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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*

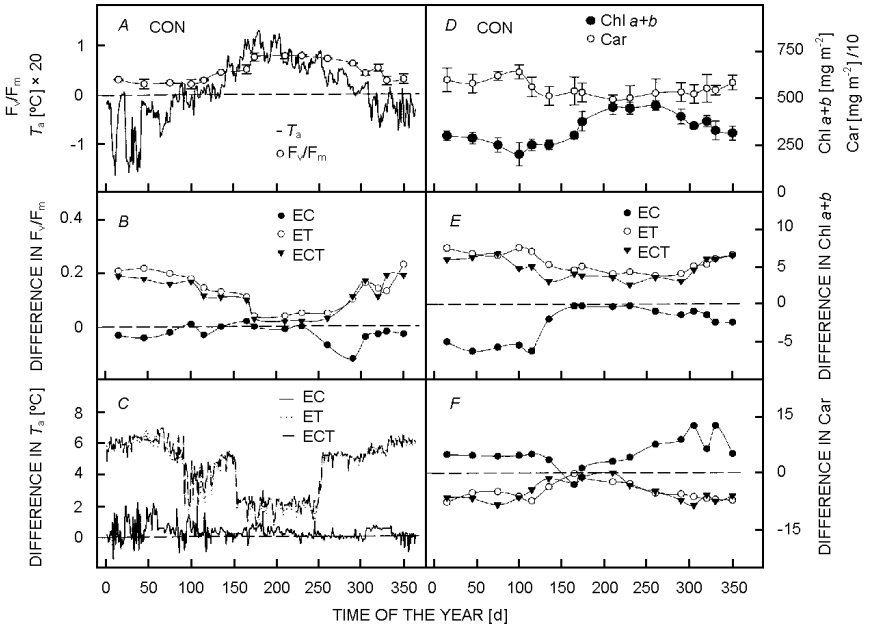

Fig. 1. Annual course of (*A*) mean daily air temperature (T_a) and the optimal photochemical efficiency of PS2 for dark-adapted needles (F_v/F_m), (*B*) differences (treatment – CON) in F_v/F_m relative to CON, (*C*) differences in T_a relative to those in the control chambers, (*D*) mean contents of 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.

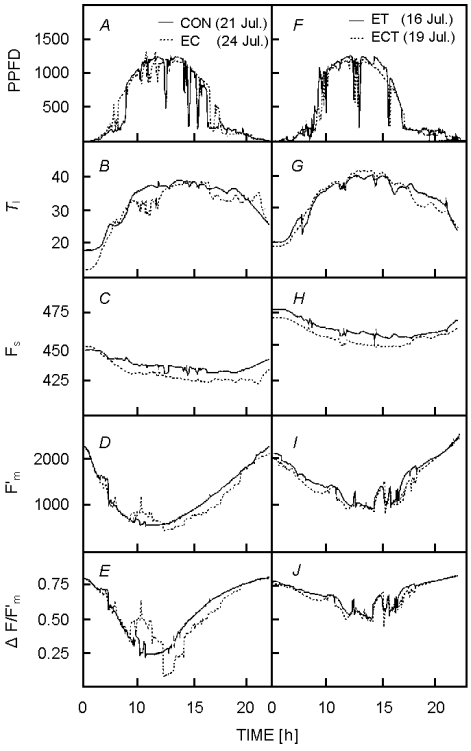

Fig. 2. Four examples of the diurnal time course of (*A, F*) PPFD [μmol m^{-2} s^{-1}], (*B, G*) leaf temperature, T_l [°C], (*C, H*) relative steady-state fluorescence (F_s), (*D, I*) maximum fluorescence (F'_m), and (*E, J*) effective photochemical efficiency of PS2 (F/F'_m) as measured *in situ* on 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

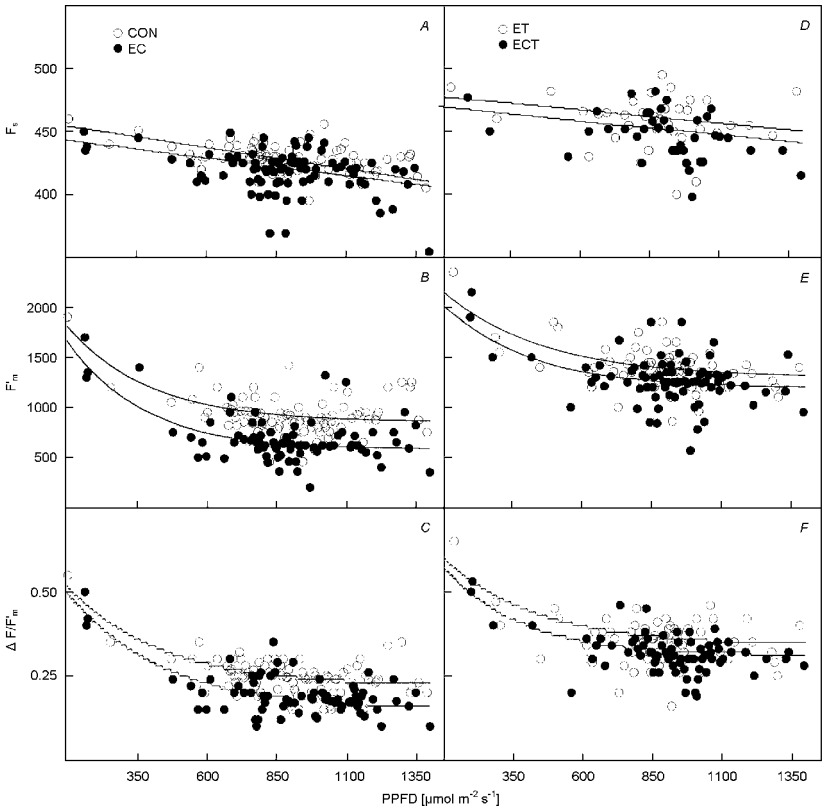

Fig. 3. Irradiance response of relative steady-state (F_s), maximum fluorescence (F'_m), and effective photochemical efficiency of PS2 ($\Delta F/F'_m$) as a function of treatment (CON, EC, ET, and ECT). Each symbol represents values with VPD>1.5 kPa for 5-d measurements in summer 2001 for each treatment (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.

References

Aiken, R.M., Smucker, A.J.M.: Root system regulation of whole plant growth. – Annu. Rev. Phytopathol. **34**: 325-326, 1996.

Besford, B., Mousseau, M., Scarascia-Mugnozza, G.: Biochemistry, physiology and biophysics of photosynthesis. – In: Jarvis, P.G. (ed.): ECOCRAFT: The Likely Impact of Rising CO_2 and Temperature on European Forests. Final Report. Pp. 29-77. University of Edinburgh, Edinburgh 1996.

Butler, W.L.: Energy distribution in the photosynthetic apparatus of photosynthesis. – Annu. Rev. Plant Physiol. **29**: 345-378, 1978.

Ceulemans, R., Jiang, X.N., Shao, B.Y.: Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO_2 levels. – Ann. Bot. **75**: 609-617, 1995.

Chang, C.W., Heggested, H.E.: Effect of ozone on photosystem II in *Spinacia oleracea* chloroplasts. – Phytochemistry **13**: 871-873, 1974.

Delgado, E., Mitchell, R.A.C., Pary, M.A.J., Driscoll, S.P., Mitchell, V.J., Lawlor, D.W.: Interacting effects of CO_2 concentration, temperature and nitrogen supply on the photosynthesis and composition of winter wheat leaves. – Plant Cell Environ. **17**: 1205-1213, 1994.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Eamus, D., Duff, G.A., Berryman, C.A.: Photosynthetic responses to temperature, light flux-density, CO_2 concentration and vapour pressure deficit in *Eucalyptus tetrodonta* grown under CO_2 enrichment. – Environ. Pollution **90**: 41-49, 1995.

Epron, D., Dreyer, E.: Photosynthesis of oak leaves under water stress: maintenance of high photochemical efficiency of photosystem II and occurrence of non-uniform CO_2 assimilation. – Tree Physiol. **13**: 107-119, 1993.

Epron, D., Dreyer, E., Picon, C., Guehl, J.M.: Relationship between CO_2-dependent O_2 evolution and photosystem II activity in oak (*Quercus petraea*) trees grown in the field and in seedlings grown in ambient or elevated CO_2. – Tree Physiol. **14**: 725-735, 1994.

Epron, D., Liozon, R., Mousseau, M.: Effects of elevated CO_2 concentration on leaf characteristics and photosynthetic capacity of beech (*Fagus sylvatica*) during the growing season. – Tree Physiol. **16**: 425-432, 1996.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Hansen, U., Schneiderheinze, J., Rank, B.: Is the lower shade tolerance of Scots pine, relative to pedunculate oak, related to the composition of photosynthetic pigments? – Photosynthetica **40**: 369-374, 2002.

Havaux, M.: Carotenoids as membrane stabilizers in chloroplasts. – Trends Plant Sci. **3**: 147-151, 1998.

Havaux, M., Strasser, R.J., Greppin, H.: A theoretical and experimental analysis of the q_p and coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. – Photosynth. Res. **27**: 41-55, 1991.

Idso, S.B., Kimball, B.A.: Downward regulation of photosynthesis and growth at high CO_2 levels. No evidence for either phenomenon in tree-year study of sour orange trees. – Plant Physiol. **96**: 990-992, 1991.

Kellomäki, S., Wang, K.-Y.: Daily and seasonal CO_2 exchange in Scots pine grown under elevated O_3 and CO_2: experiment and simulation. – Plant Ecol. **136**: 229-248, 1988.

Kellomäki, S., Wang, K.-Y.: Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. – Ann. Bot. **87**: 669-682, 2001.

Kellomäki, S., Wang, K.-Y., Lemettinen, M.: Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions. – Photosynthetica **38**: 69-81, 2000.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Leverenz, J., Öquist, G.: Quantum yields of photosynthesis at temperatures between -2°C and 35°C in a cold tolerant C_3 plant (*Pinus sylvestris*) during the course of one year. – Plant Cell Environ. **10**: 287-295, 1987.

Lewis, J.D., Olszyk, D., Tingey, D.T.: Seasonal patterns of photosynthetic light response in Douglas-fir seedlings subjected to elevated atmospheric CO_2 and temperature. – Tree Physiol. **19**: 243-252, 1999.

Lichtenthaler, H.L.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. **148**. Pp. 350-382. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto 1987.

Linder, S.: Seasonal variation of pigments in needles: A study of Scots pine and Norway spruce seedlings grown under different nursery conditions. – Stud. forest. suec. **100**: 1-37, 1972.

Long, S.P.: Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? – Plant Cell Environ. **14**: 729-739, 1991.

Long, S.P., Hutchin, P.R.: Primary production in grasslands and coniferous forests with climate change: an overview. – Ecol. Applic. **1**: 139-156, 1991.

Lundmark, T., Bergh, J., Strand, M.: Seasonal variation of maximum photochemical efficiency in boreal Norway spruce stands. – Trees **13**: 63-67, 1998.

Marek, M.V., Šprtová, M., Kalina, J.: The photosynthetic irradiance-response of Norway spruce exposed to long-term elevation of CO_2 concentration. – Photosynthetica **33**: 259-268, 1997.

Martin, B., Mårtensson, O., Öquist, G.: Effects of frost hardening and dehardening on photosynthetic electron transport and fluorescence properties in isolated chloroplasts of *Pinus sylvestris*. – Physiol. Plant. **43**: 297-305, 1978.

Michelsen, A., Jonasson, S., Sleep, D., Havoström, M., Callaghan, T.V.: Shoot biomass, ^{13}C, nitrogen and chlorophyll responses of two arctic dwarf shrubs to *in situ* shading, nutrient application and warming simulating climate change. – Oecologia **105**: 112-123, 1996.

Miller, A., Tsai, C.-H., Hemphill, D., Endres, M., Rodermel, S., Spalding, M.: Elevated CO_2 effects during leaf ontogeny. A new perspective on acclimation. – Plant Physiol. **115**: 1195-1200, 1997.

Mohammed, G.H., Binder, W.D., Gillies, S.L.: Chlorophyll

fluorescence: a review of its practical forestry applications and instrumentation. – Scand. J. Forest Res. **10**: 383-410, 1997.

Moser, E.B., Saxton, A.M., Pezeshki, S.R.: Repeated measures analysis of variance: application to tree research. – Can. J. Forest Res. **20**: 524-535, 1990.

Mousseau, M., Enoch, H.Z.: Carbon dioxide enrichment reduces shoot growth in sweet chestnut seedlings (*Castanea sativa* Mill.). – Plant Cell Environ. **12**: 927-934, 1989.

Ormrod, D., Lesser, V.M., Olszyk, D.M., Tingey, D.T.: Elevated temperature and carbon dioxide affect chlorophyll and carotenoids in Douglas-fir seedlings. – Int. J. Plant Sci. **160**: 529-534, 1999.

Powles, S.B., Björkman, O.: Photoinhibition of photosynthesis: effect on chlorophyll flluorescence at 77K in intact leaves and in chloroplast membrances of *Nerium oleander*. – Planta **156**: 97-107, 1978.

Pritchard, S.G., Peterson, C.M., Prior, S.A., Rogers, H.H.: Elevated atmospheric CO_2 differentially affects needle chloroplast ultrastructure and phloem anatomy in *Pinus palustris*: interactions with soil resource availability. – Plant Cell Environ. **20**: 461-471, 1997.

Roden, J.S., Ball, M.C.: The effect of elevated [CO_2] on growth and photosynthesis of two eucalyptus species exposed to high temperatures and water deficits. – Plant Physiol. **111**: 909-919, 1996.

Scarascia-Mugnozza, G., De Angelis, P., Matteucci, G., Valentini, R.: Long-term exposure to elevated [CO_2] in a natural *Quercus ilex* L. community: net photosynthesis and photochemical efficiency of PSII at different levels of water stress. – Plant Cell Environ. **19**: 643-654, 1996.

Strand, M., Öquist, G.: Inhibition of photosynthesis by freezing temperatures and high light levels in cold-acclimated seedlings of Scots pine (*Pinus sylvestris*). II. Effects on chlorophyll fluorescence at room temperature and 77 K. – Physiol. Plant. **65**: 117-123, 1985.

Strand, M., Öquist, G.: Effects of forest hardening, dehardening and freezing stress on *in vivo* chlorophyll fluorescence of seedings of Scots pine (*Pinus sylvestris* L.). – Plant Cell Environ. **11**: 231-238, 1988.

Surano, K.A., Daley, P.F., Houpis, J.L.J., Shinn, J.H., Hellms, J.A., Palassou, R.J., Costella, M.P.: Growth and physiological responses of *Pinus pondersa* Dougl. ex P. Laws. to long-term elevated CO_2 concentrations. – Tree Physiol. **2**: 243-259, 1986.

Taiz, L., Zeiger, E.: Plant Physiology. – Sinauer, Sunderland 1998.

Teskey, R.O.: A field-study of the effects of elevated CO_2 on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. – Plant Cell Environ. **18**: 565-573, 1997.

Tissue, D.T., Thomas, R.B., Strain, B.R.: Atmospheric CO_2 enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. – Plant Cell Environ. **20**: 1123-1134, 1997.

Valentini, R., Epron, D., De Angelis, P., Matteucci, G., Dreyer, E.: *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. – Plant Cell Environ. **18**: 631-640, 1995.

Wang, K.-Y.: Canopy CO_2 exchange of Scots pine and its seasonal variation after four-year exposure to elevated CO_2 and temperature. – Agr. Forest Meteorol. **82**: 1-27, 1996.

Wang, K.-Y., Kellomäki, S.: Effects of elevated CO_2 and soil-nitrogen supply on chlorophyll fluorescence and gas exchange in Scots pine, based on a branch-in-bag experiment. – New Phytol. **136**: 277-286, 1997.

Woolhouse, H.W.: The biochemistry and regulation of senescence in chloroplasts. – Can. J. Bot. **62**: 2934-2942, 1984.

Wullschleger, S., Norby, R.J., Hendrix, D.L.: Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. – Tree Physiol. **10**: 21-31, 1992.