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Chapter 10

Ozone affects Scots pine phenology and growth

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Abstract

An open-top chamber (OTC) fumigation experiment with 10- to 15-year-old Scots pines (*Pinus sylvestris* L.) was started in autumn 1997 at the University of Oulu (65°N, 25°E). There were six non-filtered air (NF) and six open-field (AA) control pines. The six NF + O_3 pines were exposed from summer 1998 onwards to target ozone (O_3) concentrations of ambient air +40 ppb in May, ambient air +30 ppb in June, ambient air +20 ppb in July, ambient air +10 ppb in August and ambient air in September. The accumulated O_3 exposure over a threshold of 40 ppb (AOT40) in the NF + O_3 OTCs was 12.9 ppmh in the summer of 1998, but only 1.1 and 1.8 ppmh in the summers of 1999 and 2000, respectively, because fumigation started late. The respiration of previous-year needles was increased by exposure to O_3; and they also showed a decreasing trend in net photosynthesis and an increasing trend in the internal CO_2 concentration with increasing O_3 exposure in the summer of 1998. The results on needle carbon (C) contents suggested O_3-related changes in C allocation, and the chlorophyll a + b/carotenoid ratio in the current-year needles of the NF + O_3 pines was also lower than that in the current-year needles of the NF controls in November 1999. The slightly elevated O_3 concentrations caused clear physiological responses in Scots pine needles, which may, over a longer period, result growth reductions, as was suggested by the non-significant changes in the current-year shoot (18%

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increase in main shoots vs. 19% decrease in branches) and needle (15% and 10% decrease in main shoots and branches, respectively) growth of the NF + O_3 trees recorded in late July 2000. It seems that peak O_3 episodes during early summer are harmful to subarctic Scots pines.

1. Introduction

The critical level concept based on the accumulated ozone (O_3) exposure over a threshold of 40 ppb (AOT40) has been developed in Europe to protect vegetation from O_3 damage. In the Level I approach, a critical O_3 level of 10 ppmh has been proposed for European forests, on the basis of a few dose-response studies (Kärenlampi and Skärby, 1996). The major uncertainties in defining the critical level values for O_3 relate to the choice of response parameters and species (Fuhrer et al., 1997). There is also need for a longer term perspective. Trees are long-lived organisms, and it may take years for impacts to become apparent (Fuhrer and Achermann, 1999).

Among conifers, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.) are the most important species in Scandinavia, both ecologically and economically. The O_3 sensitivity of Scots pine has been studied much less than that of Norway spruce. Recent O_3 studies on Scots pine seedlings include open-top chamber (OTC) experiments by Broadmeadow and Jackson (2000), Landolt et al. (2000) and Utriainen et al. (2000), for example. In the Liphook Forest Fumigation Experiment, seedlings were exposed under field conditions (McLeod and Skeffington, 1995), as was also done by Utriainen and Holopainen (1999). Kellomäki and Wang (1997, 1998) studied the O_3 response of 30-year-old Scots pines using OTCs, whereas Skärby et al. (1987) exposed current-year shoots of 20-year-old Scots pines *in situ*. In the present study, 10-to-15-year-old Scots pines were exposed during the growing seasons 1998, 1999, and 2000 to elevated O_3 levels in OTCs in northern Finland. Their responses were measured by gas exchange, growth, morphology, injury, pigment, and nutrient parameters during the summer and autumn to detect both direct and delayed O_3 effects. The hypothesis was that ambient or slightly elevated O_3 concentrations affect local subarctic Scots pines. One of the special aims of the project was to assess the role of high O_3 concentrations in late spring and early summer as a factor modifying the response of Scots pine to O_3.

2. Materials and methods

2.1. The experiment

The Scots pine (*Pinus sylvestris* L.) used in this study were 10 to 15 years old when they were moved from Kempele, near the city of Oulu, to the University of Oulu's experimental field (65°N, 25°E) in September 1997. The trees were balled transplants of seed origin. Twelve of the trees were planted directly in the OTCs (one tree/OTC) and the rest in an open field. Six of the trees, that had overwintered in the open field, were planted in open-field control plots in May and June 1998. Four of the six trees that had been planted in the control OTCs in September 1997 were also replaced by new trees in May–June 1998, as they seemed to have suffered some winter damage. In summary, there were six pines in the OTCs supplied with non-filtered ambient air + supplemental ozone (NF + O_3), six pines in the non-filtered control OTCs (NF), and six pines in the open-field control plots (AA). The soil around the pine roots was a mixture of humus and sand (3 : 1) from a dryish heath forest. The trees were watered from a nearby lake, but not fertilized.

A detailed description of the experimental system is given by Hirvijärvi et al. (1993). Ozone was produced from pure oxygen by an O_3 generator (Fischer Mod. 502). In the summer of 1998, the O_3 concentrations were measured with a Monitor Labs O_3 analyzer (model 8810). In the summer of 1999, an API 400 O_3 analyzer (No. 066) was used because there were problems with the old analyzer. In the summer of 2000, we hired a Dasibi Environmental Corporation O_3 analyzer (model 1008-RS). Furthermore, the walls of the OTCs were replaced by new polycarbonate ones in June 1999. Subsequently, the average values of photosynthetically active radiation (PAR) in the OTCs were only 15% lower compared with conditions in the open field. In the summer of 1998, they were 28% lower on average.

The NF + O_3 pines were exposed to ambient air +40 ppb O_3 in May 1998, after which the O_3 exposure was decreased by 10 ppb each month to mimic the natural variation in O_3 concentrations during the growing season in northern areas. As a result, the pines were exposed to only ambient air in September 1998. In the summers of 1999 and 2000, fumigation did not start until 23 June and 7 June, respectively, and the NF + O_3 pines were exposed to the same target O_3 concentrations as in the summer of 1998, i.e., ambient air +30 ppb in June, ambient air +20 ppb in July, ambient air +10 ppb in August and ambient air in September. Fumigation was carried out between 08.00 and 16.00 hours for 5 days a week. This episodic approach was chosen to mimic natural O_3 occurrence under northern conditions. A cumulative O_3 exposure index AOT40 was calculated as a sum of the hourly O_3 concentrations above the cut off of 40 ppb. The average AOT40 amounted to 12.9 ppmh (calculated for 24 hours/day) in the NF + O_3 OTCs from May–September 1998, whereas between late June and the end of September 1999, it only reached 1.1 ppmh and, between early June and the end of July 2000, 1.8 ppmh (Table 1).

Table 1. AOT40s (ppbh) for the treatments in May–September 1998, June–September 1999, and June and July 2000 on a 24-hour basis. The 1-hour minimum and maximum O_3 concentrations are given in parentheses

		Treatment		
Year	Month	NF	NF + O_3	AA
1998	May[a]	56 (1–50)	7890 (1–154)	95 (1–47)
	June	0 (0–41)	3706 (0–113)	2 (0–42)
	July	4 (0–50)	1266 (0–97)	1 (0–42)
	August	2 (0–42)	68 (1–112)	0 (0–30)
	September[b]	7 (0–44)	7 (0–44)	7 (0–44)
1999	June[a]	0 (0–41)	526 (0–72)	2 (0–42)
	July	0 (0–39)	553 (0–110)	0 (0–39)
	August	0 (0–41)	40 (0–52)	0 (0–40)
	September[b]	0 (1–38)	0 (1–38)	0 (1–38)
2000	June[a]	0 (0–34)	1244 (0–83)	0 (0–31)
	July	0 (0–30)	584 (0–71)	0 (0–35)
Total	1998	69	12937	105
	1999	0	1119	2
	2000	0	1828	0

[a] Fumigation was started on 4 May 1998, 23 June 1999, and 7 June 2000.
[b] Ozone concentrations measured in the open field.

2.2. *Growth recording*

Budburst, current-year needle growth, and current-year shoot growth were recorded from the end of May or the beginning of June onwards, each summer. Observations were made twice weekly on the main shoot and one of the branches on the 1997 whorl. Five randomly chosen needles were measured for length each time.

2.3. *Gas exchange measurements*

Measurements of net photosynthesis (P_n), respiration (R), stomatal conductance for water vapor (g_{H_2O}), and internal CO_2 concentration (c_i) were carried out five times between 16 July and 20 September 1998. Gas exchange was not measured in 1999 and 2000. Three trees were chosen from each treatment, and from each tree, a branch was chosen from the 1996 whorl. The gas exchange of current- (c) and previous-year ($c+1$) shoots was measured using a portable photosynthesis system (ADC gas analyzer model LCA 2 and ADC PLC(N) cuvette). Photosynthetically active radiation (PAR) was measured with a Li-Cor meter equipped with a SPQA 2260 sensor. Five needles from each age class on each branch were measured for length to calculate total needle surface area ($A_t = 4.2235 \times \text{length} - 15.6835$) (Flower-Ellis and Olsson, 1993).

2.4. Pigment and glutathione reductase analyses

Current-year and $c + 1$ needles for chlorophyll (chl) a and b and carotenoid (car) analyses were collected on 15 November 1999. The needles were taken from a 1997 whorl and stored in a freezer ($-72\,^{\circ}$C) until analysis. The pigments were extracted with DMSO according to Hiscox and Israelstam (1979) and the absorbances were measured at 470, 646, and 663 nm with a Beckman DU® -64 spectrophotometer. The pigment concentrations were calculated according to Wellburn (1994).

Glutathione reductase (GR) activity was determined from c and/or $c + 1$ needles collected on 11 August and 8 September 1998 by a modification of the method of Polle et al. (1990). A Beckman DU® -64 spectrophotometer was used to measure the decrease in absorbance at 340 nm, and the activity was calculated using an extinction coefficient of 6.22 $\text{mM}^{-1}\,\text{cm}^{-1}$ for NADPH.

2.5. Microscopic studies

One millimeter sections were removed from healthy looking needles (three needles/tree) from the 1997 whorl on 1 September 1998 for morphological observations. The pieces were fixed, dehydrated, and embedded according to Soikkeli (1980) and Reinikainen and Huttunen (1989). The samples were stained with toluidine blue and examined under a Nikon Optiphot-2 light microscope connected to a digital image analyzer (Microscale TM/TC, Digithurst Ltd.) by a video camera (Hitachi CCD KP-C571). The following variables were measured: needle width (vertical thickness) and thickness (horizontal thickness), total cross-sectional area, epidermal and hypodermal area, and mesophyll area. The damage in mesophyll cells was classified according to Soikkeli (1981). The samples (two or three needles/tree) for transmission electron microscopic (TEM) studies were stained with lead citrate and uranyl acetate. Because one of the earliest O_3 symptoms observed in plants is the deformation and shrinking of chloroplasts, the chloroplast ultrastructure, i.e., chloroplast size, number of plastoglobuli and swelling of thylakoids, was recorded. Thylakoid swelling was assessed according to the following classes: $0 =$ not swollen, $1 =$ slightly swollen, $2 =$ somewhat swollen, and $3 =$ markedly swollen.

2.6. Elemental analyses

The samples for elemental analyses were collected by taking c and $c + 1$ needles from several branches on the 1996 and 1997 whorls on 18 November 1999. Total foliar concentration of sulfur (S), phosphorus (P), magnesium (Mg), potassium (K), and calcium (Ca) was analyzed using a SRS 303 As X-ray fluorescence spectrometer with an Rh anode; nitrogen (N) and carbon (C) concentrations were analyzed using CE Instrument's EA 1110 CHNS-O Elemental Analyzer supplied with Eager 200 for Windows™ (Manninen and Huttunen, 2000).

2.7. *Statistical analyses*

Differences between treatments were assessed using ANOVA, Fischer's PLSD (Protected Least Significant Difference) as a *post hoc* test, Kruskal–Wallis test and Mann–Whitney test (StatView 4.1, Abacus Concepts Inc.). The data were tested for normality. No data transformation was carried out. The tree (i.e., OTC chamber and AA plot) means were calculated for each parameter before testing; they numbered $n = 6$ for each treatment, except in the case of the gas exchange measurements, where $n = 3$.

3. Results

3.1. *Photosynthesis, respiration, and stomatal responses*

The gas exchange measurements did not show any marked O_3 effects on current-year (c) needles due to the large variation between individual trees within the treatments, although the NF + O_3 pines had lower average net photosynthesis (P_n) and higher average respiration (R) than the NF ones, especially in mid-August 1998 (Fig. 1).

Previous-year ($c + 1$) needles showed an increasing trend in the P_n of the NF pines and a decreasing trend in that of the NF + O_3 pines (Fig. 2). The NF + O_3 pines had higher R than the NF pines. The difference in R between the NF + O_3 and NF trees was statistically significant ($Z = -1.964$, $p = 0.0495$) in mid-August 1998. The internal CO_2 concentration (c_i) of the NF + O_3 pines seemed to increase with decreasing P_n, whereas no trends were seen in stomatal conductance (g_{H_2O}). The AA pines always had the highest g_{H_2O}, however.

3.2. *Growth*

In late July 2000, the current-year main shoots of the NF + O_3 pines were, on average 18% longer than those of the NF pines ($Z = -1.281$, $p = 0.2002$), whereas the average current-year growth of branches in the NF + O_3 pines was 19% less than that in the NF pines ($Z = -1.922$, $p = 0.0547$) (Fig. 3). The c needles of the NF + O_3 pines were also shorter than those of the NF

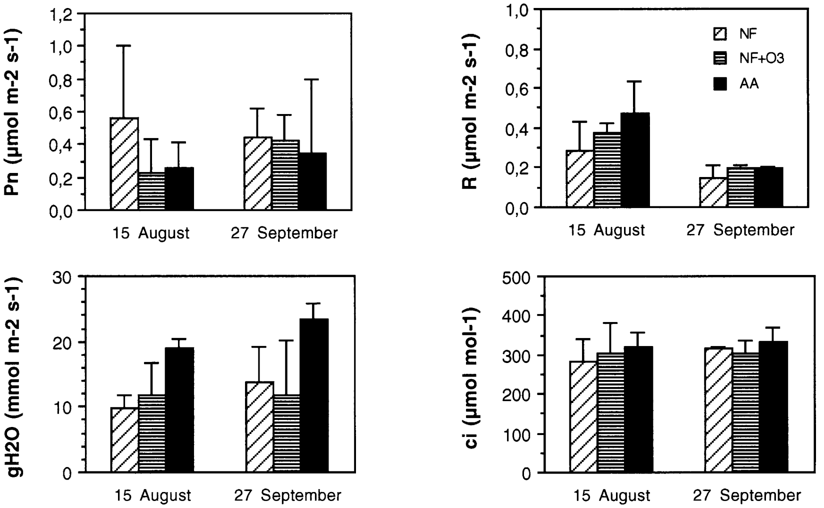

Figure 1. Net photosynthesis (P_n, µmol m^{-2} s^{-1}), respiration (R, µmol m^{-2} s^{-1}), and stomatal conductance for water vapor (g_{H_2O}, mmol m^{-2} s^{-1}) per total needle area and internal CO_2 concentration (c_i, µmol mol^{-1}) of current-year (c) needles in August and September 1998. Values are means ±SD, $n = 3$.

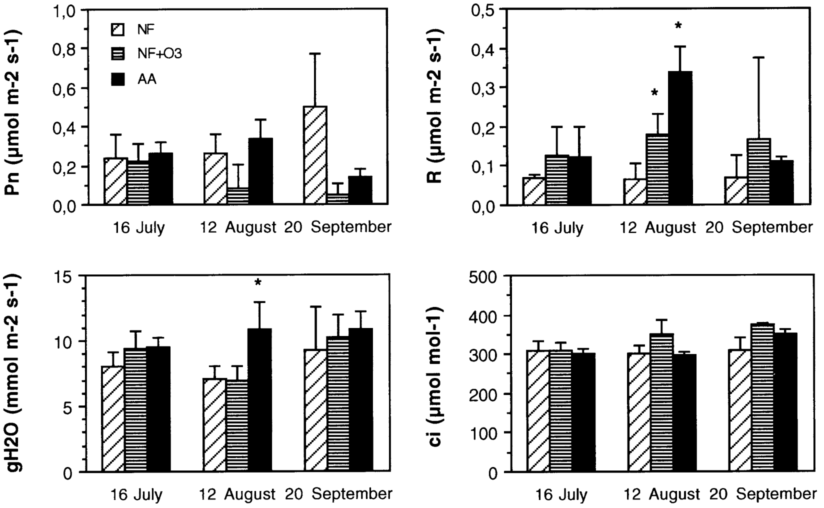

Figure 2. Net photosynthesis (P_n, µmol m^{-2} s^{-1}), respiration (R, µmol m^{-2} s^{-1}), and stomatal conductance for water vapor (g_{H_2O}, mmol m^{-2} s^{-1}) per total needle area and internal CO_2 concentration (c_i, µmol mol^{-1}) of previous-year ($c+1$) needles in July, August, and September 1998. Asterisks indicate statistically significant differences (Mann–Whitney U, $p < 0.05$) between the NF pines and the pines with the other treatments. Values are means ±SD, $n = 3$.

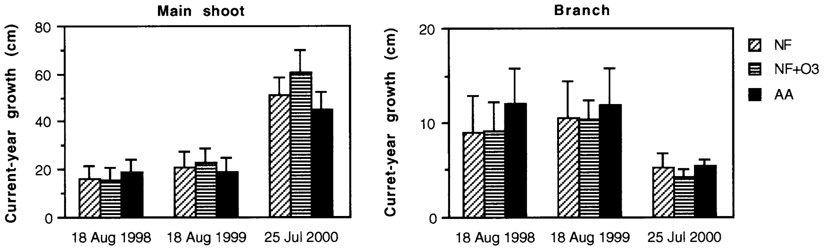

Figure 3. Current-year main shoot and branch growth in the summers of 1998–2000. Values are means ±SD, $n = 6$.

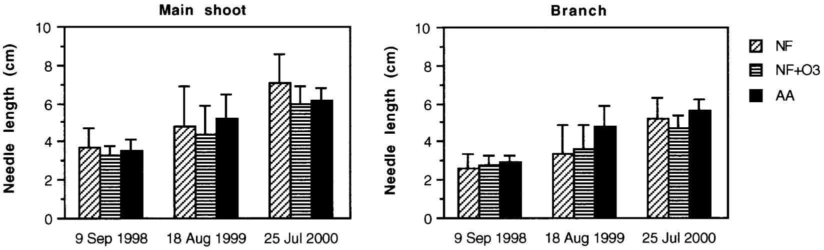

Figure 4. Current-year needle length of main shoots and branches in the summers of 1998–2000. Values are means ±SD, $n = 6$.

pines, but these differences (15% in main shoots and 10% in branches) were not statistically significant either (Fig. 4).

3.3. Microscopic studies

The microscopic studies showed no statistically significant differences in the measured parameters (Table 2). However, epidermal and hypodermal area in the c needles of the NF + O_3 pines was smaller than that in the *c* needles of the NF pines in the summer of 1998. There was no visible O_3 damage in the needles, but more injured mesophyll cells were observed in the *c* needles of the NF + O_3 pines than in those of the NF pines (data not shown). Under TEM, the NF + O_3 pines had the highest percentage of both healthy (class 0) and markedly swollen (class 3) thylakoids.

3.4. Pigment concentrations and glutathione reductase activity

The results suggested an O_3-induced decrease in the (chlorophyll a + b)/carotenoid ratio ((chl a + b)/car) of the *c* needles of the NF + O_3 pines ($p = 0.0547$) in November 1999 as a result of a decrease in the chl a + b concentration and an increase in the car concentration (Fig. 5). The difference in GR activ-

Table 2. Morphology of current-year needles in September 1998[a]

Variable	Treatment		
	NF	NF + O_3	AA
Width (mm)	1.45 ± 0.20	1.45 ± 0.19	1.43 ± 0.08
Thickness (mm)	0.62 ± 0.05	0.63 ± 0.06	0.61 ± 0.05
Cross-sectional area (mm^2)	0.78 ± 0.18	0.76 ± 0.17	0.75 ± 0.12
Epiderm + hypoderm area (mm^2)	0.096 ± 0.016	0.089 ± 0.012	0.090 ± 0.013
Mesophyll area (mm^2)	0.48 ± 0.11	0.47 ± 0.11	0.47 ± 0.08

[a] Treatment means ±SD, $n = 6$.

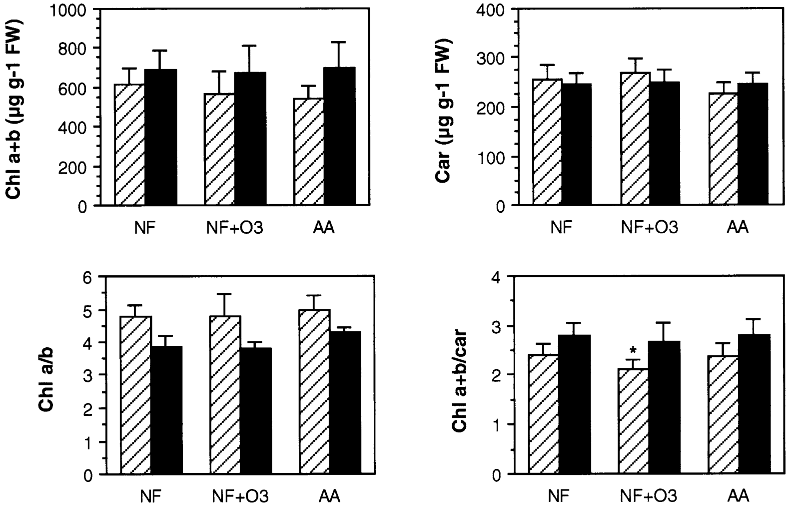

Figure 5. Chlorophyll a + b and carotenoid concentrations and ratios of chl a/b and (chl a + b)/car in current (*c*) and previous-year (*c* + 1) needles in November 1999. Hatched bars = *c* needles, black bars = *c* + 1 needles. The asterisk indicates a statistically significant difference ($p < 0.05$) between the NF + O_3 and NF pines. Values are means ±SD, $n = 6$.

ity between treatments was not statistically significant, although in the *c* and *c* + 1 needles of the NF + O_3 pines showed slightly lower average GR activity compared with the *c* and *c* + 1 needles of the NF pines (Table 3).

3.5. Carbon and nutrient concentrations

There was an O_3 effect on the needle C content, as shown by the higher C content in the *c* + 1 needles of the NF + O_3 pines than in those of either the NF

Table 3. Glutathione reductase activity (nkat g^{-1} FW) of current (c) and previous-year ($c+1$) needles in August and September 1998[a]

		Treatment		
Month	Needle age class	NF	NF + O_3	AA
August	$c+1$	5.6 ± 1.8	6.0 ± 2.8	5.4 ± 0.8
September	c	12.6 ± 3.0	11.0 ± 5.1	8.5 ± 3.2
	$c+1$	11.2 ± 4.5	10.1 ± 2.2	10.5 ± 3.3

[a] Treatment means ±SD, $n = 6$.

pines or the AA controls Table 4. All the elemental concentrations (except the Ca concentration) depended on needle age: c needles had higher N, S, P, Mg, and K concentrations than $c+1$ needles, which in turn had a higher C content than the c needles. The interaction between treatment and needle age in the case of the needle S concentrations could be explained by the differences in the foliar S concentrations of the c vs. $c+1$ needles between the OTC and AA pines.

4. Discussion

Broadmeadow and Jackson (2000) exposed seedlings of oak (*Quercus petraea* L.), ash (*Fraxinus excelsior* L.), and Scots pine to 20–80 ppb O_3 (annual 24 h AOT40s 47.8–74.1 ppmh) in a 3-year experiment. Oak was the most responsive species, with a 30% reduction in growth followed by a 15% reduction in Scots pine. Ozone had no detectable effect on ash. Chlorophyll degradation in response to O_3 was only observed in oak and it correlated with stomatal conductance (Broadmeadow et al., 1999). Landolt et al. (2000), in turn, studied the O_3 response of seedlings during one growing season (50% ambient +30 ppb; daylight hour AOT40 19.7 ppmh, 24 h AOT40 29.3 ppmh) and calculated a biomass loss of 25.5% for ash, 17.4% for beech (*Fagus sylvatica* L.), 9.9% for Scots pine, and 5.6% for Norway spruce per AOT40 increment of 10 ppmh. Utriainen and Holopainen (1999) and Utriainen et al. (2000) exposed 3-year-old Scots pine to 1.3–1.5 × ambient O_3 for three growing seasons in open-field (annual 24 h AOT40s 15.4–38.0 ppmh) and to 1.5 × ambient O_3 for two growing seasons in OTCs (growing season 24 h AOT40s 33.3 and 39.9 ppmh). Ozone had a growth-depressing effect on the current-year main shoot length after the third year (19% under elevated O_3 during the growing season and 41% under elevated O_3 during the springtime and growing season) (Utriainen and Holopainen, 1999). Slight O_3-induced yellowing and/or chlorotic mottling was observed in the $c+1$ needles in the OTC experiment (Utriainen et al., 2000). In the Liphook Project, which lasted for nearly 4 years, no major effects of O_3

Table 4. Carbon and nutrient concentrations in the c and c + 1 needles of Scots pine in November 1999[a]

Treatment	Needle age	C (%)	N (%)	S ($\mu g\, g^{-1}$)	P ($\mu g\, g^{-1}$)	Mg ($\mu g\, g^{-1}$)	K ($\mu g\, g^{-1}$)	Ca ($\mu g\, g^{-1}$)
NF	c	49.9 ± 0.4	1.39 ± 0.12	1303 ± 71	1615 ± 130	1866 ± 299	6268 ± 777	2729 ± 336
	c + 1	51.4 ± 0.4	1.25 ± 0.18	1142 ± 100	1338 ± 47	1296 ± 210	5263 ± 399	2279 ± 748
NF + O_3	c	50.3 ± 0.4	1.46 ± 0.17	1318 ± 103	1573 ± 91	1791 ± 352	5745 ± 690	2775 ± 428
	c + 1	51.9 ± 0.4	1.23 ± 0.13	1076 ± 106	1293 ± 52	1167 ± 180	4877 ± 429	2122 ± 437
AA	c	50.1 ± 0.5	1.28 ± 0.11	1205 ± 99	1587 ± 49	1571 ± 223	5743 ± 433	2606 ± 439
	c + 1	51.2 ± 0.4	1.25 ± 0.11	1198 ± 103	1456 ± 113	1286 ± 316	5208 ± 637	2601 ± 771
Treatment		*	ns	ns	ns	ns	ns	ns
Needle age		***	***	***	***	***	***	ns
Treatment × needle age		ns	ns	*	ns	ns	ns	ns

[a] Treatment means ± SD.

Significances of ANOVA ($n = 6$) for the of treatment effects and needle age, or the interaction between them, on the element concentrations are given as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = non-significant.

were observed on Scots pine, Norway spruce, or Sitka spruce (*Picea sitchensis* [Bong.] Carr.) at average AOTs of 28.8–30.7 ppmh (McLeod and Skeffington, 1995).

Scots pine may be considered moderately sensitive to O_3 based on the seedling studies cited above. The marked variation in the growth parameters between individual young trees in late July 2000 makes it difficult to demonstrate any systematic O_3 response. Furthermore, the increasing trends in needle and main shoot length from the summer of 1998 to the summer of 2000 may only reflect the rooting and adaptation of the trees to the experimental site. If so, then the growth results from the 1998 vs. 2000 summers may suggest that high O_3 levels have little effect on Scots pines when the trees have root damage or soil water availability is limited due to climatic factors (McLaughlin and Downing, 1995), whereas under normal conditions Scots pine is sensitive to O_3. Actually, marked differences might have been found merely by increasing the number of needles studied. The results of Laakso (2001), based on ten needles/tree rather than five needles/tree, as in this study, showed a statistically significant decrease in the length of *c* needles attached to the branches of our $NF + O_3$ pines in the summer of 2000 ($p = 0.037$). Given the formidable costs of free-air and mature-tree fumigation and the number of replications needed to detect small biological changes over short-term experimentation, Samuelson and Kelly (2001) recommend that research on cause-effect relationships in forest trees should apply the rigorous statistical and monitoring protocol developed by Schreuder and Thomas (1991).

At any rate, the reduced growth of *c* needles may be attributed to the decreased net photosynthesis and increased respiration under elevated O_3. Kellomäki and Wang (1997, 1998) exposed naturally grown 30-year-old Scots pines to doubled ambient O_3 in OTCs. In the third year of O_3 treatment, the doubled ambient O_3 (69 ppb; average daylight hour AOT40 19.2 ppmh) significantly reduced the photosynthetic rate, the specific growth rate of needles undergoing early expansion, and the needle N concentration in the late stage, but increased the apparent respiration rates in the late stage. An increase in dark respiration was also found by Skärby et al. (1987), who exposed current-year shoots of 20-year-old pines to 60–200 ppb in branch chambers for 1 month. The TREGRO simulations by Constable and Retzlaff (1997) showed that, regardless of O_3 exposure and peak O_3 episode occurrence, a peak O_3 episode in August caused the greatest reduction in C gain in yellow poplar, whereas a peak O_3 episode in July caused the greatest reduction in the C gain of loblolly pine. In other words, maximum O_3 response was observed when the peak O_3 episode occurred at or near the completion of the annual foliage production phenophase.

Age-dependent differences in O_3 uptake, anatomy, and detoxification as well as injury repair appear to be of paramount importance (Matyssek and Innes, 1999). Older needles of Norway spruce have been found to react more negatively to O_3 stress than young needles (Skärby et al., 1995), as was also suggested by the present results. The average net photosynthetic rate of the $c + 1$ needles in the NF + O_3 pines was only 10% that of the $c + 1$ needles in the NF pines in September 1998, and their average respiration rate was 2.3-fold compared to the NF control. This means that the O_3 effect is small in young trees and becomes greater in old trees, mainly because of the different proportions of the needle age classes (Skärby et al., 1995). It also means that a loss of 2- to 3-year-old needles in Scots pine influences the remaining crown more significantly than a loss of 7- to 10-year-old needles in Norway spruce (Langebartels et al., 1997).

Glutathione reductase activity did not suggest any changes in the antioxidative status of the NF + O_3 pines in September 1998. According to Foyer et al. (1994), glutathione, ascorbate, and superoxide dismutase defenses are often not responsive until visible injury occurs. There were no visible foliar O_3 injuries in the NF + O_3 pines. On the other hand, the reduced chl a + b concentration and the increased car concentration, i.e., the reduced $(\mathrm{chl\,a} + \mathrm{b})/\mathrm{car}$ ratio, in c needles of the NF + O_3 pines in November 1999 compared with pigment concentrations and ratio in c needles of the NF pines point to O_3-related oxidative stress. Carotenoids provide one line of defence against oxidative stress (Young and Britton, 1990; Polle and Rennenberg, 1994). The lower $(\mathrm{chl\,a} + \mathrm{b})/\mathrm{car}$ ratio in c needles of the NF + O_3 pines than in those of the NF pines in November 1999 may be considered a memory effect that develops in early autumn in Scots pine (Langebartels et al., 1997). It has been suggested that a reduction in the proportion of surface structures is an acclimation reaction to elevated O_3 levels (Günthardt-Goerg et al., 1993; Pääkkönen et al., 1993, 1995). It may, however, merely indicate restricted resources as a result of increased respiration and decreased net photosynthesis.

Foliar nutrient concentrations do not explain any of the observed changes. The higher C content of $c+1$ needles of the NF + O_3 pines compared with $c+1$ needles of the NF pines in November 1999 may indicate changes in carbohydrate allocation. It seems that O_3 sensitivity is strongly affected by (genetic) variation in stomatal conductance for water vapor ($g_{\mathrm{H_2O}}$) and C allocation to fine root biomass (Constable and Taylor, 1997; Skärby et al., 1998; Samuelson and Kelly, 2001). The possible decrease in carbohydrate allocation to roots and the change in the root/shoot ratio can be verified after final harvesting (in summer 2001). The average $g_{\mathrm{H_2O}}$ of $c + 1$ needles of the NF + O_3 pines was higher than that of $c + 1$ needles of the NF pines in the summer of 1998.

Samuelson and Kelly (2001) point out that is unclear to what degree the higher O_3 uptake rates in seedlings are offset by the production of new foliage, as there is some variation in the shoot phenology of *Pinus* species between juvenile and mature trees (Clark et al., 1995). Scots pine has a determinate growth pattern, and as the O_3 concentrations decreased towards the end of the growing season, the developing needles always experienced peak O_3 episodes. This may explain the observed effects of low AOT40s in this study. This also means that elevated O_3 concentrations, especially occurring in early summer in northern areas, may have harmful effects on Scots pine.

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