide; F_m, maximal fluorescence; F_v, variable fluorescence; Mes, morpholinoethane sulfonic acid; OEE, oxygen-enhancing extrinsic; PMSF, phenylmethysulfonyl fluoride; PS, photosystem; SDS, sodium dodecyl sulfate.
Acknowledgments: Plasmid pPND1 was a generous gift from Dr. P.J. Nixon. Spruce seedlings were donated by Pacific Regeneration Technologies, Victoria B.C. We thank Dr. B.R. Green for a helpful review of this manuscript. Support from NSERC (E.L.C.) and the Science Council of British Columbia (P.A.K.E.) is gratefully acknowledged.

Additional key words: chlorophyll fluorescence induction; gymnosperm; oxygen-enhancing extrinsic polypeptides; oxygen evolution; *Picea glauca* (Moench) Voss × *P. engelmanni* Parry hybrid complex; *Triton X-100*.

Introduction

The impact of environmental stress on physiology and composition of photosynthetic membranes has been the subject of much attention. During photoinhibition, for example, changes in electron transport and thylakoid protein dynamics evoked by an array of stresses including water deprivation have been modeled from studies using green algae and angiosperm species (Aro *et al.* 1993). In particular, regulation of PS2 activity during stress may involve changes in the OEE polypeptides.

Evidence that control of PS2 activity, through changes in water oxidation, can be effected by the OEE polypeptides is provided by numerous examples in which environmental stresses cause loss or dissociation of OEE polypeptides in angiosperm species. In response to freezing (Shen *et al.* 1990), chilling (Wang *et al.* 1992), or high irradiance (Aro *et al.* 1993), changes in OEE protein levels and/or membrane association have been documented. In contrast to other nuclear-encoded chloroplast proteins, there are pools of OEE polypeptides that are free in the lumen, suggesting a persistent regulatory role (Ettinger and Theg 1991). Such a population of free OEE proteins may be a component of the constitutive repair cycle of the labile PS2 centres (Aro *et al.* 1993). The effect of such water stress *per se* on OEE polypeptides is not known, although water stress increases the susceptibility of PS2 to photoinhibition (Powles 1984).

Generally, less attention has been paid to the effect of environmental stress in photosynthetic membranes of conifers, although here too, the negative effect of environmental stress on electron transport and amounts of specific proteins have been documented. For example, seasonal stress (Öquist and Strand 1986, Bolhàr-Nordenkampf and Lechner 1988, Strand and Öquist 1988) and pollution damage (Dietz *et al.* 1988, Evans *et al.* 1992, Ruth and Weisel 1993) have been associated with lowered electron transport. Changes in protein synthetic capacity (Schmitz *et al.* 1993) and in amounts or activities of various thylakoid proteins such as cytochrome *f* and the D1 reaction centre protein (Wild *et al.* 1988, Flammersfeld and Wild 1992, Godde 1992, Godde and Buchhold 1992, Lütz *et al.* 1992) have also been reported for pollution-damaged Norway spruce.

For conifers like spruce, water stress is a frequent environmental constraint on photosynthesis, causing an array of responses well-characterized at the whole plant level (Binder *et al.* 1989, Grossnickle 1989) and membrane level (Eastman and Camm 1995). Although analysis of chlorophyll (Chl) fluorescence induction kinetics (Schreiber *et al.* 1986) has provided evidence that PS2 activity is inhibited at the donor side (Toivonen and Vidaver 1988), the biochemical processes leading to inhibition of water oxidizing ability during water stress have not been explored previously in a gymnosperm species. In our study we looked at the effects of severe drought stress on electron transport through PS2 in thylakoids isolated from one-year

old seedlings of spruce. We then estimated levels of photosynthetic proteins in well-watered and droughted plants, using antibodies recognizing OEE proteins, D1, cytochrome *f*, ATP synthetase,and Chl-protein complex CP2.

Materials and methods

One-year-old interior spruce [*Picea glauca* (Moench) Voss × *P. engelmanni* Parry hybrid complex] seedlings grown in styrofoam block containers were maintained in a greenhouse under natural photoperiod through September 1993 to May 1994. Buds remained dormant throughout this period as indicated by the absence of new shoot growth.

To induce water stress, water was withheld from subsets of the young trees until predawn shoot water potential was ≤-3.5 MPa measured with a pressure chamber model *3015* (*Soilmoisture Equip. Corp.*, Santa Barbara, CA, U.S.A.). For each set of stressed trees analyzed, an adjacent, regularly watered group was used for comparison. Stress was also assessed by measurement of variable fluorescence/maximal fluorescence (F_v/F_m) after 10 min dark adaptation, using previously described parameters (Eastman and Camm 1995). A minimum of five plants was used to calculate an average value of F_v/F_m.

After determination of water status, needles were harvested from well-watered or drought-stressed seedlings by freezing the shoots in liquid nitrogen. The needle tissue was crushed to a fine powder and homogenized in 50 mM Tricine-NaOH (pH 7.6) containing 500 mM saccharose, 10 mM NaCl, 5 mM $MgCl_2$, 2 % soluble polyvinylpyrrolidone *PVP-40*, 0.1 % BSA, and 1 mM PMSF. After filtering the resultant brei through cheesecloth, the filtrate was centrifuged at 2500×*g* for less than 2 min. The chloroplast pellets were washed 5 or more times by resuspension in the isolation buffer without *PVP-40* or BSA, followed by 2 min centrifugations at 2500× *g*. The final thylakoid pellet was resuspended in thylakoid storage buffer (25 mM Hepes-NaOH, pH 7.6, containing 1 M saccharose, 10 mM NaCl, and 5 mM $MgCl_2$), and stored at -80 °C. For immunoprobing, additional protease inhibitors (1 mM benzamidine and 1 g m^{-3} leupeptin) were included in all solutions. The Chl content was determined according to Arnon (1949) or Lichtenthaler and Wellburn (1983).

To prepare detergent fractionated membranes, thylakoids were thawed on ice and *Triton X-100* added to a final detergent:Chl ratio of 15:1. Samples were incubated in the dark for 20 min on ice with gentle agitation, then diluted with thylakoid storage buffer, and briefly centrifuged at 2500×*g*. The supernatant was centrifuged at 39 000 ×*g* for 30 min, and the final membrane pellet resuspended in 25 mM Mes-NaOH (pH 6) containing 2 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. For immunoprobing, additional protease inhibitors (1 mM benzamidine and 1 g m^{-3} leupeptin) were included in the final storage buffer.

Photosynthetic oxygen evolution by thylakoids or *Triton*-treated thylakoids equivalent to 40 μg Chl was measured at 22 °C using a Clark-type oxygen electrode (*Hansatech* model *DW1*) upon irradiance with 1000 μmol m^{-2} s^{-1} "white light"

(*Kodak Ektagraphic III E* projector equipped with a 300 W tungsten halogen lamp). The reaction mixture (2 cm^3) consisted of 25 mM Mes-NaOH (pH 6.0) containing 500 mM saccharose, and 10 mM NaCl. As an electron acceptor from PS2, 500 μM DCBQ was used. Oxygen production was measured with and without the addition of 15 mM $CaCl_2$. All measurements were performed in triplicate.

The capacity of thylakoids or *Triton*-treated membranes (equivalent to 30 μg Chl) to photoreduce DCIP was measured at 590 nm with a *Cary 210* (*Varian*) spectrophotometer. The reaction mixture (2 cm^3) was the same as that used for oxygen evolution with the addition of 60 μM DCIP. Measurements made with or without the addition of 15 mM $CaCl_2$ and with or without 500 μM DPC were performed in triplicate.

Membrane preparations were solubilized in 70 mM Tris-HCl (pH 6.8) containing 20 % glycerol, 2 % sodium dodecyl sulfate (SDS), and 10 % β-mercaptoethanol by heating at 90 °C for 3 min. Membrane polypeptides, loaded on the basis of Chl, were resolved on T=12 % or 14 %, C=2.7 % polyacrylamide gels. Gels were stained with Coomassie Brilliant Blue *G-250* or transferred to nitrocellulose (0.45 μm). Polyclonal antibodies were used to immunodecorate the protein blots. Goat antirabbit antibody conjugated with alkaline phosphatase, fast red (1,5-naphthalenedisulfonate salt), and sodium napthal AS-MX phosphate were used for detection. Immunoblots were scanned with an *XRS* scanner using *BioImage* software (*Visage* version *4.6Q, Millipore Corp.* 1993) for quantification.

OEE1 protein, isolated from spinach chloroplasts (Dunahay *et al.* 1984) following the method of Yamamoto and Kubota (1987) and further purified by preparative SDS-PAGE gels, was used to immunize rabbits. The immune serum cross-reacted with proteins of 33 kDa in chloroplast preparations from spinach, lettuce, larch, pine, and spruce. The OEE2 antibody has been previously described (Rashid *et al.* 1994). For production of the D1 antibody, a full length D1-β-galactosidase fusion protein generated by transformation of *E. coli* with plasmid pPND1 (Nixon *et al.* 1987), and purified by preparative SDS-PAGE gels was used to immunize rabbits. The serum cross reacted with the D1 proteins in spinach and lettuce, identified by a verified D1 antibody (gift of Dr. L. McIntosh, Michigan State University, MI, U.S.A.) and protein bands of slighter lower mobility in larch, pine, and spruce. The antibody to cytochrome *f* was a gift of Dr. R. Malkin (University of California at Berkley, CA, U.S.A.). Antibodies to CP2 and ATP synthetase were gifts of Dr. B.R. Green (University of British Columbia, Vancouver, Canada).

Results and discussion

One-year-old spruce seedlings grown under natural irradiation were deprived of water until the average predawn shoot water potential was less than -3.5 MPa, and the average maximal quantum yield (F_v/F_m) was between 0.3 and 0.5, indicating that these plants were significantly photoinhibited. We have previously demonstrated that stomatal conductance and photosynthetic gas exchange are minimal at predawn shoot

water potentials below -1 MPa (Eastman and Camm 1995) in water-stressed spruce seedlings. Gas exchange approaches zero at water potentials below -2.86 MPa (Brix 1979). Well-watered control seedlings had an average predawn shoot water potential of -0.5 MPa and F_v/F_m of 0.8.

In thylakoid membranes from water-stressed spruce seedlings, the rate of oxygen evolution using DCBQ as an electron acceptor from PS2 ($H_2O \rightarrow DCBQ$) was only 40 % that of membranes isolated from well-watered plants (Table 1). A reduction in activity may be a consequence of loss of the requisite Ca^{2+} cofactor associated with the OEE2 polypeptide or disruption of the reaction centre Mn complex shielded by OEE1 (Enami *et al.* 1994). In this case the fact that addition of 15 mM $CaCl_2$ to stressed thylakoids restored activity to only 53 % of control indicated that only some of the stress-induced activity loss could be accounted by Ca^{2+} cofactor associated with the OEE2 polypeptide.

Photoreduction of DCIP with water as the electron donor ($H_2O \rightarrow DCIP$) was reduced by 50 % in stressed thylakoids (Table 1). Again addition of $CaCl_2$ increased PS2 activity in stressed membranes, confirming loss of Ca^{2+} cofactor associated with the OEE2 polypeptide. Addition of DPC as an electron donor, in the absence of added $CaCl_2$, augmented electron transport capacity in thylakoids from stressed plants, but only to 63 % of control levels. This observation suggested that a proportion of the reaction centres were impaired beyond the OEE.

Table 1. Oxygen evolution ($H_2O \rightarrow DCBQ$) [mmol(O_2) kg^{-1}(Chl) s^{-1}] and DCIP photoreduction [mmol(DCIP) kg^{-1}(Chl) s^{-1}] activities of thylakoid membranes and *Triton X-100* treated thylakoids isolated from well-watered and water-stressed spruce, *Picea glauca*, seedlings. Values are means ± standard error.

Activity	Thylakoids well-watered	water-stressed	*Triton*-treated well-watered	water-stressed
Oxygen evolution	114±6	46±1	63±7	32±1
+15 mM $CaCl_2$	-	61±3	63±6	45±3
DCIP photoreduction	63±4	30±2	70±4	18±1
+15 mM $CaCl_2$	64±1	36±1	71±2	24±1
+0.5 mM DPC	66±6	42±3	71±3	45±4

To verify that exogenously added cofactors, electron donors and acceptors had access to the PS2 reaction center, oxygen evolution and electron transport were also measured in membrane preparations partially disrupted with the detergent *Triton*, using the method of Rashid and Camm (1995) (Table 1). *Triton* treatment resulted in the loss of some O_2-evolving activity even in preparations from well-watered plants. In membranes from water-stressed plants, oxygen evolving activity was reduced compared to well-watered controls, and little of the activity was restored by $CaCl_2$. PS2 activity using DCIP as electron acceptor was very low in *Triton*-treated membranes from stressed thylakoids. In this case, addition of DPC significantly enhanced DCIP photoreduction in stressed, *Triton*-treated membranes. Although

activity was not restored to the control levels, the DPC stimulation suggested that a donor-side inactivation, possibly resulting from dissociation of the OEE1 protein, was exacerbated by the *Triton* treatment. Thus, drought stress affects PS2 in at least two ways, *i.e.*, by perturbation of the Ca^{2+} cofactor associated with the OEE2 polypeptide, and by direct effects on the reaction centre.

We examined levels of photosynthetic membrane proteins in thylakoid membranes from stressed and well-watered seedlings to see if these were consistent with observed changes in electron transport. No change in Chl *a*/*b* ratios was detected, indicating that a selective depletion of PS1 did not occur. Immunoblots of membrane samples were probed with antibodies (Fig. 1*A*) to OEE1 and OEE2. A control of a Coomassie-stained gel in which the same amount of Chl-containing membranes were loaded in each lane (Fig. 1*B*) offered visual evidence of a decrease in levels of OEE2 in membranes from water-stressed seedlings, particularly visible in *Triton*-treated membranes.

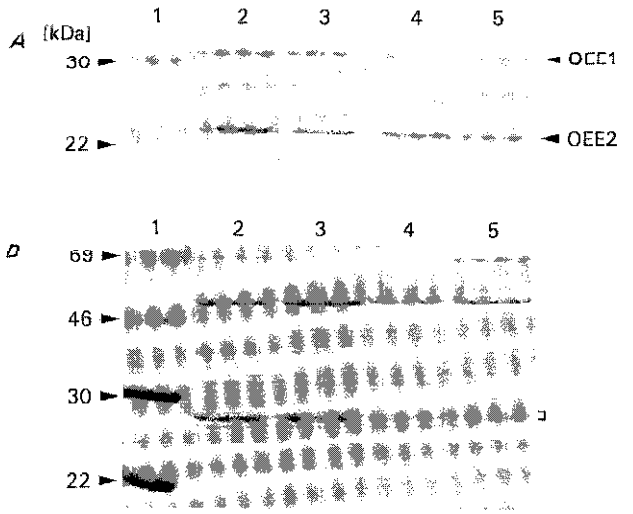

Fig. 1. *A*: Protein immunoblot of SDS-PAGE separation of spruce thylakoids (lanes *2* and *3*) and *Triton*-fractionated membranes (lanes *4* and *5*) from well-watered (lanes *2* and *4*) and water-stressed (lanes *3* and *5*) spruce trees, probed with a mixture of antibodies for OEE1 and OEE2. *B*: Coommassie brilliant blue stained SDS-PAGE separation. Lanes as for *A*. Each lane represents membranes containing 250 ng of Chl. Molecular masses [kDa] of standards (lane *1*) are indicated. The *cab* protein zone used for data correction is indicated by a square bracket.

To further investigate the effect of drought stress in seedlings on protein levels in thylakoids and *Triton*-treated membranes, we imposed drought stress on three additional sets of seedlings from the same original batch. Because there was variation from experiment to experiment, it was very important to ensure comparison of equivalent membrane samples. Quantification was made with correction factors derived from scanning of the major *cab* protein zone (23-25 kDa) in Coomassie-stained gels (Fig. 1*B*). Further, to verify this normalization, blots were also probed

with an antibody specific for CP2 (LHC2). There was little or no change in the relative amount of CP2 or ATP synthetase in thylakoids or *Triton*-treated membranes (Table 2). The absence of alterations in ATP synthetase content indicates that in water-stressed and, consequently, photoinhibited, spruce a generalized loss of thylakoid proteins was not evident.

Different water stress experiments produced somewhat variable results with respect to quantification of OEE1 in the thylakoid preparations. For example, the experiment illustrated in Fig. 1*A* showed little stress-induced change in OEE1 levels, contrasting with the preparations documented in Table 2 in which water stress caused a 40 % decline in OEE1. The variability may reflect changes in thylakoid integrity during needle aging.

Table 2. Relative amounts of protein detected by immunodecoration of protein blots in thylakoids and *Triton*-treated thylakoid membranes isolated from water-stressed spruce, *Picea glauca*, seedlings. Amounts are expressed as relative percentage of amounts detected in thylakoids and *Triton*-treated membranes, respectively, isolated from well-watered plants. Values are means ± standard error from at least three water-stress experiments.

Protein	Thylakoids	*Triton*-treated membranes
OEE1	59.1±3.9	58.6± 8.1
OEE2	59.8±4.1	37.3± 5.2
D1	69.4±1.8	73.6± 6.6
cytochrome *f*	71.0±9.8	40.0± 4.8
ATP synthatase	71.3±5.9	89.7±12.3
CP2	92.1±5.1	84.2± 5.6

In all experiments, the content of OEE2 in membranes from stressed plants was consistently lower than in control membranes. In addition, treatment with *Triton* resulted in greater loss of OEE2 from stressed membranes than control membranes (Fig. 1*A*, Table 2), suggesting that the association of OEE2 with the membrane was more labile after stress. These observations on OEE1 and OEE2 support the hypothesis that the diminished water oxidation capacity in these membranes may be effected in part by OEE2 dissociation from the reaction centre, causing a reduction in water oxidation. A more severe stress or diminished capacity for repair would lead to removal of the OEE1 from the membrane and cessation of water oxidation.

As postulated from the incomplete reactivation of DCIP photoreduction by DPC, there were significantly reduced levels of the D1 reaction centre protein in stressed membranes (Table 2). We note that immunoquantification of D1 does not distinguish between inactive PS2 centres containing photoinactivated D1 and remaining active centres (Smith *et al.* 1990). The low concentrations of cytochrome *f* (Table 2) in the *Triton*-treated membranes indicated that water stress increased the susceptibility of these intrinsic proteins to detergent extraction, possibly due to protein damage. In pollution damaged spruce needles, decreases in D1 concentrations, estimated by atrazine binding, and cytochrome *f* levels, determined by spectrophotometric

quantitation, were cited as evidence that these components were damaged at an early phase of stress (Wild *et al.* 1988). Another possible explanation for the increased detergent lability of these intrinsic proteins could be a result of alterations in the lipid environment. In *P. abies*, drought stress causes a significant increase in monoterpenes (Kainulainen *et al.* 1992), compounds which affect the distribution of fatty acids in polar and neutral lipids in spruce seedlings (Frosch *et al.* 1990). Free fatty acids such as linolenic acid reduce PS2 activity *in vitro* by causing release of Mn and loss of OEE polypeptides (Garstka and Kaniuga 1988). Additionally, oxygen radicals generated in thylakoid membranes during photoinhibition (Hideg *et al.* 1994) are implicated in a range of effects such as Chl oxidation and degradation of D1 (Rintamäki *et al.* 1994).

In conclusion, we report decreased PS2 activity in water-stressed plants concurrent with changes in specific intrinsic and extrinsic proteins including both chloroplast and nuclear-encoded thylakoid constituents involved in electron transport. Loss of electron transport activity is a consequence of reduced oxygen-evolving capacity and additional damage within the reaction centre. Changes in OEE1 appeared to be related to the severity of the imposed stress whereas amounts of OEE2 were consistently lower in all membrane preparations from stressed plants. In *Triton*-treated membranes, a further reduction in OEE2 levels coupled with reduced levels of cytochrome *f* and intact D1 suggest that these proteins become specifically destabilized during water stress.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-5, 1949.

Aro, E.-M., Virgin, I., Andersson, B.: Photoinhibition of photosystem II. Inactivation, protein damage and turnover. - Biochim. biophys. Acta **1143**: 113-134, 1993.

Binder, W.D., Spittlehouse, D.L., Draper, D.A.: Ecophysiology: post-planting physiological results and their implications for growing spruce. - In: Scrivener, B.A., MacKinnon, J.A. (ed.): FRDA Report 030. Pp. 33-35, Northern Silviculture Committee, Prince George 1989.

Bolhár-Nordenkampf, H.R., Lechner, E.G.: Temperature and light dependent modifications of chlorophyll fluorescence kinetics in spruce needles during winter. - Photosynth. Res. **18**: 287-298, 1988.

Brix, H.: Effects of plant water stress on photosynthesis and survival of four conifers. - Can. J. Forest Res. **9**: 160-165, 1979.

Dietz, B., Moors, I., Flammersfeld, U., Rühle, W., Wild, A.: Investigation on the photosynthetic membranes of spruce needles in relation to the occurrence of novel forest decline. I. The photosynthetic electron transport. - Z. Naturforsch. **43c**: 581-588, 1988.

Dunahay, T.G., Staehelin, L.A., Seibert, M., Ogilvie, P.D., Berg, S.P.: Structural, biochemical and biophysical characteristics of four oxygen-evolving Photosystem II preparations from spinach. - Biochim. biophys. Acta **764**: 179-193, 1984.

Eastman, P.A.K., Camm, E.L.: Regulation of photosynthesis in interior spruce during water stress: changes in gas exchange and chlorophyll fluorescence. - Tree Physiol. **15**: 229-235, 1995.

Enami, I., Tomo, T., Kitamura, M., Katoh, S.: Immobilization of the three extrinsic proteins in spinach oxygen-evolving Photosystem II membranes: roles of the proteins in stabilization of binding of Mn and Ca^{2+}. - Biochim. biophys. Acta **1185**: 75-80, 1994.

Ettinger, W.F., Theg, S.M.: Physiologically active chloroplasts contain pools of unassembled extrinsic proteins of the photosynthetic oxygen-evolving enzyme complex in the thylakoid lumen. - J. Cell Biol. **115**: 321-328, 1991.

Evans, E.H., Brown, R.G., Wellburn, A.R.: Chlorophyll fluorescence decay profiles of O_3-exposed spruce needles as measured by time-correlated single photon counting. - New Phytol. **122**: 501-506, 1992.

Flammersfeld, U., Wild, A.: Changes in the constitution of thylakoid membranes in spruce needles during an open-top chamber experiment. - Bot. Acta **105**: 348-354, 1992.

Frosch, S., Tremolieres, A., Wagner, E.: Rearrangement of fatty acids in lipid classes spruce seedlings during β-pinene fumigation. - Plant Physiol. Biochem. **28**: 567-576, 1990.

Garstka, M., Kaniuga, Z.: Linolenic acid-induced release of Mn, polypeptides and inactivation of oxygen evolution in photosystem II particles. - FEBS Lett. **232**: 372-376, 1988.

Godde, D.: Photosynthesis in relation to modern forest decline; content and turnover of the D-1 reaction centre polypeptide of photosystem 2 in spruce trees (*Picea abies*). - Photosynthetica **27**: 217-230, 1992.

Godde, D., Buchhold, J.: Effect of long term fumigation with ozone on the turnover of the D-1 reaction center polypeptide of photosystem II in spruce (*Picea abies*). Physiol. Plant. **86**: 568-574, 1992.

Grossnickle, S.C.: Shoot phenology and water relations of *Picea glauca*. - Can. J. Forest Res. **19**: 1287-1290, 1989.

Hideg, É., Spetea, C., Vass, I.: Singlet oxygen production in thylakoid membranes during photoinhibition as detected by EPR spectroscopy. - Photosynth. Res. **39**: 191-199, 1994.

Kainulainen, P., Oksanen, J., Palomaki, V., Holopainen, T.: Effect of drought and waterlogging on needle monoterpenes of *Picea abies*. - Can. J. Bot. **70**: 1613-1616, 1992.

Lichtenthaler, H.K., Wellburn, A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. - Biochem. Soc. Trans. **11**: 591-592, 1983.

Lütz, C., Steiger, A., Godde, D.: Influence of air pollutants and nutrient deficiency on D-1 protein content and photosynthesis in young spruce trees. - Physiol. Plant. **85**: 611-617, 1992.

Nixon, P.J., Dyer, T.A., Barber, J., Hunter, C.N.: The production of monospecific antibodies to D1 and D2 polypeptides of photosystem II. - In: Biggins, J. (ed.): Progress in Photosynthesis Research. Vol. 3. Pp. 779-782. Martinus Nijhoff Publ., Dordrecht - Boston - Lancaster 1987.

Öquist, G., Strand, M.: Effects of frost hardening on photosynthetic quantum yield, chlorophyll organization, and energy distribution between the two photosystems in Scots pine. - Can. J. Bot. **64**: 748-753, 1986.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. - Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Rashid, A., Camm, E.L.: Activities and stability of native and *Triton X-100*-fractionated thylakoid membranes from white spruce and spinach. - Photosynthetica **31**: 389-398, 1995.

Rashid, A., Camm, E.L., Ekramaddoullah, A.K.M.: Molecular mechanism of action of Pb^{2+} and Zn^{2+} on water oxidizing complex of photosystem II. - FEBS Lett. **350**: 296-298, 1994.

Rintamäki, E., Salo, R., Aro, E.-M.: Rapid turnover of the D1 reaction center protein of photosystem II as a protection against photoinhibition in a moss, *Ceratodon purpureus* (Hedw.) Brid. - Planta **193**: 520-529, 1994.

Ruth, B., Weisel, B.: Investigations on the photosynthetic system of spruce affected by forest decline and ozone fumigation in closed chambers. - Environ. Pollut. **79**: 31-35, 1993.

Schmitz, H., Loffel, U., Weidner, M.: The rate of protein synthesis in needles of Norway spruce (*Picea abies*): light stimulation, regulation through photophosphorylation, stress enhancement. - Physiol. Plant. **87**: 237-247, 1993.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. - Photosynth. Res. **10**: 51-62, 1986.

Shen, J.-R., Terashima, I., Katoh, S.: Cause for dark, chilling-induced inactivation of photosynthetic oxygen-evolving system in cucumber leaves. - Plant Physiol. **93**: 1354-1357, 1990.

Smith, B.M., Morrissey, P.J., Guenther, J.E., Nemson, J.A., Harrison, M.A., Allen, J.F., Melis, A.: Response of the photosynthetic apparatus in *Dunaliella salina* (green algae) to irradiance stress. - Plant Physiol. **93**: 1433-1440, 1990.

Strand, M., Öquist, G.: Effects of frost hardening, dehardening and freezing stress on *in vivo* chlorophyll fluorescence of seedlings of Scots pine (*Pinus sylvestris* L.) - Plant Cell Environ. **11**: 211-218, 1988.

Toivonen, P., Vidaver, W.: Variable chlorophyll *a* fluorescence and CO_2 uptake in water-stressed white spruce seedlings. - Plant Physiol. **86**: 744-748, 1988.

Wang, W.-Q., Chapman, D.J., Barber, J.: Effect of cold treatments on the binding stability of photosystem II extrinsic proteins and an associated increase in susceptibility to photoinhibition. - Plant Physiol. **99**: 21-25, 1992.

Wild, A., Flammersfeld, U., Moors, I., Dietz, B., Rühle, W.: Investigation on the photosynthetic membranes of spruce needles in relation to the occurrence of novel forest decline. II. The content of Q_B-protein, cytochrome *f*, and P-700. - Z. Naturforsch. **43c**: 589-595, 1988.

Yamamoto, Y., Kubota, F.: Specific release of the extrinsic 18-kDa protein from spinach Photosystem-II particles by the treatment with NaCl and methanol and its application for large-scale purification of the three extrinsic proteins of Photosystem II without chromatography. - Biochim. biophys. Acta **893**: 579-583, 1987.