First-Year Results on the Effects of Elevated Atmospheric CO_2 and O_3 Concentrations on Needle Ultrastructure and Gas Exchange Responses of Scots Pine Saplings

Virpi Palomäki, Kaisa Laitinen, Toini Holopainen and Seppo Kellomäki

Palomäki, V., Laitinen, K., Holopainen, T. & Kellomäki, S. 1996. First-year results on the effects of elevated atmospheric CO_2 and O_3 concentrations on needle ultrastructure and gas exchange responses of Scots pine saplings. Silva Fennica 30(2–3): 123–134.

The effects of realistically elevated O_3 and CO_2 concentrations on the needle ultrastructure and photosynthesis of ca. 20 year-old Scots pine (*Pinus sylvestris* L.) saplings were studied during one growth period in open-top field chambers situated on a natural pine heath at Mekrijärvi, in eastern Finland. The experiment included six different treatments: chamberless control, filtered air, ambient air and elevated O_3, CO_2 and O_3 + CO_2. Significant increases in the size of chloroplast and starch grains were recorded in the current-year needles of the saplings exposed to elevated CO_2. These responses were especially clear in the saplings exposed to elevated O_3 + CO_2 concentrations. These treatments also delayed the winter hardening process in cells. In the shoots treated with O_3, CO_2 and combined O_3 + CO_2 the P_{max} was decreased on average by 50 % (ambient CO_2) and 40 % (700 ppm CO_2). Photosynthetic efficiency was decreased by 60 % in all the treated shoots measured under ambient conditions and by 30 % in the CO_2 and O_3 + CO_2 treated shoots under 700 ppm. The effect of all the treatments on photosynthesis was depressive which was probably related to evident accumulation of starch in the chloroplasts of the pines treated with CO_2 and combined O_3 + CO_2. But in O_3 treated pines, which did not accumulate starch in comparison to pines subjected to ambient air conditions, some injuries may be already present in the photosynthetic machinery.

Keywords CO_2, O_3, Scots pine, needle ultrastructure, gas exchange, climate change
Authors' addresses *Palomäki & Holopainen:* University of Kuopio, Department of Ecology and Environmental Science, P.O.Box 1627, FIN-70211 Kuopio, Finland. *Laitinen & Kellomäki:* University of Joensuu, Faculty of Forestry, P.O. Box 111, FIN-80101 Joensuu, Finland. **Fax** Palomäki + 358-17-163230 **E-mail** virpi.palomaki@uku.fi
Accepted June 18, 1996

List of symbols

P_n Net photosynthesis rate, μmol m^{-2} s^{-1}
θ Convexity of light response curve; dimensionless parameter with values of $0 \leq \theta \leq 1$
α initial slope of light response curve; photosynthetic efficiency, mol mol $photon^{-1}$
I photon flux density, μmol m^{-2} s^{-1}
I_c light compensation point of photosynthesis, μmol m^{-2} s^{-1}
P_m maximum rate of photosynthesis at saturating photon flux density, μmol m^{-2} s^{-1}
I_s light saturation point, μmol m^{-2} s^{-1}

1. Introduction

The concentrations of atmospheric CO_2 and O_3 are forecasted to rise to levels approximately double the present by the year 2100 (Dickinson 1986). The incorporation of ozone studies into climate change research has proved to be necessary since the ozone concentrations measured in Finland (Laurila and Lättilä 1994), especially in spring and early summer, momentarily exceed the levels believed to be harmful to sensitive forest vegetation. The effects of elevated CO_2 and O_3 concentrations on the photosynthesis of plants have been widely studied and they have been reported to be either stimulative or depressive depending on the dose, exposure time and environmental conditions (Bazzaz 1990, Bunce 1992, Weber et al. 1993, Ceulemans and Mousseau 1994, Flagler et al. 1994, Runeckles and Krupa 1994). Ultrastructural study of needles has proved to be a sensitive method revealing early stimulative or harmfull effects of ozone in several conifer species (Sutinen et al. 1990, Rantanen et al. 1994, Anttonen et al. 1995, Holopainen et al. 1996), but very little is so far known on the effects of elevated CO_2 alone or in combination with O_3.

The objective in this research is to study the effects of realistically elevated O_3 and CO_2 concentrations alone and in combination on the needle ultrastructure and photosynthesis of Scots pine saplings grown in open-top field chambers situated on a natural pine heath in eastern Finland.

2. Materials and Methods

2.1 Experimental Area

The experimental area is situated in a naturally regenerated stand of Scots pine (*Pinus sylvestris* L.) close to the Mekrijärvi Research Station (62°47'N, 30°58'E, 145 m a.s.l.) of the University of Joensuu in Finland. The heights of the saplings chosen for the experiment varied between 2.5 m and 3.4 m (mean 3.0 m) and their ages between 14 years and 24 years (mean 19). The mean stem diameter at 1.3 m above ground level was 5 cm and the number of stems was 2500 per hectare. The stand had not been fertilised during the lifespan of the experimental pines. The forest type of the site is of *Vaccinium* type (Cajander 1949) and soil is sandy moraine.

2.2 Experimental Design

Each experimental pine (20) was surrounded by an open-top chamber (about 10 m^3 volume, width 1.7 m and height 3.5 m) made of plastic sheet. A circular tube of perforated PVC membrane (diameter 250 mm) was located at the bottom of each chamber for distribution of air and experimental gases. The total air volume in the chambers changed once a minute. Five different treatments (four replicates) were arranged in the chambers: filtered air, ambient air, elevated O_3, elevated CO_2, elevated O_3 + CO_2. In addition five similar saplings serve as chamberless controls. Saplings for each treatments were selected randomly. CO_2 concentrations approx. doubled (ranges between 600 ppm and 800 ppm) compared to the ambient, and O_3 concentrations between 40 ppb (September) and 70 ppb (June) were maintained in the chambers. The total O_3 doses received by the saplings during the growth period and critical doses exceeding 40 ppb threshold concentration (AOT 40) are shown in Table 1. The exposure and controlling systems for the gases are computer-controlled. Exposure was started on 2 June and ended on 23 September in 1994. The time of exposure was 16 h per day (from 6.00 to 22.00).

Table 1. The total doses (>0 ppb) and critical doses exeeding 40 ppb (AOT 40) of O_3 in O_3 exposed chambers, filtered chambers and ambient air over the growth period of 1994.

Treatment	Total dose	Critical dose AOT 40
O_3	109 651	16 499
Filtered air	59 243	203
Ambient air	80 977	1 274

2.3 Needle Sampling and Microscopical Studies

Needle samples for microscopical studies were collected three times (9 August, 19 September, and 1 November) during the study year of 1994, from the first-order laterals on the fourth whorl from the stem apex both from current and previous year shoots separately from each sapling. The samples were directly placed in 2 % glutaraldehyde fixative prepared in phosphate buffer (pH 7.0, 0.1 M). Approx. 1 mm piece was cut from a region 1/3 behind the tip of each needle and prefixed for 20 h in glutaraldehyde fixative followed by postfixation in 1 % OsO_4 solution for 6 h at +6°C. After fixation, samples were dehydrated in a graded ethanol series and embedded in epon LX-112. Blocks were polymerized for one day at +37°C followed by three days at +60°C. Sections for electron microscopy (2 replicates) were stained with uranylacetate and lead citrate. Observations on the condition of cell organelles and cytoplasm were made using an electron microscope (JEOL JEM 1200 EX). The size of the starch grains and chloroplasts in the mesophyll cells were measured from electron micrographs (×10 000, 20–25 per series).

2.4 Gas Exchange Measurements

The gas exchange measurements were conducted using a portable open gas analysing system ADC LCA-4 (Analytical Development Co. Ltd. UK.) and employing a Parkinson leaf chamber for conifers (PLC4) with a portable light unit (PLU-002) and neutral density filters. The gas exchange results are given according to the silhouette shoot area (m^2) determined from still-video images (model Canon ION Still Video C-Camera RC-260, PAL High-Band). The images of the shoots were taken in the field in the direction parallel to the light beams from the light source.

Photosynthesis in saturated light was measured simultaneusly with needle sampling in August and September. The measurements were made under ambient CO_2 concentration (310–350 ppm) of the current-year shoots, i.e. of the same shoots as the needle samples prior to needle sampling.

The light response curves for fully developed current-year shoots, one shoot in each pine, were measured during the period 10–29 August, after one growing season of exposure to the treatments. The gas exchange measurements were conducted on the first-order laterals of the fourth whorl from the stem apex. The temperature of the shoot cuvette during measurements varied between 16 and 20°C. The measurements for each shoot were done under 350 ppm (ambient) and 700 ppm concentrations of CO_2. The CO_2 concentrations for the measurements were obtained from standard cylinders of CO_2 (AGA Ltd. Sweden).

2.5 Analysis of Gas Exchange Measurements

The non-rectangular hyperbolic equation (1) presented by Thornley and Johnson (1990) was fitted to the light response curve measurements conducted of a single shoot for each tree for the purpose of obtaining the values for the parameters.

$$P_n = \frac{1}{2\Theta}\left\{\alpha(I-I_c)+P_m-\sqrt{(\alpha(I-I_c)+P_m)^2-4\Theta\alpha P_m(I-I_c)}\right\} \quad (1)$$

Respectively, the light saturation point was calculated using the following equation (2).

$$I_s = \frac{P_m}{\alpha}\left[\frac{\rho-\Theta}{1-\rho}+\Theta(1+\rho)\right]+I_c \quad (2)$$

$\rho = P_n / P_m$ with the fixed value 0.7. The dark respiration was also calculated from the data.

2.6. Statistical Analysis

The data of the size of the starch grains and chloroplasts were subjected to analysis of variance (ANOVA). The individual means were compared by the Duncan's multiple range test and using general factorial ANOVA test (SPSS-PC programmes).

The means and standard deviations for the sampling of photosynthetic values and for the values of the different parameters were calculated. Furthermore, the photosynthetic values connected to sampling of needles and the model parameters were tested using the SPSS general factorial ANOVA test and the non-parametric Mann-Whitney U-test.

3. Results

3.1 Needle Ultrastructure

In the beginning of August, a significant increase in the size of chloroplasts in the saplings exposed to filtered air, elevated CO_2 and elevated $O_3 + CO_2$ concentrations compared to ambient air were measured (Table 2). Correspondingly starch grain size increased in saplings exposed to filtered air, elevated CO_2 and elevated $O_3 + CO_2$ concentrations in the current-year needles of the saplings (Fig. 1). In September, the starch grains were small or absent, and significant differences between treatments were no longer observable. In November, some delay in the appearance in the structural changes related to winter-hardening in the needles were observed. Especially in the needles of ambient air treatment and O_3 exposed saplings, chloroplasts were aggregated together and small vacuoles appeared in the cytoplasm, which is typical in winter condition. In the treatments, which caused accumulation of starch in summer (CO_2 and $O_3 + CO_2$), the chloroplasts were situated adjacent to the cell wall which is characteristic in summer condition (Fig. 2).

Table 2. The area of chloroplasts (mm^2) and starch grains (means±SD) measured from EM- photographs taken from the mesophyll tissue of current-year needles in saplings treated with elevated O_3 and CO_2 alone and in combination, filtered air, ambient air (air) and open air (out control) in August 1994 (a) and the main effects and interactions of the elevated O_3 and CO_2 (b).

2a.

Treatment	Chloroplast area	Starch grain area
O_3	10.6±2.5ab	5.6±2.6ab
$O_3 + CO_2$	15.9±5.9c	11.9±5.8c
CO_2	11.6±4.4ab	7.8±3.9b
Filtered air	12.4±3.6b	7.9±3.8b
Air	10.8±3.9ab	6.0±4.4ab
Out control	9.9±1.9a	4.9±3.9a
F	7.3001	10.1737
P	0.0000	0.0000

Means in the same column followed by different letters are significantly different (P<0.05).

2b.

	df	SS	F	P
Chloroplast area				
O_3	1	1335.067	5.433	.022
CO_2	1	2661.191	10.830	.001
$O_3 + CO_2$	1	1564.882	6.368	.013
Residual	92	22607.111		
Starch grain area				
O_3	1	1173.241	4.803	.031
CO_2	1	4896.553	20.047	.000
$O_3 + CO_2$	1	1686.082	6.903	.010

3.2 Photosynthesis Measurements During Sampling of the Needles

Compared to the chamber control (AIR = ambient air), the light saturated photosynthesis (P_{max}) decreased in all of the exposed pines, both in August and in September (Fig. 3, Table 3 and 4). The decrease was greatest in the shoots exposed to the filtered air and O_3 treatments and varied from 45 % and 40 % in August to 31 % and 17 % in September, respectively. In the CO_2 exposed shoots, the decrease was 27 % and 14 %, but in the $O_3 + CO_2$ exposed shoots only 6 % and 1 %, respectively.

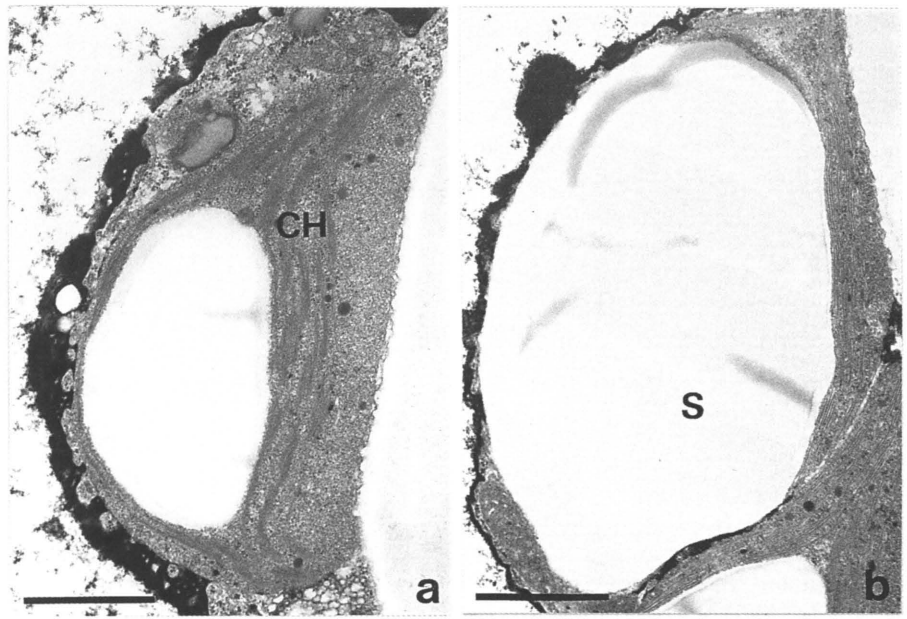

Fig. 1. (a) Normal structure of mesophyll chloroplasts (CH) in the needles of saplings subjected to ambient air treatment in August. (b) Accumulation of starch (S) in a chloroplast in the needles of elevated $O_3 + CO_2$ treatments in August.

3.3. Light Response Curves

In the O_3, CO_2 and combined $O_3 + CO_2$ treated shoots the *maximum rate of photosynthesis* (Fig. 4a) decreased by 44–59 % measured under ambient and 30–47 % under 700 ppm of CO_2 as compared to the chamber control (AIR = ambient air) (Tables 5 and 6). Respectively, the decrease was 12 % and 44 % in the filtered air treatment of shoots.

Compared to the values of the chamber control (AIR), *dark respiration* (Fig. 4b) decreased by 39–67 % in the shoots exposed to O_3, $O_3 + CO_2$, CO_2 and filtered air treatments when measured under the ambient CO_2 level. Respectively, under CO_2 concentration of 700 ppm, dark respiration increased by 13–84 % in shoots subjected to the $O_3 + CO_2$, CO_2 and filtered air treatments as was also the case with the chamberless control (out control) shoots (Table 5 and 6). In the O_3 exposed shoots under the 700 ppm concentration of CO_2, the dark respiration rate decreased by 48 %.

Compared to the values of the chamber control *light compensation* (Fig. 4c) increased by 115 % and 130 % when measured under the ambient and by 63 % and 163 % when measured under the concentration of 700 ppm of CO_2 in $O_3 + CO_2$ and CO_2 exposed shoots (Table 4). In the O_3 exposed shoots there was a great increase in the parameter value (287 %) measured under the ambient but a decrease (76 %) measured under 700 ppm concentration of CO_2. In the filtered air treatment there was an increase of 88 % in light compensation when measured under the 700 ppm CO_2 concentration.

Light saturation increased in the O_3, $O_3 + CO_2$, CO_2 and filtered air treated shoots. The increase was 24–90 % when measured under the ambient and 16–105 % when measured under a CO_2 concentration of 700 ppm (Fig. 4d, Table 4).

Photosynthetic efficiency i.e. the initial slope

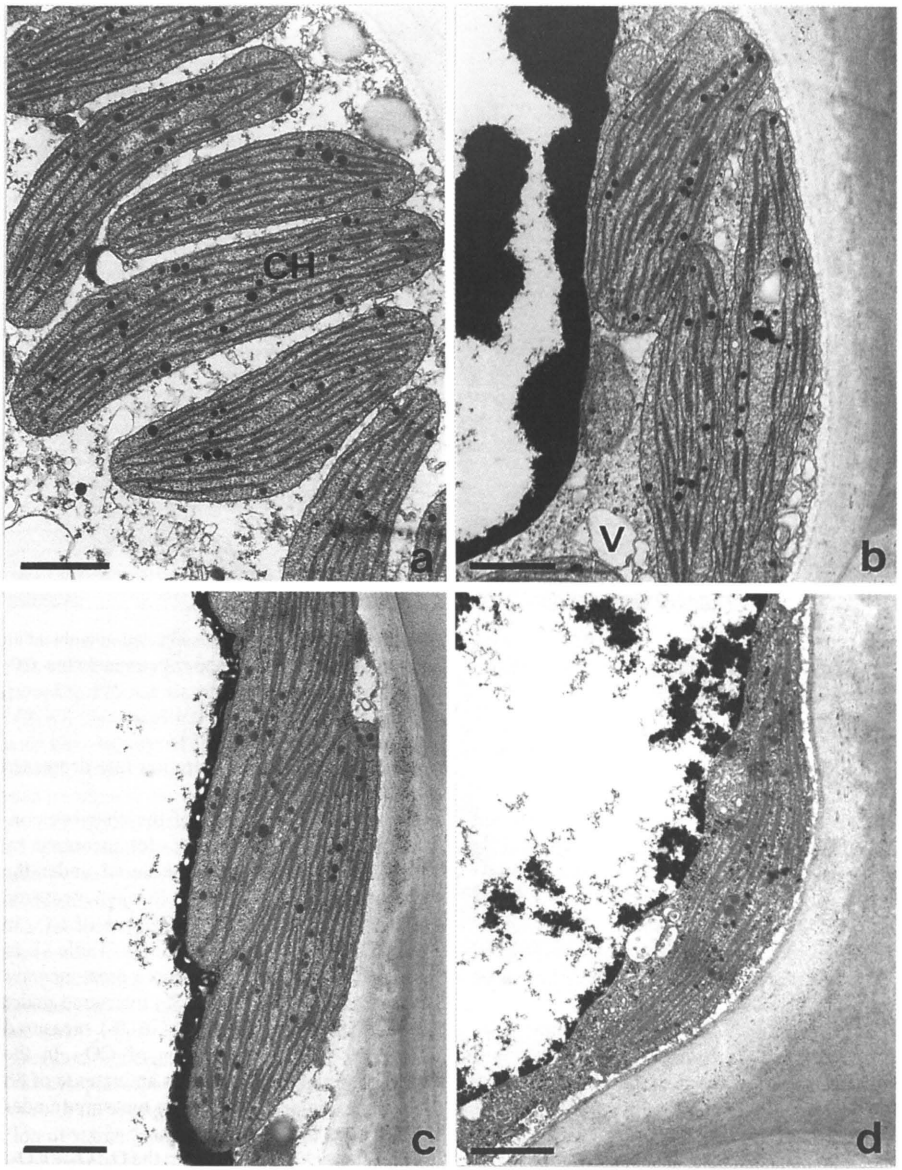

Fig. 2. Mesophyll cell from the needles of samples subjected to different treatments and sampled in November. a–b. The chloroplasts (CH) in needles treated with ambient air (a) and O_3 (b) have aggregated and small vacuoles (V) have appeared in the cytoplasm; this is typical for the winter situation. c–d. The chroloplasts are situated adjacent to the cell wall in needles treated with elevated CO_2 (c) and O_3 + CO_2 (d); this is typical in summer.

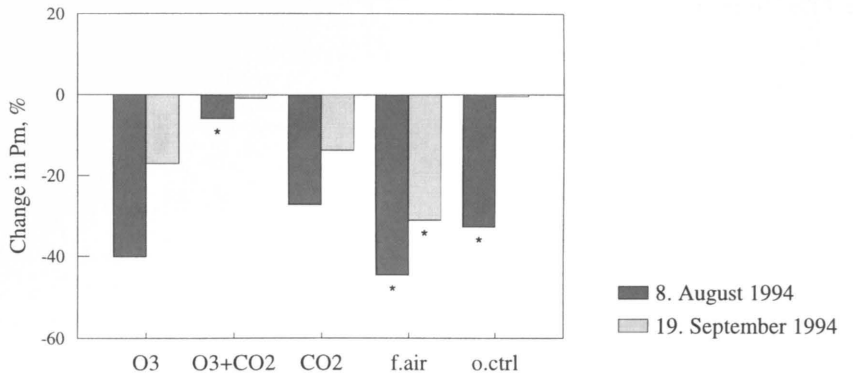

Fig. 3. The relative change (%) in the maximum rate of photosynthesis as compared to the value for the chamber control in August and September; * denotes a statistically significant difference ($p<0.05$).

Table 3. The maximum rate of photosynthesis (μmol m^{-2} s^{-1}, means±SD) measured in connection with needle sampling in August and September.

Treatment	August	September
O_3	16.67± 2.79	12.83±7.91
O_3 + CO_2	26.19±11.76	15.35±5.21
CO_2	20.29±11.34	13.35±6.49
Filtered air	15.46± 5.42	10.67±2.08
Air	27.85± 6.15	15.47±4.34
Out control	18.73± 8.52	15.41±5.52

(Fig. 4e), decreased in the O_3, O_3 + CO_2, CO_2 and filtered air treated shoots. Compared to the chamber control, the decrease was 52–62 % when measured under the ambient CO_2 concentration (Table 4a). When measured under a concentration of 700 ppm, the decrease was 8–38 % (Table 4b).

Convexity of the curve (Fig. 4f) decreased in the O_3, O_3 + CO_2, CO_2 and filtered air treated shoots compared to the chamber control. The decrease was 10–52 % when measured under the ambient concentration and 11–67 % under a CO_2 concentration of 700 ppm (Table 4).

Table 4. The statistical analysis for the needle sampling photosynthesis data. The main effects and interactions (ANOVA) of the treatments elevated O_3 and CO_2 (a) and the Mann-Whitney U -test for chamber control (air) vs. out control and filtered air (b). * denotes a statistically significant difference ($p<0.05$).

4a.

	df	SS	F
August			
O_3	1	85.26	1.08
CO_2	1	11.71	0.15
O_3 + CO_2	1	892.54	11.26*
Within and residual	45	3566.82	
September			
O_3	1	0.85	0.02
CO_2	1	0.34	0.01
O_3 + CO_2	1	45.22	1.15
Within and residual	30	1179.89	

4b.

vs. air	Time	Z	2-Tailed P
Out control	August	–2.4398*	0.0147
	September	–0.2407	0.8098
Filtered air	August	–3.6373*	0.0003
	September	–2.5019*	0.0124

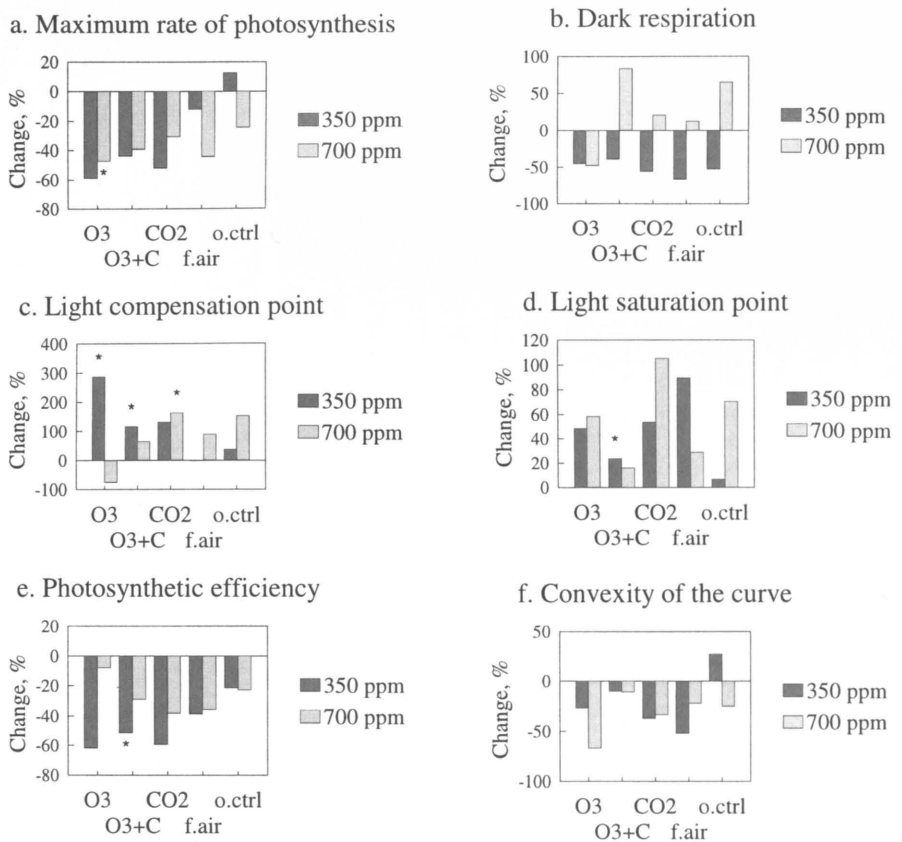

Fig. 4. The relative change (%) in the parameter value as compared to the value in the chamber control (AIR) under 350 ppm and 700 ppm; a) maximum photosynthesis, b) dark respiration rate, c) light compensation point, d) light saturation point, e) photosynthetic efficiency, f) convexity of light response curve; * denotes a statistically significant difference ($p<0.05$).

4. Discussion

The effects of elevated concentration of CO_2 and O_3 on different species of plants have been widely studied in different exposure levels and durations (Barnes et al. 1990, Bunce 1992, Ceulemans and Mousseau 1994, Flagler et al. 1994, Clark et al. 1995). In this study we report the effects of elevated CO_2, O_3 and their combination to the needle ultrastructure and photosynthesis of Scots pine after one growing season exposure.

The effects of elevated CO_2 and O_3 and their combination on photosynthesis appeared to be depressive. The reduction could be related to the accumulation of starch in chloroplasts in plants subjected to the CO_2 and combined O_3 + CO_2 treatments, because chloroplasts may have been saturated by starch earlier in the summer (e.g. Carmi and Shomer 1979). However, the reduction of net photosynthesis was lowest and starch accumulation most clear in combined O_3 + CO_2 exposure in the simultaneous photosynthesis and microscopical studies. In earlier studies Carmi

Table 5. The values for the parameters (meansSD), P_m = maximum photosynthesis, R_d = dark respiration rate, I_c = light compensation point, I_s = light saturation point, α = photosynthetic efficiency, θ = convexity of the light response curve, under the treatments measured under 350 ppm (a) and 700 ppm (b).

5a. 350 ppm Treatment	P_m	R_d	I_c	I_s	α	θ
O_3	12.57± 3.04	1.97±0.91	156±30	969±247	0.0248±0.0077	0.378±0.175
O_3 + CO_2	17.16±10.48	2.19±1.20	87±60	807±145	0.0315±0.0170	0.463±0.205
CO_2	14.67± 9.10	1.60±0.78	93±23	1072±227	0.0263±0.0133	0.324±0.119
Filtered air	26.95±11.89	1.20±0.32	40±14	1237±268	0.0399±0.0044	0.248±0.080
Air	30.53±10.92	3.58±4.33	40±34	652±224	0.0650±0.0253	0.515±0.321
Out control	34.40±12.66	1.72±0.57	55±36	695±128	0.0510±0.0137	0.665±0.173
5b. 700 ppm Treatment	P_m	R_d	I_c	I_s	α	θ
O_3	33.83±12.67	1.02±0.76	8± 6	875±550	0.0843±0.0523	0.257±0.343a
O_3 + CO_2	38.88± 3.81	3.59±2.47	52±33	640±157	0.0650±0.0276	0.700±0.023ab
CO_2	44.40± 9.74	2.36±1.29	84±19	1134±798	0.0566±0.0033	0.525±0.369ab
Filtered air	35.67± 1.32	2.21±0.84	60±27	713±342	0.0589±0.0089	0.612±0.278ab
Air	64.03±13.71	1.96±1.24	32± 5	553± 99	0.0914±0.0079	0.785±0.042b
Out control	48.39±21.30	3.23±1.88	81±39	941±669	0.0707±0.0197	0.588±0.225ab

and Shomer (1979) reported accumulation of starch in bean exposed to elevated CO_2 concentration but Robertson and Leech (1995) measured reduction in starch accumulation in wheat.

Our results indicating depression of photosynthesis after one growing season under elevated CO_2 concentration are contrary to what is reported in an earlier study with long-term exposure (Wang et al. 1995) made in the same area in the open top chambers with the same species using the same measuring technique. Wang et al. (1995) reported slightly enhanced photosynthesis (14 %) and decreased dark respiration (20 %) for a Scots pine grown for two years under doubled CO_2 concentrations compared to the chamber control. In general, variable results have been obtained when studying the effect of elevated CO_2 concentrations, but down-regulation in photosynthesis and decrease in dark respiration after long-term exposure has often been reported (Bunce 1992, 1995, Ceulemans and Mousseau 1994) in deciduous species. In conifers both increase or no acclimation of photosynthesis has been reported in long-term studies. In seedlings of *Pinus radiata* Hollinger (1987) reported no difference in assimilation after 120 days at 340 or 640 ppm of CO_2. Correspondingly, Teskey (1995) reported no change in assimilation of *Pinus taeda* after 157 days under elevated CO_2, as also a decrease in respiration after elevated CO_2 treatment. Tissue et al. (1993) studied *Pinus taeda* seedlings after two years under elevated CO_2 and reported an increase in assimilation, but only when the seedlings received supplemental nitrogen.

In the present study, O_3 exposure did not induce initial starch accumulation to the chloroplasts, which has been observed in several other fumigation studies with slightly elevated O_3 concentrations (e.g. Luethy-Krause and Landolt 1990, Rantanen et al. 1994). Environmental conditions e.g. limited nutrient availability may have reduced the stimulative effect of low O_3 concentration in the present experiment (Wallin et al. 1990, Rantanen et al. 1994). In the present study the depression of photosynthesis may be an early indication of the harmful effect of O_3, although the O_3 exposure did not cause advanced injury symptoms like increased density of chloroplasts stroma and clearly decreased size of chloroplasts and starch grains (e.g. Sutinen et al. 1990) in the needle ultrastructure during the study

Table 6. The statistical analysis for the light response parameters (P_m = maximum photosynthesis, R_d = dark respiration rate, I_c = light compensation point, I_s = light saturation point, α = photosynthetic efficiency, θ = convexity of the light response curve) measured under 350 ppm and 700 ppm. The main effects and interactions (ANOVA) of the treatments elevated O_3 and CO_2 (a) and the Mann-Whitney U-test for chamber control (air) vs. out control and filtered air (b). * denotes a statistically significant difference ($p<0.05$).

6a.

Treatment	df	P_m SS	P_m F	R_d SS	R_d F	I_c SS	I_c F	I_s SS	I_s F	α SS	α F	θ SS	θ F
350 ppm													
O_3	1	205.01	2.35	0	0	10349.28	5.99*	12612.01	0.29	0	3.23	0	0
CO_2	1	109.88	1.25	1.06	0.30	232.04	0.13	30317.01	0.69	0	2.70	0.01	0.18
O_3+CO_2	1	356.61	4.11	1.18	0.33	12793.22	7.40*	224960.5	5.13*	0	5.44*	0.07	1.25
Within and residual	10	872.53		35.56		17282.54		438571.1		0		0.53	
700 ppm													
O_3	1	850.59	10.99*	0.02	0.01	2106.96	5.02	19415.76	0.07	0	0	0.08	1.13
CO_2	1	141.88	1.83	6.49	2.68	6188.72	14.74*	80460.93	0.29	0	1.93	0.02	0.31
O_3+CO_2	1	406.32	5.25	2.98	1.23	37.41	0.09	444253.0	1.61	0	0.16	0.33	4.51
Within and residual	7	541.76		16.96		2938.93		1935782		0.01		0.51	

6b.

vs. air	P_m Z	P_m 2-Tailed P	R_d Z	R_d 2-Tailed P	I_c Z	I_c 2-Tailed P	I_s Z	I_s 2-Tailed P	α Z	α 2-Tailed P	θ Z	θ 2-Tailed P
350 ppm												
Out control	–0.5774	0.5637	–0.2887	0.7728	–0.5774	0.5637	–0.2887	0.7728	–0.8660	0.3865	–0.8660	0.3865
Filtered air	–0.4629	0.6434	–0.9258	0.3545	–0.9258	0.3545	–1.8516	0.0641	–0.9258	0.3545	–0.9258	0.3545
700 ppm												
Out control	–0.3873	0.6985	–0.7746	0.4386	–1.5492	0.1213	–0.3873	0.6985	–1.5492	0.1213	–1.5492	0.1213
Filtered air	–1.8516	0.0641	0	1	–1.8516	0.0641	–0.4629	0.6434	–1.8516	0.0641	–1.3887	0.1649

period, which lasted over one summer. The early decrease in assimilation may be associated with changes in Rubisco functioning as reported by Pell et al. (1992), who also reported a decline in photosynthetic efficiency in several species after exposure to O_3.

In long-term O_3 exposures a reduction in assimilation rate has been a common response as reported by Barnes et al. (1990) and Wallin et al. (1990) in *Picea abies* and by Flagler et al. (1994) in *Pinus echinata* and by Weber et al. (1993) and Clark et al. (1995) in *Pinus ponderosa*. Both Barnes et al. (1990) and Wallin et al. (1990) found increased dark respiration in *Picea abies* after long-term O_3 exposure. As in this study Barnes et al. (1990) reported an increase in light compensation in *Picea abies* after long-term O_3 exposure.

In the present study the elevated CO_2 concentration alone and in combination with O_3 appeared to delay the structural winter-hardening process (e.g. Soikkeli 1980) in the cells of Scots pine needles, which needs further attention involving frost tolerance measurements. If winter hardening process is continuously delayed, this may have serious consequences for the frost tolerance of the trees in harsh continental climate, like in Eastern Finland.

The results presented in this study are rather preliminary, since the experiment is planned to last at least two more years and the tree responses will be studied with several methods in the future. The results already available indicate, however, that slightly elevated ozone concentrations may have negative effects on the photosynthetic efficiency of naturally growing young Scots pines. After longer duration of O_3 exposure, decreased photosynthesis may be followed by structural injuries of chloroplast (Sutinen et al. 1990, Wallin et al. 1990) also in the present experiment. Although the accumulation of starch was increased in new needles of the seedlings grown at higher CO_2 level , there was no direct evidence that elevated CO_2 provided additional protection against harmful effects of O_3. This is in agreement with the observations of Barnes et al. (1995) dealing with Norway spruce.

Acknowledgements

This work belongs to a subproject of the SILMU research programme and financed by the Academy of Finland. Special thanks are due to Mr Alpo Hassinen, Mr Matti Lemettinen, Mr Timo Oksanen and Mrs Mirja Korhonen for technical assistance.

References

Anttonen, S., Herranen, J., Peura, P. & Kärenlampi, L. 1995. Fatty acid and ultrastructure of ozone-exposed Aleppo pine (Pinus halepensis Mill.) needles. Environmental Pollution 87: 235–242.

Barnes, J.D., Eamus, D. & Brown, K.A. 1990. The influence of ozone, acid mist and soil nutrient status on Norway spruce [Picea abies (L.) Karst.]. II Photosynthesis, dark respiration and soluble carbohydrates of trees during late autumn. New Phytologist 115: 149–156.

— , Pfirrmann, T., Steiner, K., Lütz, C., Busch, U., Küchenhoff, H. & Payer, H.-D. 1995. Effects of elevated CO_2, elevated O_3 and potassium deficiency on Norway spruce [Picea abies (L.) Karst.]: seasonal changes in photosynthesis and non-structural carbohydrate content. Plant, Cell and Environment 18: 1345–1357.

Bazzaz, F.A. 1990. The response of natural ecosystems to rising global CO_2 levels. Ann. Rev. Ecol. Syst. 21: 167–196.

Bunce, J.A. 1992. Stomatal conductance, photosynthesis and respiration of temperate deciduous tree seedlings grown outdoors at an elevated concentration of carbon dioxide. Plant, Cell and Environment 15: 541–549.

— 1995. The effect of carbon dioxide concentration on respiration of growing and mature soybean leaves. Plant, Cell and Environment 18: 575–581.

Cajander, A.K. 1949. Forest types and their significance. Acta Forestalia Fennica 56. 69 p.

Carmi, A. & Shomer, I. 1979. Starch accumulation and photosynthetic activity in primary leaves of bean (Phaseolus vulgaris L.). Annals of Botany 44: 479–484.

Ceulemans, R. & Mousseau, M. 1994. Tansley review No.71. Effects of elevated atmospheric CO_2 on woody plants. New Phytologist 127: 425–446.

Clark, C.S., Weber, J.A., Lee, E.H. & Hogsett, W.E. 1995. Accentuation of gas exchange gradients in flushes of ponderosa pine exposed to ozone. Tree Physiology 15(3): 181–190.

Dickinson, R.E. 1986. Impact of human activities on climate-framework. In: Clark, W.C. & Munn, R.E.(eds.). Sustainable development of the biosphere. IIASA,Laxenburg.

Flagler, R.B., Lock, J.E. & Elsik, C.G. 1994. Leaf-level and whole-plant gas exchange charasteristics of shortleaf pine exposed to ozone and simulated acid rain. Tree Physiology 14: 361–374.

Hollinger, D.Y. 1987. Gas exchange and dry matter allocation responses to elevation of atmospheric CO_2 concentration in seedlings of three tree species. Tree Physiology 3: 193–202.

Holopainen, T., Anttonen, S., Palomäki, V., Kainulainen, P. & Holopainen, J.K. 1996. Needle ultrastructure and starch concentration in Scots pine and Norway spruce after ozone fumigation. Canadian Journal of Botany 74: 67–76.

Laurila, T. & Lättilä, H. 1994. Surface ozone exposures measured in Finland. Atmospheric Environment 28: 103–114

Luethy-Krause, B. & Landolt, W. 1990. Effects of ozone on starch accumulation in Norway spruce (Picea abies). Trees 4: 107–110.

Pell, E.J., Eckardt, N. & Enyedi, A.J. 1992. Timing of ozone stress and resulting status of ribulose biphosphate carboxylase/oxygenase and associated net photosynthesis. New Phytologist 120: 397–405.

Rantanen, L., Palomäki, V. & Holopainen, T. 1994. Interactions between O_3 exposure and nutrient status of trees: effects on nutrient content and uptake, growth, mycorrhiza and needle ultrastructure. New Phytologist 128: 679–687.

Robertson, E.J. & Leech, R.M. 1995. Significant changes in cell and chloroplast development in young wheat leaves (Triticum aestivum cv Hereward) grown in elevated CO_2. Plant Physiology 107: 63–71.

Runeckles, V.C. & Krupa, S.V. 1994. The impact of UV-radiation and ozone on terrestrial vegetation. Environmental Pollution 83: 191–213.

Soikkeli, S. 1980. Ultrastructure of the mesophyll in Scots pine and Norway spruce: seasonal variation and molarity of the fixative buffer. Protoplasma 103: 241–252.

Sutinen, S., Skärby, L., Wallin, G. & Sellden, G. 1990. Long-term exposure of Norway spruce, Picea abies (L.) Karst., to ozone in open-top chambers. New Phytologist 115: 345–355.

Teskey, R.O. 1995. 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 and Environment 18: 565–573.

Thornley, J.H.M. & Johnson, I.R. 1990. Plant and crop modelling. A mathematical approach to plant and crop physiology. Clarendon Press, Oxford. 669 p.

Tissue, D.T., Thomas, R.B. & Strain B.R. 1993. Long-term effects of elevated CO_2 and nutrients on photosynthesis and rubisco in loblolly pine seedlings. Plant, Cell and Environment 16: 859–865.

Wallin, G. Skärby, L. & Selldén, G. 1990. Long-term exposure of Norway spruce, Picea abies (L.) Karst., to ozone in open-top chambers. I. Effects on the capacity of net photosynthesis, dark respiration and leaf conductance of shoots of different ages. New Phytologist 115: 335–344.

Wang, K. Kellomäki, S. & Laitinen, K. 1995. Effects of needle age, long-term temperature and CO_2 treatments on the photosynthesis of Scots pine. Tree Physiology 15: 211–218.

Weber, J.A., Clark, C.S. & Hogsett, W.E. 1993. Analysis of the relationships among O_3 uptake, conductance, and photosynthesis in needles of Pinus ponderosa. Tree Physiology 13: 157–172.

Total of 28 references