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Anti-oxidative effect of elevated CO_2 concentration in the air on maize hybrids subjected to severe chill

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Abstract

Elevated CO_2 concentration (700 cm^3 m^{-3}, EC) inhibited chill-dependent (7 °C) depression of net photosynthetic rate of two maize hybrids with different sensitivity to low temperature. The rate of superoxide radical formation in leaves, leaf membrane injury, and the decrease in maximal quantum efficiency of photosystem 2 were successfully diminished by the treatment. The protective effect of EC toward stress conditions was prolonged at the recovery phase (20 °C). The genotypic impact on studied parameters was also notable.

Additional key words: ascorbate peroxidase; catalase; photoinhibition; photosynthetic induction; membrane injury; superoxide dismutase; *Zea mays*.

Introduction

In the present millennium, global atmospheric carbon dioxide concentration will continue to rise, probably up to *ca*. 700 cm^3 m^{-3} by the end of the recent century (Houghton *et al.* 1996). This might influence various physiological processes of plants. On the other hand, because maize is a C_4 plant of tropical origin, it is extremely sensitive to chill (temperatures 0–15 °C), although it has been grown even in cool temperate zones because of its high yield. Photosynthesis of C_4 species may, as C_3 species do, respond to elevated [CO_2] (EC) in the atmosphere (Ziska *et al.* 1999). CO_2 leak rate from the bundle sheath cell walls is relatively high (Brown and Byrd 1993). This diminishes the concentration of this gas within RuBPCO, and the reduced affinity of the enzyme to CO_2 in low temperature (Leegood and Edwards 1996, Pittermann and Sage 2000), predisposes C_4 plants to photoinhibition (Pittermann and Sage 2001). Chill also enhances the generation of reactive oxygen species, *e.g.* superoxide radical $O_2^{\bullet -}$, in relation to the cell antioxidative capacity, and this leads to oxidative stress (Saruyama and Tanida 1995, Wise 1995), especially in the bun-dle sheath (Kingston-Smith and Foyer 2000).

As a result of the increase of CO_2 assimilation and dark phase of photosynthesis, faster photosynthetic electron transfer may diminish the intensity of reactive oxygen species formation in green tissues (Schwanz *et al.* 1996). However, the influence of EC on anti-oxidative system is not clear (Reising and Schreiber 1992, Roden and Ball 1996, Azevedo *et al.* 1998).

Materials and methods

Caryopses (*Nasiona Kobierzyc*, Kobierzyce, Poland) of two maize (*Zea mays*) hybrid genotypes KOC 9431 (chill-resistant) and K103×K85 (chill-sensitive) were used in the experiment. The susceptibility of seedlings to low temperature was estimated in glasshouse experiments. Conditioned caryopses (50 % *Thiuram*) were sown into plastic pots (volume 2 000 cm^3) with a mixture of peat, organic soil, and sand (3 : 1 : 1, v/v).

Preliminary growing was performed in air-conditioned glasshouse with CO_2 concentration of *ca*. 350 cm^3 m^{-3}, thermoperiod 20/17 °C (day/night), photoperiod 16/9 °C (day/night), air humidity *ca*. 60 %, and additional lightening during cloudy days. Chilling was performed on plants that reached the phase of fully developed 3rd leaf. Pots containing the plants of both genotypes were divided into groups subjected to elevated (700 cm^3 m^{-3}, EC)

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Abbreviations: APX – ascorbate peroxidase; CAT – catalase; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase, SOD – superoxide dismutase.

and ambient (350 cm^3 m^{-3}, AC) [CO_2], put into containers, and exposed to 7 °C for 11 d, then recovered for 1 d (20 °C at AC). Infrared gas analysers (*LCA-2*; *Analytical Development Co.*, Hoddesdon, UK) monitored the CO_2 concentration. To prevent the rise in temperature, cooled air was pumped into each container.

All physiological and biochemical parameters were measured on the 3rd leaf of individual plant (biological replicate). *In situ* superoxide radical generation was estimated on leaf segments taken from the middle part of leaf using the nitroblue tetrazolium (NBT) method (Doke and Ohashi 1988). Electrolyte leakage was measured conductometrically (Markowski and Skrudlik 1995) on leaf discs. Gas exchange was assayed at 20 °C using the infrared gas analyser (*LCA-2*; *Analytical Development Co.*) operated within the open system with the fixation chamber *PLC(N)*. Chlorophyll *a* fluorescence was measured at 20 °C using the plant stress meter (*BioMonitor AB*, Umeå, Sweden). The parameter F_v/F_m was obtained after dark adaptation for 15 min. The saturating irradiance was 600 μmol(quantum) m^{-2} s^{-1}. Anti-oxidative enzyme activities were quantified on dialysed crude extract. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to McCord and Fridovich (1969). One unit was defined as the amount of enzyme required for 50 % inhibition of cytochrome *c* reduction. Catalase (CAT; EC 1.11.1.6) activity was determined by monitoring the disappearance of H_2O_2 at 240 nm (Aebi 1984). Ascorbate peroxidase activity (APX; EC 1.11.1.11) was assayed according to Nakano and Asada (1981). Soluble protein content was measured according to Bradford (1976) using bovine serum albumin for calibration.

The statistical significance of differences was evaluated by variance analysis using the Student *t*-test (two means) or Duncan multiple range test (3 means).

Results

EC inhibited chill-dependent depression of net photosynthetic rate (P_N) (Fig. 1*A*), especially in leaves of chill-resistant genotype KOC 9431. The effect of CO_2 treatment on this hybrid was distinct not only during chilling (11 Ch), but also during the recovery of plants at 20 °C (1 R). Seedlings subjected to EC showed 4-fold higher P_N when compared to AC plants.

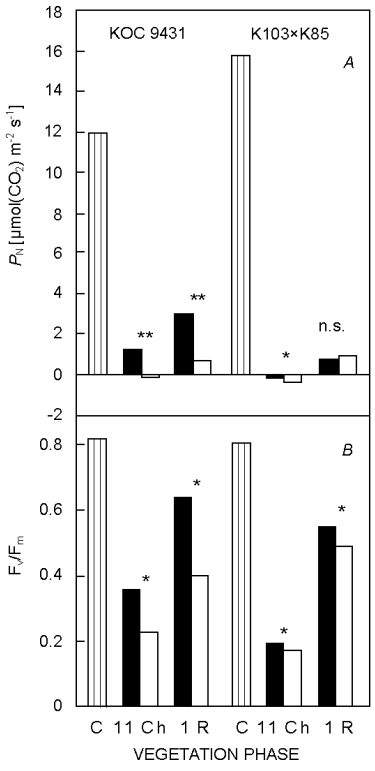

Fig. 1. Direct response (during chilling) and after-effect of elevated growth CO_2 concentration (*full columns*: 700 cm^3 m^{-3}, *empty columns*: 350 cm^3 m^{-3}) on (*A*) net photosynthetic rate (P_N) and (*B*) maximal quantum efficiency of PS2 (F_v/F_m) of maize leaves: C – before chilling (20 °C), 11 Ch – chilling (7 °C) at different CO_2 concentration, 1 R – recovery (20 °C). Measurements at 20 °C and 350 $cm^3(CO_2)$ m^{-3}. $^{*, **}$ – differences significant at $p = 0.05$ and $p = 0.01$, respectively; n.s. – differences not significant.

EC diminished the rate of superoxide radical formation in leaves in comparison to the AC control (Fig. 2). The presence of $O_2^{\bullet -}$ in all series of leaves was visible as purple and/or navy-blue dots. The dots were more abundant in leaves of plants kept at AC. Irrespective of the genotype and treatment, formation of superoxide occurred predominantly on the edges where the segment was cut off from the leaf, due to fast tissue infiltration by atmospheric oxygen.

Activities of SOD, CAT, and APX were not affected by the CO_2 concentration (Fig. 3*A,B,C*), although a decreasing tendency of CAT activity during chilling was

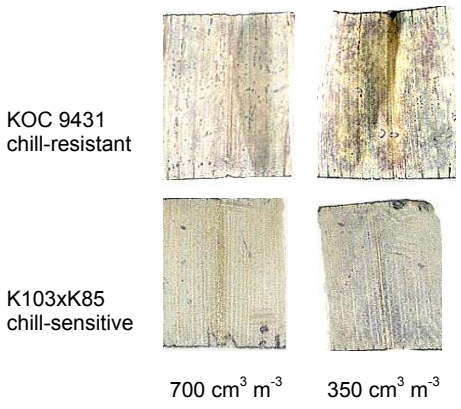

Fig. 2. The effect of elevated growth CO_2 concentration during 11-d chilling (7 °C) on superoxide radical formation measured *in situ* in leaves of maize hybrids. Means of 6 biological replicates.

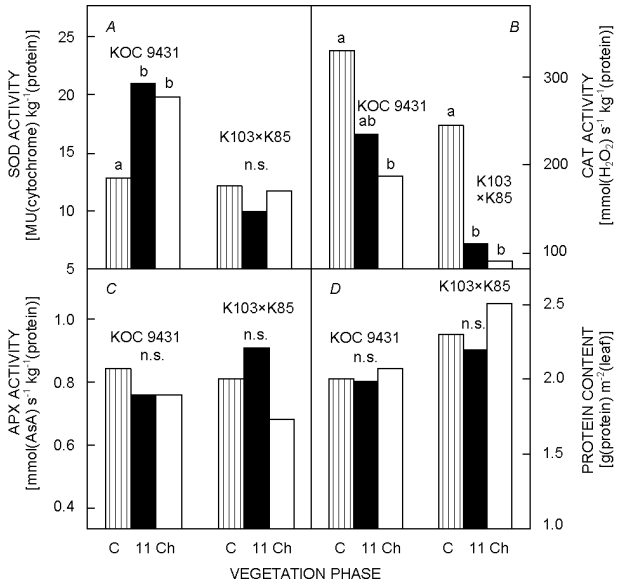

Fig. 3. Direct response (during chilling) and the after-effect of elevated growth CO_2 concentration on activities of superoxide dismutase (*A*), catalase (*B*), ascorbate peroxidase (*C*), and content of soluble protein (*D*) in maize leaves: C – before chilling (20 °C), 11 Ch – chilling (7 °C) with different CO_2 concentration (*full columns*: 700 cm^3 m^{-3}, *empty columns*: 350 cm^3 m^{-3}). n.s. or the same letters = no significant difference according to Duncan's test; $p = 0.05$. Means of 6-8 replicates.

slightly (not significantly) diminished with EC. However, the effect of the genotype on anti-oxidative activity was distinct. SOD and CAT activities were *ca*. 40 % higher in leaves of chill-resistant (KOC 9431) than of chill-sensitive (K103×K85) hybrid (Fig. 3*A,B*). Chilling durably stimulated SOD activity in KOC 9431 leaves. Typical for the low-temperature stress, the drop in CAT activity was smaller in leaves of this genotype (the range of activity 0.7 of the value obtained before chilling) than in K103×K85 leaves (0.4). The pattern of changes in enzymatic activity was not accompanied by the changes in protein pool (Fig. 3*D*). Interestingly, the chill-resistant hybrid showed lower protein content than the chill-sensitive one.

A positive effect on leaf membranes was noticed. The electrolyte leakage from the tissue, a parameter reflecting membrane injury, was significantly lower in samples of plants subjected to EC than AC. Chill resistance of KOC 9431 genotype was reflected by lower electrolyte leakage (9.02 and 11.90 %) when compared to chill-sensitive K103×K85 (12.8 and 15.6 %) for EC and AC, respectively.

The enrichment of air with CO_2 successfully inhibited also the decrease in F_v/F_m ratio (maximal quantum efficiency of PS2) (Fig. 1*B*), both after chilling and during 1-d recovery, especially in leaves of the chill-resistant genotype KOC 9431.

Discussion

Anti-oxidative effect of elevated atmospheric CO_2 on maize, observed as the diminished rate of superoxide radical formation in leaves of both tested hybrids, may result from increasing CO_2 assimilation by plants, overcoming the RuBPCO limitation in low temperature (Pittermann and Sage 2001). The activity of SOD, the enzyme scavenging the $O_2^{\bullet-}$, was not affected by the CO_2 treatment. The affinity of SOD to its substrate is very high, and activity is substrate-controlled (Scandalios 1993). The structure of the enzyme is very stable at unfavourable temperatures, maintaining high activity (Jahnke *et al.* 1991, Burke and Oliver 1992, Miszalski *et al.* 1998). In maize tissues, H_2O_2, a product of $O_2^{\bullet-}$ dismutation, is scavenged by CAT in mitochondria, peroxisomes, glyoxysomes, and cytosol (Prasad *et al.* 1994a,b), and by APX, present mainly in chloroplasts and cytosol (Chen and Asada 1989, Miyake and Asada 1992, Doulis *et al.* 1997). CAT is extremely prone to the interaction of chill and irradiance (Feierabend *et al.* 1992), and inhibition of its activity in photoinhibitory conditions was observed in numerous ecophysiological studies (Prasad *et al.* 1994b, Streb and Feierabend 1995, Skrudlik *et al.* 2000). Chill-involved overproduction of H_2O_2, dependently on its concentration, may act as an intracellular signal or intoxicate the cell (Prasad *et al.* 1994a,b, Foyer *et al.* 1997). Due to the higher superoxide formation by leaves of plants grown at AC, and to dramatic drop in CAT activity caused by long and severe chilling, those plants may overproduce H_2O_2, and this process may be suppressed by EC. This might result indirectly in the lower extents of leaf membrane injury and photoinhibition of photosystem 2, PS2 (F_v/F_m parameter). Both lipid and protein constituents of membranes (Fridovich 1978, Halliwell and Gutteridge 1986, Scandalios 1993), as proteins of PS2, especially D1 protein (Hideg *et al.* 1994, Huner *et al.* 1998), are prone to peroxidation. The diminished photoinhibition may also result from the adjustment of photosynthetic electron transfer to increasing CO_2 assimilation (Hurry *et al.* 1992, Huner *et al.* 1993). Interestingly, the protective effect of EC on maximal quantum efficiency of PS2 toward stress was prolonged in the recovery phase, and the pattern of F_v/F_m was similar to the time-course of CAT activity.

Irrespective of the CO_2 influence on chilled plants, the genotypic impact on studied parameters, especially on SOD and CAT activity, was notable. Our results confirm those of Jahnke *et al.* (1991), Saruyama and Tanida (1995), or Hodges *et al.* (1997), who postulated the tolerance of chill-sensitive cultivars with more cold stable anti-oxidative enzymes, especially scavengers of H_2O_2. Due to the polymorphism of SOD, CAT, and APX, one can suggest various mechanisms of activation and transcription processes of individual isoforms (Miyake and Asada 1992, Scandalios 1993, Prasad *et al*. 1994a,b). But

at long-term and severe chilling stress of the present experiment this might not play the crucial role.

In face of continuous global climate changes we conclude that the increase in atmospheric [CO_2] seems to be one of the protective factors for maize grown in cold temperate regimes.

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Baker, A., Graham, I.A. (ed.): **Plant Peroxisomes.** (**Biochemistry, Cell Biology and Biotechnical Applications.)** – Kluwer Academic Publishers, Dordrecht – Boston – London 2002. ISBN 1-4020-0587-3. 505 pp., € 180.00, USD 155.00, GBP 110.00.

In the two decades since the last comprehensive work on plant peroxisomes appeared in 1983 the scientific approaches have changed beyond all recognition. The best proof of this is the fact that the majority of literature cited in this book was published during the last ten years. The accelerating pace of plant research in the post-genomic era has led us to appreciate the fact that peroxisomes have many important roles in plant cells including reserve mo-bilisation, nitrogen assimilation, the metabolism of plant hormones, defence against stress, *etc.*, all of which are vital for normal plant development.

The fifteen chapters in three sections of this book, written by 26 leading experts in this field from 9 countries, survey peroxisomal metabolic pathways, protein targeting and biogenesis of the organelle, and prospects for the manipulation of peroxisomal function for biotechnological purposes.

The first section surveys the functions of plant peroxisomes. Chapter 1 provides a personal historical perspective of the pioneering work in this field and provides a link between the past and the present. Chapters 2, 3, and 5 are comprehensive reviews of fatty acid oxidation, the glyoxylate cycle, and the photorespiratory pathway, integrating older observations with new data arising from mutants, genome, and EST sequences about gene families, expression, and function. Chapter 5 also deals with the compartmentalisation of pathways in the context of glycolate and glycerate pathways of photorespiration. Chapter 4 deals with the complexities of catalase function and biogenesis with multiple genes regulated at different levels, giving rise to a wide range of isoforms to defend plants against oxidative stress. Chapter 6 addresses the role of root nodule peroxisomes in nitrogen fixation in tropical legumes. Chapter 7 addresses the role of peroxisomes as a source of reactive oxygen species (ROS) and ROS related signal molecules and as a first line of defence against these potentially harmful species. Chapter 8 deals with the composition and function of the peroxisome membrane. The last chapter of this section describes several peroxisome-deficient mutants including those that are disrupted in specific aspects of peroxisome metabo-lism and peroxisome biogenesis.

The second section deals with peroxisome biogenesis and protein import in detail. Chapter 10 discusses the origin and differentiation of peroxisomes and reviews the current knowledge of the targeting and insertion of peroxisome membrane proteins. Chapter 11 deals with the targeting and import of peroxisome matrix proteins. Chapter 12 deals with genes required for peroxisome biogenesis in other organisms and their counterparts in plants. And with the question whether these components for peroxisome biogenesis are universal.

The third section considers our potential to exploit the growing knowledge of peroxisome biogenesis and function for biotechnical ends. Chapter 13 deals with the current possibilities for engineering plant peroxisomes to increase plant resistance to stress. Chapter 14 addresses the problem of futile cycling through β-oxidation as a potential barrier to increased yields of novel oils in transgenic plants. Chapter 15 reviews the use of peroxisomes as a compartment for the synthesis of biodegradable plastics and other novel biopolymers.

The aim of this book is to draw together the current state of the art as a convenient starting point for anyone who wishes to know about plant peroxisomes. There is another aspect to this book. Its English could serve as a textbook of wonderfully clear, concise English in this field.

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Modifications in photosynthetic pigments and chlorophyll fluorescence in 20-year-old pine trees after a four-year exposure to carbon dioxide and temperature elevation

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Abstract

Changes in pigment composition and chlorophyll (Chl) fluorescence parameters were studied in 20 year-old Scots pine (*Pinus sylvestris* L.) trees grown in environment-controlled chambers and subjected to ambient conditions (CON), doubled ambient CO_2 concentration (EC), elevated temperature (ambient +2–6 °C, ET), or a combination of EC and ET (ECT) for four years. EC did not significantly alter the optimal photochemical efficiency of photosystem 2 (PS2; F_v/F_m), or Chl *a*+*b* content during the main growth season (days 150–240) but it reduced F_v/F_m and the Chl *a*+*b* content and increased the ratio of total carotenoids to Chl *a*+*b* during the 'off season'. By contrast, ET significantly enhanced the efficiency of PS2 in terms of increases in F_v/F_m and Chl *a*+*b* content throughout the year, but with more pronounced enhancement in the 'off season'. The reduction in F_v/F_m during autumn could be associated with the CO_2-induced earlier yellowing of the leaves, whereas the temperature-stimulated increase in the photochemical efficiency of PS2 during the 'off season' could be attributed to the maintenance of a high sink capacity. The pigment and fluorescence responses in the case of ECT showed a similar pattern to that for ET, implying the importance of the temperature factor in future climate changes in the boreal zone.

Additional key words: carotenoids; diurnal course; environment chambers; irradiance; leaf temperature; photosynthetic photon flux density; photosystem 2; *Pinus sylvestris*; seasonal course; specific leaf area.

Introduction

Rising atmospheric CO_2 concentration [CO_2], elevated air temperature, and their interaction on trees have been the most intensively studied environmental problems in recent times, while changes in CO_2 exchange and its underlying biochemical regulation are at the core of the effects of CO_2 on plants. Research in past decades has been focused on tree growth, photosynthesis, biomass production, and allocation under these conditions, the main emphasis with regard to photosynthesis being placed on net carbon uptake, altered ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity, and the production of primary assimilates during the growth season. Little attention in global climate studies has been paid to the functioning of light-harvesting complexes, either photosystems or electron carriers in the thylakoid membranes of trees growing under long-term conditions that simulate global change.

Under the boreal climatic conditions, most of the

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Abbreviations: Car – carotenoid; Chl – chlorophyll; $\Delta F/F'_m$ – effective photochemical efficiency; F_0 – initial fluorescence; F_m – maximal fluorescence; F_s – steady state fluorescence; F_v/F_m – optimal photochemical efficiency of PS2; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
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needles on confers must literally survive years of fluctuat-ing temperatures (varying from +35 to –40 °C in Finland, Wang 1996) and temperature (Hansen *et al*. 2002), implying two important things that we must face when studying the responses of trees to CO_2: acclimation of the photosynthetic apparatus to the prevailing temperature, and the response of trees to CO_2 at different base-line temperatures. A low temperature, often accompanied by high irradiance in spring and autumn, can lead to cold acclimation in trees, which could involve changes in membrane composition and RuBPCO content, so that photosynthesis is depressed (Strand and Öquist 1985, 1988, Leverenz and Öquist 1987). Elevated temperatures may therefore greatly reduce the period during which natural acclimation results in depression of photosynthetic capacity and allow trees to utilise photons intercepted during the spring and autumn with greater efficiency (Long and Hutchin 1991). In contrast, too little is known about interactions between elevated [CO_2] and low tem-perature. In view of the dependence of elevated [CO_2] on the base-line temperature, models for the biochemistry of C_3 photosynthesis predict much larger CO_2 stimulation of photosynthesis at higher temperatures and little benefit from CO_2 enrichment at low temperatures (<15 °C) (Long 1991). This implies that increased [CO_2] will have diffe-rent impacts on photosynthesis during the growing season and during the 'off season'. Some long-term field experi-ments have indeed supported these predictions, but there are exceptions and considerable variations in the respon-ses. In *Pinus taeda*, for example, the relative stimulation of photosynthetic rate over several seasons was correlated with temperature in one study (Tissue *et al.* 1997) but not in another (Teskey 1997). The information available concerning the interaction of elevated [CO_2] with the base-line temperature is still rather limited. Nevertheless, the influence of the CO_2 and temperature factors individually on trees is superimposed on the seasonal variations, so that it is difficult to predict what the resultant changes in annual photosynthesis will be under future conditions of climate change.

The *in vivo* chlorophyll (Chl) fluorescence signatures provide basic information on the functioning of the photo-synthetic apparatus and on the capacity and performance of photosynthesis, whereas leaf pigments, including Chl and Cars, are directly related to light harvesting in photo-synthesis, excess energy dissipation, and the inactivation of stress-related toxic products (Havaux 1998, Taiz and Zeiger 1998). Consequently, pigment and fluorescence analyses have been used to evaluate plant responses to environmental stresses (Mohammed *et al.* 1995). Epron *et al.* (1996) found that the Chl content per unit N decreased with time in beech saplings grown under [CO_2] enrich-ment, suggesting that less N may be invested in the light harvesting complex. In *Eucalyptus tetrodonta*, however, the Chl content was reduced by elevated [CO_2], although the rates of light-saturated photosynthesis remained higher (Eamus *et al*. 1995). In most ECOCRAFT (likely impact of elevated [CO_2] and temperature on European forests) studies, elevated [CO_2] failed to affect the optimal photochemical efficiency of photosystem 2 (PS2; F_v/F_m) (Besford *et al.* 1996), but either a significant in-crease (Ceulemans *et al.* 1995) or a decrease (Roden and Ball 1996, Scarascia-Mugnozza *et al*. 1996) have been reported. By comparison with elevated [CO_2], the effects of long-term temperature elevation on the pigment con-tent and functioning of PS2 with rising growth tempera-tures have less often been reported in tree species. Studies of Douglas fir seedlings pointed to a positive relationship of enhanced contents of needle pigments and increased [CO_2] uptake to elevated temperature (Lewis *et al.* 1999, Ormrod *et al.* 1999). This was consistent with findings in an evergreen arctic dwarf shrub (*Cassiope tetragona*) (Michelsen *et al.* 1996), but in contrast to the situation in winter wheat (*Triticum aestivum*) due to the temperature-induced enhancement of leaf senescence (Delgado *et al.* 1994). Aiken and Smucker (1996) suggest in their review that under more favourable temperature conditions the roots would synthesise and export more cytokinin to the foliage to promote pigment synthesis. Thus the responses of photochemical processes to elevated [CO_2] or tempera-ture probably reflect other feedback regulation mecha-nisms in whole-tree growth and sink/source capacity as well as differences in species, genotype, and experimental conditions.

Individual Scots pine, *Pinus sylvestris* L., trees grown in environment-controlled chambers were subjected here to two [CO_2] and two temperature levels. The Chl *a* fluo-rescence and pigment compositions of 1-year-old needles were measured in the fifth year of exposure (2001). Specific objectives were to determine whether naturally growing trees of Scots pine adjust, over time, their photosynthetic activity to elevated [CO_2] and temperature, and to analyse the effect of possible interactions between treatments and naturally occurring weather stresses on these trees.

Materials and methods

Tree growth conditions: The experiments were done in a naturally-seeded stand of Scots pine located near the Mekrijärvi Research Station (62º47′N, 30º58′E, 145 m a.s.l.), University of Joensuu, Finland. The trees were approximately 20 years old, with a mean height of 3.5 m. Soil is a sandy loam with a water-retention value of 40 mm at field capacity and 20 mm at the wilting point for the top of 30 cm.

Sixteen trees of approximately the same crown size and height were chosen and enclosed individually in

closed-top chambers in the field in 1996. Four treatments of combinations of [CO_2] and temperature were conducted: (*1*) ambient temperature and [CO_2] (CON); (*2*) elevated [CO_2] (EC); (*3*) elevated temperature (ET); (*4*) elevated [CO_2] and temperature (ECT). Each treatment had four replicates.

The chambers are approximately cylindrical, with eight walls, an internal volume of approximately 26.5 m^3 and a ground area of 5.9 m^2. The four walls facing south and west are constructed of special heating glass with a thin resistance element converting electricity into heat (K-glass + *AS Green*, *Eglas Oy*, Imatra, Finland) and the four north and east-facing walls of dual-layer acrylic sheets. A fan blower through a duct fed unfiltered air into the chamber approximately 3.5 m above the ground, and the airflow was determined periodically with a hot wire anemometer and adjusted with a butterfly valve. A computer-controlled heat exchanger linked to a refrigeration unit (*CAJ-4511YHR*, *L'Unite-Hermetique*, Barentin, France) was installed in the top of each chamber. The computer-controlled heating and cooling system, together with a set of magnetoelectric valves (controlling the pure CO_2 supply), enabled temperature and [CO_2] inside the chambers to be adjusted automatically to follow ambient conditions, or to achieve a specified enrichment in CO_2 (+350 μmol mol^{-1}) and/or rise in temperature (+2 ºC during the 'main growth season', *i.e.* days 150–240 and +6 ºC during the 'off season'). The [CO_2] was enriched all day throughout the year. Performance of the chamber system has been detailed in Kellomäki *et al*. (2000).

Measurements of fluorescence and pigment contents: Chl *a* fluorescence was measured in attached one-year-old needles on the shoot in a secondary whorl at the top of the crown using a portable Chl fluorometer (*MINI-PARM*, *H. Walz*, Effeltrich, Germany). Two groups of measurements were made in the year 2001, measurements on dark-adapted needles monthly throughout the year to estimate the optimal photochemical efficiency of PS2 (F_v/F_m) and PPFD-response curves, and a total of five natural daily courses under contrasting weather conditions monitored in the summer of 2001 for each treatment. For all the measure-ments, the three needles in the middle of the shoot were fastened side by side on a strip of transparent tape, attached to a "Distance Clip", and oriented so that their curved surfaces were fully exposed to irradiation during the day. Care was also taken to avoid shading of the leaf surface, and the PPFD close to the leaf surface was measured with a micro-quantum sensor calibrated against a quantum sensor (*LI-190SB*, *LI-COR*, Lincoln, NE, USA) during the daily course of measurements. The fibre-optic tip, with a 2 mm active diameter (*MINI-PAM/F1*) was maintained at a distance of 6 mm from the needle surface at an angle of 60º by means of the "Distance Clip", and fluorescence was excited with a modulated red radiation of *ca.* 2 μmol m^{-2} s^{-1} by setting a pulse-width of 3 μs and a frequency of 20 kHz. A saturating radiation pulse (0.8 s) of *ca.* 8 000 μmol m^{-2} s^{-1} was provided by an 8 V/20 W halogen lamp (*Bellaphot*, *Osram*). The initial (F_0) and maximal (F_m) fluorescence of the needles after 20 min of dark acclimation were recorded. The fluorescence at the steady state (F_s) and at the delivery of the saturation pulse (F'_m) were determined after pro-longed irradiation. The measurements were then used to calculate the optimal photochemical efficiency of PS2 (Butler 1978), $F_v/F_m = (F_m - F_0)/F_m$ and its effective photochemical efficiency (Genty *et al.* 1989), $\Delta F/F'_m = (F'_m - F_s)/F'_m$.

After the fluorescence measurement, a total of twelve needles were collected from the shoot adjacent to the one used for the fluorescence measurements, enclosed in plastic bags, and immediately stored on ice. Six of these needles were used to determine the relationships between fresh mass, dry mass (80 ºC for 48 h), and projected area (*WinRHIZO*™, *Regent Instruments*, Quebec, Canada), and the remaining ones for pigment analysis. The fresh needles were extracted with a constant volume of 10 cm^3 of 100 % methanol in a mortar with pestle, some of the crude extract being transferred to 2 cm^3 Eppendorf tubes for centrifugation at 600 rps for 10 min to clarify the extract. The absorbance was determined with a recording spectro-photometer (*U3200*, *Hitachi*, Ibaraki, Japan). The concentrations of Chl *a* and *b* and total Cars were calculated per fresh mass using the equations and absorption coefficients of Lichtenthaler (1987).

Statistical analyses: It was assumed that any significant responses to the treatments were the result of the growth temperatures and [CO_2] and not to unknown chamber effects. Repeated-measures analysis of variance (Moser *et al.* 1990) was used to test the effects of the growth conditions (CON, EC, ET, and ECT) and date of measurement on pigments and fluorescence parameters during the season. One-way ANOVA was used to test differences in the parameters between the control and enriched treatments on any specific date using the means of the four replicate chambers.

Results

Contents of Chl and Cars: The Chl *a*+*b* content in the one-year-old needles showed a clear dependence on season, with its lowest value in early April, and remained almost constant during the main growth season (days 150-240) independent of the treatments (*right panels* in Fig. 1). By comparison, Cars showed slight variations over the year. The treatments significantly affected both the Chl *a*+*b* and carotenoid (Car) content, but did not alter the ratio of

Chl *a/b* (Table 1). In view of the seasonal differences in photosynthetic pigments, the treatments might have a more pronounced impact on pigment content in the 'off season' than in the main growth season. In terms of the seasonal average, ET increased Chl *a+b* by 8.8 % (p = 0.044) and 21.6 % (p = 0.001) in the growth season and the off season, respectively, but reduced Cars by 4.2 % (p = 0.058) and 11.3 % (p = 0.034), respectively. By contrast, EC significantly reduced Chl *a+b* by 14.7 % (p = 0.022) but increased Cars by 10.1 % (p = 0.036) during the off season, whereas it produced only a marginal reduction in Chl *a+b* (–1.4 %, p = 0.070) and Cars (2.6 %, p = 0.075) during the growing season. In addition, specific leaf area values (SLA in Table 1) were lower in EC during the off season, whereas ET led to no significant change in SLA at any time in the year. Even so, statistics indicated that the treatment-induced changes in pigment content relative to area were not significantly different from those calculated relative to dry mass.

Table 1. Seasonal means (MGS = main growing season, days 150-240) of photosynthetic pigment ratios and specific leaf area (SLA) [cm^2 kg^{-1}(dry mass)] in one-year-old needles of Scots pine trees grown in different environments (CON, EC, ET, and ECT), and their statistical significance. The effects of the treatments on the variables were analysed by multi-factor *ANOVA*. Different letters within a column indicate statistically different from each other value at p = 0.05. Means of measurements from four sample trees represent the same treatment, and the standard error is given in parentheses.

Treatment	Chl *a/b*		Cars/Chl *a+b*		SLA	
	MGS	Off season	MGS	Off season	MGS	Off season
CON	4.25 (0.18)*a*	5.34 (0.26)*a*	0.284 (0.005)*a*	0.312(0.009)*a*	41.2 (1.01)*a*	40.7(0.82)*a*
EC	4.39 (0.21)*a*	5.52 (0.31)*a*	0.307 (0.010)*a*	0.359 (0.011)*b*	40.3 (1.03)*a*	38.1(1.01)*b*
ET	4.18 (0.16)*a*	5.11 (0.17)*a*	0.266 (0.006)*a*	0.276 (0.008)*c*	42.4 (0.96)*a*	39.9(0.98)*ab*
ECT	4.21 (0.22)*a*	5.19 (0.27)*a*	0.257 (0.008)*a*	0.280 (0.013)*c*	42.1 (1.04)*a*	40.1(0.75)*ab*

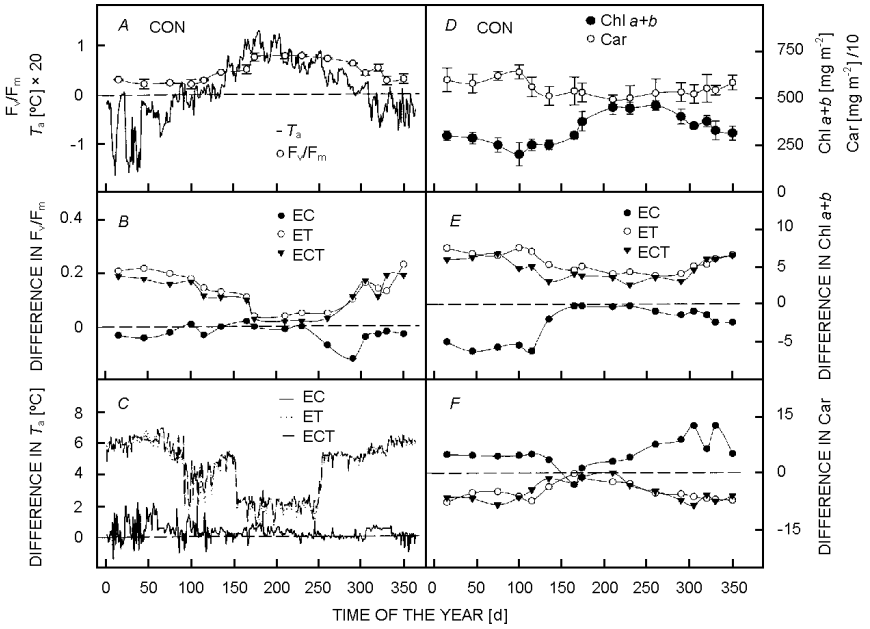

Fig. 1. Annual course of (*A*) mean daily air temperature (T_a) and the optimal photochemical efficiency of PS2 for dark-adapted needles (F_v/F_m), (*B*) differences (treatment – CON) in F_v/F_m relative to CON, (*C*) differences in T_a relative to those in the control chambers, (*D*) mean contents of chlorophyll (Chl) *a+b* and carotenoids (Car), (*E*) differences in Chl *a+b* relative to CON, and (*F*) differences in content of Cars relative to CON. The plots are based on measurements made on four trees (chambers) for each treatment.

Optimal photochemical efficiency of PS2*:* The annual course of optimal photochemical efficiency of PS2 (F_v/F_m) was related to some extent to that of the daily means of air temperature (Fig. 1*A*). In the case of EC, F_v/F_m averaged 0.78 during the growing season and 0.36 during the off season, gained its lowest value, 0.21, at the end of March, began to recover in early May, and reached full recovery in late June. The factor-enhanced treatments led to following changes in F_v/F_m relative to CON: (*1*) the recovery of F_v/F_m began earlier (around the end of April) in the temperature-elevated chambers, but showed no change in the EC chambers; (*2*) the treatments did not significantly alter the

values of F_v/F_m during the growing season, although EC slightly reduced F_v/F_m (–1.4 %; p = 0.073) and ET increased it (4.8 %; p = 0.061) (Fig. 1*B*); (*3*) ET-induced increases in F_v/F_m during the off season (41 %, p = 0.023) coincided very well with the increases in growth temperature in the chambers (Fig. 1*C*); (*4*) EC depressed F_v/F_m in general (–8.7 %, p = 0.042), but this was especially pronounced in September and October (–31 %, p = 0.001); (*5*) regardless of the treatment, significant changes in F_v/F_m were due more to modification in the maximum fluorescence (F_m) than of the initial fluorescence (F_0); (*6*) ECT-induced modification of F_v/F_m was similar to that observed with ET.

Diurnal course of fluorescence: Four examples of the diurnal course of fluorescence parameters represent similar weather conditions but different treatments (Fig. 2). In general, they referred to typical sunny days in summer with temperature and irradiation stress. The morning records of F_s and F'_m fluorescence were very similar for trees growing in CON and EC (Fig. 2*C,D*). Regardless of the treatment, a sharp diurnal decline in F'_m coinciding with increase in PPFD was always observed on sunny days. Recovery began at the end of the afternoon, levels close to the morning values being reached after sunset, and transient decreases in PPFD caused by clouds being accompanied by rapid increases in F'_m. F_s displayed less marked trends than F'_m, although there was a slight diurnal decrease. Since the depressions in F'_m were more pronounced than those in F_s, the effective photochemical efficiencies of PS2 ($\Delta F/F'_m$) showed parallel patterns with F'_m (Fig. 2*D,E,I,J*). The temperature-elevated treatments significantly increased $\Delta F/F'_m$ throughout the day relative to CON (p = 0.042),
whereas EC significantly enhanced the decreases in $\Delta F/F'_m$ in the afternoon (p = 0.039).

To quantify the responses of fluorescence parameters to irradiation and water stresses, we selected data with VPD>1.5 kPa from 5-d-series of measurements made in summer 2001 and plotted the relationship between PPFD and F_s, F'_m, and $\Delta F/F'_m$ for each treatment (Fig. 3). There were a few points in the figures where PPFD was lower than 400 μmol m^{-2} s^{-1} (for CON and EC) or 600 μmol m^{-2} s^{-1} (for ET and ECT), because of the demand for the critical VPD. The regressions indicated that a linear fitting was significant for F_s, with r^2 varying from 0.74 to 0.86 and p<0.05 (Fig. 3*A,D*), and exponential relations were significant for F'_m and $\Delta F/F'_m$, with r^2 varying from 0.69 to 0.77 and p<0.05 (Fig. 3*B,C,E,F*). An interesting finding was that the treatment-induced differences in fluorescence parameters appeared to be almost constant when PPFD was over 600 μmol m^{-2} s^{-1}. Based on the average of data with PPFD from 600 to 1 400 μmol m^{-2} s^{-1}, EC reduced F_s, F'_m, and $\Delta F/F'_m$ by 2.6, 24.8, and 17.6 %, respectively, while ET increased F_s, F'_m, and $\Delta F/F'_m$ by 6.1, 32.2, and 28.3 %, respectively.

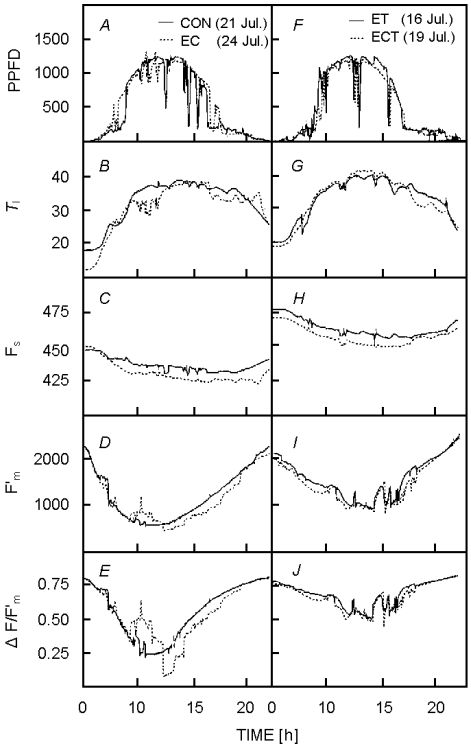

Fig. 2. Four examples of the diurnal time course of (*A, F*) PPFD [μmol m^{-2} s^{-1}], (*B, G*) leaf temperature, T_l [°C], (*C, H*) relative steady-state fluorescence (F_s), (*D, I*) maximum fluorescence (F'_m), and (*E, J*) effective photochemical efficiency of PS2 (F/F'_m) as measured *in situ* on sunlit needles of Scots pine on 21st (CON), 24th (EC), 16th (ET), and 19th (ECT) July 2001.

Discussion

The seasonal variations of the Chl *a*+*b* and fluorescence parameters correlated well with earlier reports in the photosynthetic performance for Scots pine or other pine trees (Linder 1972, Martin *et al.* 1978, Leverenz and Öquist 1987, Wang 1996, Lundmark *et al.* 1998). They exhibited a gradual decline during late summer and autumn, marked inhibition during the winter, and a rapid recovery in early spring. A new finding was that elevated [CO_2] or elevated temperature resulted in marked differences in PS2 photochemical efficiency between treatments and that these were superimposed on the seasonal variations (Fig. 1).

Elevated [CO_2] did not significantly reduce the maximum photochemical efficiency of PS2 (F_v/F_m) during the growing season (Fig. 1). This gives some support to earlier finding that, even in a resource-limited environment, trees are capable of fixing more carbon in a doubled ambient [CO_2] environment, at least during a few

years following the initiation of [CO_2] treatment (Idso and Kimball 1991, Scarascia-Mugnozza *et al*. 1996, Marek *et al.* 1997, Wang and Kellomäki 1997). The maintenance of high photosynthetic capacity in the Scots pine after four years of exposure may be related to the CO_2-induced enhancement in the active sink such as leaf area and root absorption (Wang and Kellomäki 1997, Kellomäki and Wang 2001). Nevertheless, the above explanation does not fully preclude a response of Scots pine to [CO_2]. For example, lower minimum daily values of the photochemical efficiency of PS2 ($\Delta F/F'_m$ in Fig. 2) were observed on most days in summer in the case of EC, while a reduction in F_v/F_m relative to CON was also evident in the 'off season', particularly in September and October (Fig. 1). This implies that the effect of elevated [CO_2] on photosynthetic performance of Scots pines depends greatly on season.

EC reduced the mean of F_v/F_m by 31 % for the dark-adapted needles in August and September and by 12 % in other months of the year (Fig. 1). As reported in the literature, a reduction in F_v/F_m can be related to two different processes: enhanced non-radiative energy loss and/or the occurrence of damage to PS2 reaction centres. The first process reduces both F_0 and F_m (Butler 1978), while the second one would cause a marked decrease only in F_m (Powles and Björkman 1978). The needles used for our measurements of F_v/F_m were dark-adapted for 20 min, and the measuring radiation was applied in the "burst mode". Thus all the reaction centres in PS2 should be open. A very brief but strong saturation-irradiance pulse was used to induce F_m. It would thus seem to be impossible for a 38 % depression in F_m to be fully attributed to the enhanced non-radiative energy loss, or to inactivation in the PS2 reaction centre complex, as found in the enhanced O_3 experiment (Chang and Heggested 1974). An alternative interpretation for the simultaneous decrease in F_0 and F_m is that it is due to the CO_2-induced changes in photosynthetic pigment content (Fig. 1), *i.e.* Chl *a*+*b* decreased by 24 % in August and September whereas the ratio of Cars/Chl *a*+*b* increased significantly. Although decreases in photosynthetic pigments in response to elevated [CO_2] have been observed in many tree species (Mousseau and Enoch 1989, Wullschleger *et al*. 1992, Marek *et al.* 1997, Ormrod *et al*. 1999), the mechanisms are unclear. Surano *et al*. (1986) reported that ponderosa pines growing in an elevated [CO_2] had accelerated needle abscission and chlorosis, while Pritchard *et al.* (1997) found that *Pinus palustris* chloroplasts exhibited under elevated [CO_2] stress symptoms, increased amount of plastoglobuli, and shorter grana. In potato and tobacco plants the whole-plant development was

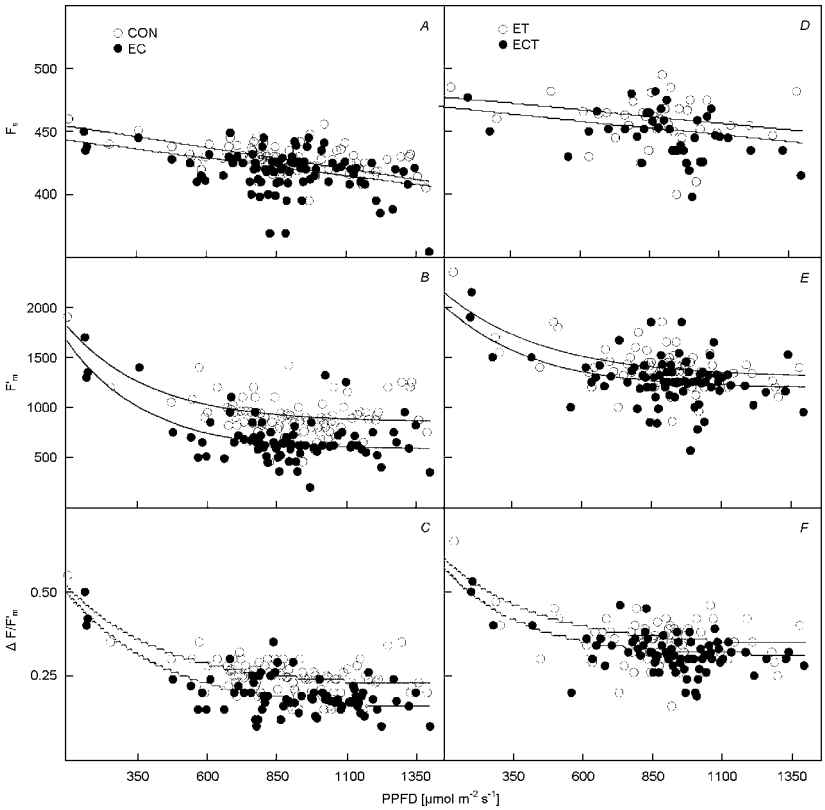

Fig. 3. Irradiance response of relative steady-state (F_s), maximum fluorescence (F'_m), and effective photochemical efficiency of PS2 ($\Delta F/F'_m$) as a function of treatment (CON, EC, ET, and ECT). Each symbol represents values with VPD>1.5 kPa for 5-d measurements in summer 2001 for each treatment (see Fig. 2). Lines are fitted by a linear function for F_s (*A, D*) and an exponential function for F'_m (*B, E*) and $\Delta F/F'_m$ (*C, F*).

faster in elevated [CO_2] than in normal [CO_2] and there was an earlier onset of the natural decline in photosynthetic rates associated with plant senescence (Miller *et al.* 1997). Our earlier gas exchange measurements on a shoot of Scots pine showed that elevated [CO_2] enhanced the photosynthetic capacity in the main growth season but slightly decreased it in late autumn (Kellomäki and Wang 1988). Such changes in both structural and functional components of chloroplasts are similar to those observed during senescence of leaves (Woolhouse 1984). Consequently, there are reasons to speculate that the acclimation of F_v/F_m to elevated [CO_2] during late autumn may be the result of accelerated leaf development.

The fluorescence parameter $\Delta F/F'_m$ depends on the relative change in steady-state fluorescence (F_s) and the maximum fluorescence at the steady state (F'_m). As the values of F_s were not affected significantly by the treatments at all PPFD levels, the CO_2-induced decreases in $\Delta F/F'_m$ must have resulted from the reduction in F'_m (Fig. 3). This suggests that F_s may be relatively independent of environmental variations, whereas F'_m is more sensitive to growing conditions (Havaux *et al.* 1991). Transient diurnal decreases in $\Delta F/F'_m$ in response to increasing irradiance are a general feature of photosynthesis in natural environments (see review by Demmig-Adams and Adams 1992). The depression is much greater on days with marked water stress, as observed in several tree species (Epron and Dreyer 1993, Epron *et al*. 1994, Valentini *et al*. 1995). Such changes are often related to thermal de-excitation of PS2 (Strand and Öquist 1985), and maintain a balance between light-driven linear electron flow and requirements of reducing power for both carboxylation and oxygenation of RuBP (Krause and Weis 1991). The CO_2-induced decrease in the efficiency of open PS2 centres can thus make an important contribution to the greater CO_2-induced depression of $\Delta F/F'_m$ at high VPD.

The temperature-induced enhancement of F_v/F_m in the main growth season arose mainly from the greater increase in F_m than in F_0, and was coupled with a corresponding increase in photosynthetic pigments (Fig. 1). This suggests that elevated temperature enhanced the functioning of PS2, by promoting a high absorption rate in the needles that affects F_0, and stimulating energy cycling between the reaction centre and the Chl pool that affects F_m (Havaux *et al*. 1991).

Given the weather conditions prevailing in Finland, the autumn and winter-induced depression in PS2 observed in Scots pines growing under natural conditions was most related to low-temperature-induced photoinhibition of PS2, accompanied by a loss of the reaction centre (Strand and Öquist 1985, 1988, Leverenz and Öquist 1987). In the 'off season', therefore, elevated temperature could reduce the period during which natural acclimation results in the depression of photoinhibition. This would lead to an increase in the ability of leaves to respond to irradiance during the early spring and autumn, and apparently to an increase in F_v/F_m (Fig. 1). Similarly, the increased Chl *a*+*b* content and decreased ratio of Cars/Chl *a*+*b* during the 'off season' might imply that the elevation in growth temperature provided nearer optimal conditions for pigment biosynthesis (Aiken and Smucker 1996). Our earlier measurements on the same experimental trees showed that elevated temperature increased photon-saturated net photosynthetic rates in the early spring and late autumn (Kellomäki and Wang 1988), validating a positive relationship between enhanced needle pigments and increased [CO_2] uptake or maximum efficiency of PS2 under elevated temperature.

The responses to the combined treatment of elevated [CO_2] and temperature in terms of $\Delta F/F'_m$, F_v/F_m, and photosynthetic pigments were almost the same as those brought about by elevated temperature alone (Fig. 1 and Table 1). No interactive effects of [CO_2] and temperature on pigments were also observed in Douglas fir needles, but a significant decrease in pigment contents was found in the experiment of Ormrod *et al*. (1999). The diversity in the results could be related to species differences or differences in the availability of soil water and nutrient, exposure time, *etc*. However, it must be recognised that (*1*) the temperature elevation used in this experiment was markedly high in winter (a mean increase of 6 °C); this may have important consequences for processes other than water loss, *e.g*. leaf development, whole-tree sink activity, root absorption, *etc*.; and (*2*) the lower ambient temperatures in this experimental site than in other study sites may imply that an equal rise in growth ambient temperature will have greater physiological and ecological consequences for plants in the boreal zone than in other zones.

In conclusion, acclimation of pigments and fluorescence features in the needles of Scots pine to elevated [CO_2] or temperature under boreal conditions depended greatly on the season. Elevated [CO_2] did not significantly modify the optimal photochemical efficiency of PS2 during the main growing season, but it did reduce during the 'off season' and increase the sensitivity of PS2 to high VPD during the main growing season. A mean increase of 2–6 °C in growth temperature significantly enhanced the efficiency of PS2 in terms of increases in F_v/F_m, $\Delta F/F'_m$, and Chl *a*+*b* content throughout the year, but with a greater increase in the 'off season' than the main growing season. Increased Chl content may be advantageous for the efficient usage of radiation energy, and this could be an important reason why trees had a higher potential for photosynthetic capacity during growing season. The results suggest that any interpretation for responses in photochemical reactions to elevated [CO_2] or temperature should combine seasonal acclimation of the trees to particular climate conditions with changes in the sink and sources of whole tree.

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The problem of air quality has become a global issue that must be addressed in an international context. The present book summarizes and integrates more than a decade of atmospheric chemistry research, carried out under the auspices of the International Global Atmospheric Chemistry (IGAC) Project of the International Geosphere-Biosphere Programme (IGBP). This volume was initiated, prepared, and reviewed at workshops held in Toulouse (France, 1998) and in Aspen (Colorado, 2000), and at a meeting of the lead authors in Ispra (Italy, 2001). The book contains seven chapters written by 149 leaders in the field of atmospheric chemistry research from Australia, Brazil, Canada, Chile, Finland, France, Germany, Greece, Italy, Japan, The Netherlands, New Zealand, Norway, Russia, South Africa, Sweden, United Kingdom, and the USA.

The first chapter deals with the changes in the chemical composition of the atmosphere and potential impacts (atmospheric chemistry and life on Earth, past changes in atmospheric chemical composition, causes of atmospheric changes, impacts of changes in atmospheric composition, *etc*.). Biosphere-atmosphere interactions are discussed in the next chapter that deals with key biogenic gases or families and their relevance to atmospheric chemistry, paleoclimatic perspective on methane and dimethylsulphide, atmospheric compounds as nutrients or toxins, approaches for studying exchange, terrestrial highlights (exchange of trace gases and aerosols from terrestrial ecosystems, methane production and consumption, biomass burning, wet deposition in the tropics), marine highlights (air-water gas exchange, marine biogenic emissions, atmospheric deposition on marine and estuarine systems), achievements and remaining research challenges, *etc*.

Chapter 3 is devoted to atmospheric photooxidants (ozone precursors, photochemistry in the troposphere, transport and mixing processes, climatology of tropospheric ozone, long-range transport of pollution and impact on the ozone budget, principal achievements and remaining uncertainties, *etc.*). Chapter 4 deals with tropospheric aerosols (present state of knowledge, recent developments and approaches, highlights and remaining challenges, *etc*.).

Advances in laboratory and field measurements are summarized in chapter 5 (measurements of isotopes, use of lidar on airborne chemistry missions, flux measurements, aerosol analyses, satellite instruments for tropospheric chemistry, long-term measurements, *etc.*). Modelling is discussed in the further chapter (model types, components, evaluation, applications, *etc.*).

An integrated view of the cause and impacts of atmospheric changes is dealt with in the last chapter. The main questions to be solved are, *e.g*., What determines the chemical composition of the atmosphere?, How have human activities altered atmospheric composition, and the global atmospheric budgets of carbon, nitrogen, and sulphur?, What controls tropospheric ozone?, Is the "Cleansing Efficiency" of the atmosphere changing?, How does atmospheric chemistry affect the biosphere and food production, and human health?, What is the connection between atmospheric composition and climate?, How might chemical composition evolve in the future, and what should the research strategy be to address unresolved questions?, *etc.*

An appendix presents lists of authors and reviewers, IGAC foci and activities and their convenors, 2001 (Biosphere-atmosphere interaction, Oxidants and photochemistry, Atmospheric aerosols, Capacity building, Fundamentals and cross-cutting activities), IGAC field campaigns (short-term, multi-year), publications (books, journal papers, IGAC scientific conference proceedings, IGAC programmatic and other publications), and a list of acronyms used in the field. The volume is accompanied by a list of more than 1750 references which, together with carefully prepared subject index (almost 2000 items), will surely be welcome by any reader. The book is carefully edited and produced in the excellent quality of Springer's books. I would like to recommend the volume to all scientists, postgraduate students, and teachers engaged in the field.

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