

Open-top Chamber Fumigation System for Exposure of Field Grown *Pinus sylvestris* to Elevated Carbon Dioxide and Ozone Concentration

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Palomäki, V., Hassinen, A., Lemettinen, M., Oksanen, T., Helmisaari, H.-S., Holopainen, J., Kellomäki S. and Holopainen, T. 1998. Open-top chamber fumigation system for exposure of field grown *Pinus sylvestris* to elevated carbon dioxide and ozone concentration. *Silva Fennica* 32(3): 205–214.

An open-top chamber fumigation system was built in a young Scots pine stand to study the effects of realistic elevated ozone (O₃) and carbon dioxide (CO₂) concentrations and their combination on trees in natural conditions. Doubled CO₂ concentration compared to present ambient concentration, and O₃ concentration between 40 ppb and 70 ppb in the first study year (1994) and doubled O₃ concentration in years 1995 and 1996 were the target concentrations in the chambers. The O₃ concentration in the chambers was successfully maintained close to the target concentration and differences between chambers were small. The mean CO₂ concentration in the CO₂ treatment was ca. 100 ppm below the target, but was maintained at this level throughout the growing season. Two degrees higher mean air temperature and slightly lower light intensity compared to open air were measured in the chambers. The operation of the fumigation system was satisfactory during the three study years and repeatability of the gas treatments can be regarded good in this low cost exposure system.

Key words open-top chamber, O₃, CO₂, *Pinus sylvestris*

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Received 18 September 1997 **Accepted** 6 July 1998

1. Introduction

Atmospheric carbon dioxide and ozone concentrations have increased during the last decades, and they have been forecast to rise to a level of twice the present concentration by the year 2100 (Dickinson 1986, Runeckles and Krupa 1994). The increase in the carbon dioxide concentration may, at least transiently, stimulate the metabolic processes of plants leading to increased growth and production (Dahlman et al. 1985, Rogers et al. 1994). On the other hand, the effects of ozone have been mainly estimated to cause damage to vegetation (Krupa and Manning 1988, Kickert and Krupa 1990). The incorporation of ozone studies into climate change research has proved necessary, since the measured ozone concentrations in Finland (Laurila and Lättilä 1994) have been high, especially in spring and early summer, temporarily exceeding the levels that may be harmful to sensitive forest vegetation (e.g. Pääkkönen et al. 1995, Skärby and Karlsson 1996).

For studying forest plants at realistic elevated concentrations of ozone and carbon dioxide in near natural conditions we built an open-top field chamber system enclosing Scots pine saplings. This system can avoid or decline many chamber effects, which are often problematic in closed chamber systems (e.g. Lucas et al. 1987, Allen et al. 1992, Bernier et al. 1994). Conditions are more natural, because plants are growing in the natural soil and receive natural rain and light. In any case, some chamber effects like suppressed illumination, slightly higher temperature and abnormal air velocity have been measured in open-top chambers (Heagle et al. 1973, Heagle et al. 1979, Hirvijärvi et al. 1993, Janous et al. 1996). However, an open field fumigation system, which avoids these problems (Wulff et al. 1992, Lewin et al. 1994), could not be used in this forest stand experiment, due to high amount and cost of carbon dioxide needed in such systems.

In this study naturally growing Scots pine saplings were exposed during three whole growing seasons (1994–1996) to elevated or filtered ozone and elevated carbon dioxide concentrations that were aimed to follow the natural exposure pattern. Design of open-top chamber and computer-controlled method for dispensing and monitor-

ing gases in chambers is described in the present article. Results on ozone and carbon dioxide concentrations, air velocity, light intensity and temperature and comparisons between separate chambers are also reported and discussed.

2. Materials and Methods

2.1 Study Area and Field Chambers

The open-top field fumigation system was built in a pine stand at Mekrijärvi in eastern Finland (62°47'N, 30°58'E). The study stand is situated on a dry heath, where naturally reproduced 15–30 year-old Scots pine (*Pinus sylvestris*) saplings are growing with a density of about 2500 stems per hectare. The forest type of the stand is *Vaccinium* type and the soil is a sandy moraine type, which has not been fertilized during the growing period of the experimental pines. Chambers were situated randomly at the experimental stand area of about 1 hectare (Fig. 1). The height of the saplings chosen in the experiment varied between 2.5 m and 3.4 m (mean 3.0 in 1993), the age ranged between 14 and 24 years (mean 19 years), and the mean stem diameter at 1.3 m above ground level was 5 cm.

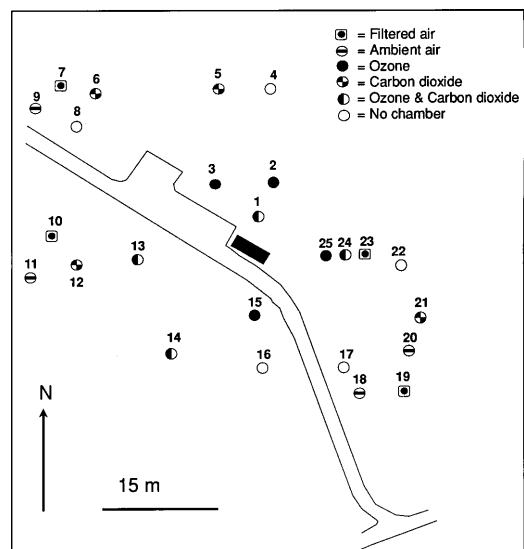


Fig. 1. A map of the experimental area.



Fig. 2. A photograph of the chamberless control (a) and the fumigation chamber (b).

Four replicate chambers for each treatment [ambient air, filtered air, elevated ozone (O_3), elevated carbon dioxide (CO_2) and elevated ozone and carbon dioxide combined] were built, each surrounding one sapling (Fig. 2). The chambers were rectangular (base 1.7×1.7 m, height 3.5 m, volume 10.1 m^3) built with a wood frame and a transparent plastic sheet used in greenhouses. The trees in the chambers were not additionally watered since most part of the root systems remained undisturbed and could receive water from the natural rainfall inside and around the chambers. At the bottom of the chamber a circular perforated (50 holes, diameter 20 mm) LD polyethylene tube (diameter 194 mm) was placed for dispersing air and studied gases. Air was blown through this tube into the chamber using a blower (160 XL, $1.9 \text{ m}^3/\text{s}$) adjusted to replace the total air volume of the chamber once per minute.

The saplings were exposed to studied gases between 1 June and 30 September 1994, be-

tween 15 May and 30 September 1995 and between 20 May and 18 September 1996. The time of exposure was 16 h per day (from 6.00 to 22.00).

2.2 Dispensing and Monitoring Systems

2.2.1 Ozone

Ozone was produced from pure oxygen with an ozone generator (Fischer 500, 5 g/h), diluted with clean air and distributed via polyamide pipes (6/4 mm) to the eight O_3 fumigation chambers (Fig. 3). The O_3 dispensing system was computer-controlled by comparing the measured O_3 concentration to the chosen target concentration and adjusting the O_3 flow into the chambers with valves (Hoke 1300) steered by a stepmotor. The gas sample was sucked in turn from every O_3 fumigation chamber, one filtered chamber and open

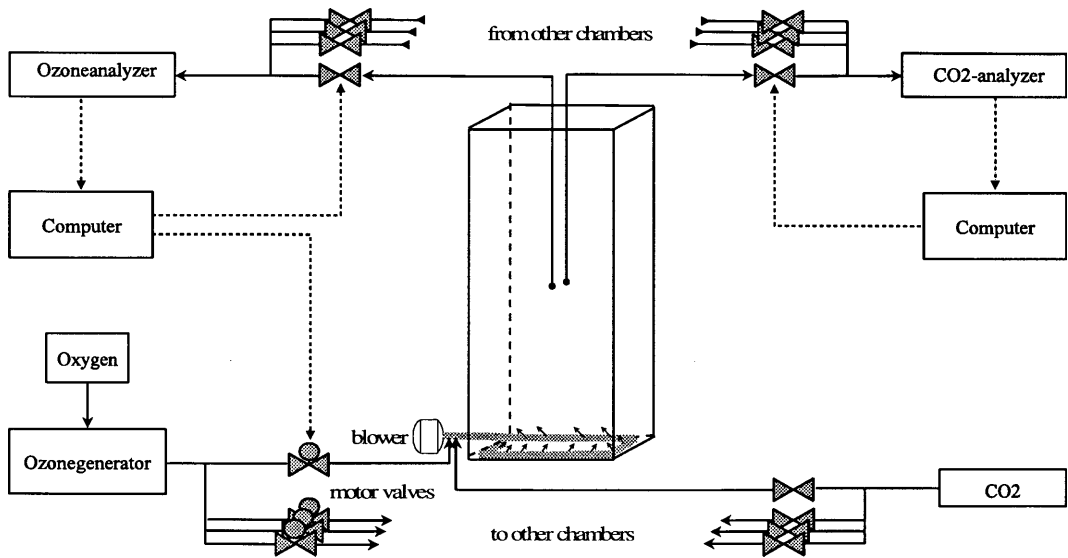


Fig. 3. A schematic picture of the structure of open-top chamber and O₃ and CO₂ dispersing and monitoring system.

air to the ozone analyser (Dasibi 1008RS, precision 0.001 ppm with a 50 s response time of 99 % of final value) via teflon pipes (6/4 mm). Gas sample continuously flowed in the sampling pipes keeping sample fresh and magnet valves (Joucomatic 3-way solenoid valve) opened in turn leading the gas sample to the analyzer. The response time of measurement of one chamber was 75 s, so one measuring cycle (9 chambers and open air concentrations) took about 13 minutes. The magnet valves were closed by the control programme when the measured O₃ concentration was higher than 120 ppb or lower than 10 ppb and during hard wind ($> 4 \text{ ms}^{-1}$) preventing the large fluctuation of the gas concentrations. In the first year of operation 1994, the O₃ concentration in the chambers was maintained at 70 ppb in June, 60 ppb in July, 50 in August and 40 in September. In the following growing seasons 1995 and 1996, the target concentration was double the ambient. The fixed O₃ sampling point was at 2 m above the base of the chamber, close to the sampling trunk. For comparison measurements were done in the heights of 1 m and 2.5 m in the beginning of the experiment. The ambient O₃ concentration was measured in a similar way close to the trunk of a sapling growing in open air.

2.2.2 Carbon Dioxide

In CO₂ treatments pure CO₂ was dispensed via polyethylene pipes (16/12 mm) to the eight CO₂ fumigation chambers (Fig. 3). The level of CO₂ concentration was maintained in the chamber by a flowmeter (Kytölä, LH-AE41-R). The gas sample was sucked via polyethylene pipes (6/8 mm) from every CO₂ fumigation chamber and distributed by magnetic valves twice per hour to the CO₂ analyzer (Siemens 7MB1300-0BA00, total error $\pm 5 \%$), which was calibrated regularly by calibration gases. The operation of the analyzer is based on absorption measurements of infrared radiation. As with O₃ the sample gas flowed all the time in the sampling pipes keeping the sample fresh. The CO₂ concentration was measured at the height of 2 m from the bottom of the chamber. The target elevated CO₂ concentration was twice the ambient CO₂ concentration. Ambient CO₂ concentration was measured at the height of 2 m close to separate tree outside the chamber.

2.3 Filtering System

In the filtering experiment, ambient air was led through a filter (Climecon PD-18-300; Purafil CP: 50 % Al_2O_3 and KMnO_4 , 50 % charcoal) before it was blown into the chamber via perforated plastic tube. Ozone concentration was continuously measured from one filtered air chamber during the whole experiment.

2.4 Air Temperature, Air Velocity and Light Measurements

Air temperature was measured once per hour at the height of 2 m using Datascan 7320 meter with thermistor coupled to a computer by RS-232 cable. Temperature was measured during years 1994 and 1995 from four experimental chambers having different light conditions and from open air. During the year 1996 temperature was measured from ten chambers and from outside the chambers (3 points).

Air velocity was measured from three heights (0.8, 1.5 and 2.5 m) in the chambers on the 13th and 14th of July 1994 using thermo-anemometer (Alnor GGA23S). Light intensity was measured at the height of 2 m, always from the same direction in the chambers on the 16th, 24th and 31st of July 1996 using quantum/radiometer/photometer (Li-Cor, LI-185B) with probe (Li-Cor, Q 3672). The weather conditions and the time of measurements were different in each measuring occasions. In addition, photon flux was continuously recorded together with the photosynthesis measurements (Kellomäki and Wang 1997).

Air temperature, air velocity and light intensity measurements were analysed with one-way analysis of variance, and individual means were compared using tukey's multiple range test (SPSS-PC statistical package).

2.5 Experiments

Two experiments were arranged in the chambers. In the first experiment, the effects of exposures on naturally growing Scots pine saplings were studied during growing seasons of 1994–

1996 (Palomäki et al. 1996). In the second experiment, three-year-old Scots pine seedlings growing in plastic pots were placed in the chambers (6 seedlings/chamber) ca. 60 cm above ground (Fig. 2) and exposed to O_3 and CO_2 alone and combination during growing seasons of 1995 and 1996.

3. Results and Discussion

3.1 Ozone Concentrations

The O_3 concentration in the fumigation chambers followed rather well the target concentration, which was at a fixed level in the year 1994, and doubled ambient O_3 concentration in years 1995 and 1996 (Fig. 4). The total day average concentration remained lower than the fixed level or doubled ambient O_3 concentration, because fumigation was stopped during the night (8 h). Few occasional high O_3 peaks, usually due to starting of fumigation in the morning or to hard wind, remaining below 120 ppb were measured. Standard deviations (SD) of O_3 concentrations measured during one hour in the fumigation chambers varied in general between 3.0 and 9.0 ppb being on an average 5.0. When high occasional concentrations were measured SD was about 20. SD in ambient air measurements varied between 0.5 and 2.5 ppb.

The O_3 concentration in the height of 1 m was ca. 10 ppb higher and in the height of 2.5 m ca. 10 ppb lower compared to the concentration measured from the fixed measurement point at the height of 2 m.

The total O_3 dose received in fumigated chambers in the growing seasons 1994, 1995 and 1996 were 1.36, 1.53 and 1.57 times the ambient O_3 concentration, respectively (Table 1). Critical doses (e.g. Ashmore and Davison 1996, Skärby and Karlsson 1996) exceeding 30 ppb and 40 ppb threshold concentration (AOT 30 and AOT 40, Table 1, Fig. 5) were clearly higher in fumigation chambers compared to ambient air. According to earlier studies the doses (AOT40) received in the present study could cause visible O_3 -injuries and decrease growth especially in birch but possibly also in conifers (Pääkkönen et

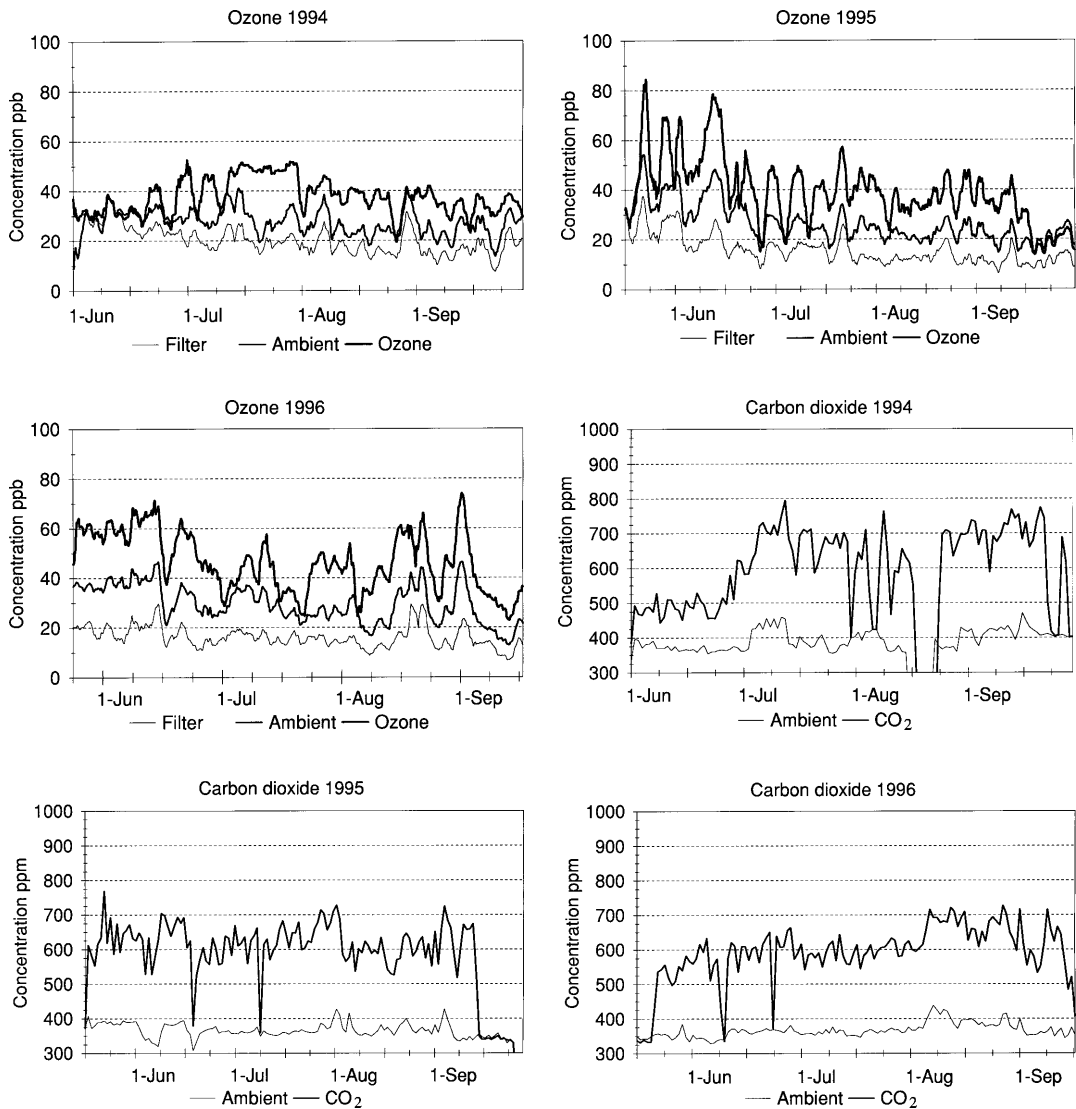


Fig. 4. Mean ozone concentrations in ozone exposure chambers, in a filtered air chamber and in open air and mean carbon dioxide concentrations (6–22) in CO₂ exposure chambers and in open air during growing periods 1994–1996.

al. 1995, Skärby and Karsson 1996).

The differences in O₃ concentration between the chambers were in general small (Fig. 5). The most remarkable difference was the high O₃ level in chamber 2 in year 1994. However, this difference was not observed during later years and therefore the repeatability of the O₃ treatments can be regarded good.

3.2 Filtering

The O₃ concentration followed the ambient air concentration in filtered chambers (Fig. 4). The total O₃ dose received in the filtered chamber was 0.73, 0.57 and 0.55 times the ambient O₃ concentration for the years 1994, 1995 and 1996, respectively (Table 1, Figs. 4–5). The dose was

Table 1. Mean ozone doses (AOT0, AOT30, AOT40; ppm-h) in ozone fumigation (n = 8) and filtered chambers and in open air and mean carbon dioxide concentration (ppm) in CO₂ fumigation chambers (n = 8) and in open air in growing seasons of 1994–1996.

	AOT0	AOT30	AOT40	CO ₂
<i>1994</i>				
Elevated	110	32	16	579
Filtered	59	2	0	
Ambient	81	8	1	374
<i>1995</i>				
Elevated	124	49	33	589
Filtered	46	1	0	
Ambient	81	10	3	365
<i>1996</i>				
Elevated	135	57	4	593
Filtered	47	1	0	
Ambient	86	13	3	367

higher in the year 1994, because the system was tested during early summer, and it was not working all the time. Using filter air O₃ concentration decreased ca. 10–20 ppb measured from the height of 2 m (Fig. 4), which was ca. 30–50 % from ambient air concentration. Similar (30 %) reductions of O₃ concentrations have been measured in earlier studies in open-top chambers (e.g. Mikkelsen and Ro-Poulsen 1994).

3.3 CO₂ Concentration

The mean CO₂ concentration in the fumigation chambers followed rather well the ambient air CO₂ concentration (Fig. 4), which varied depending the time of the day and between days. Similar variation in ambient air CO₂ concentration has also been measured in other studies (e.g. Skelly et al. 1996). During few days, especially in 1994, the fumigation system was cut off due to technical reasons and the concentration was at ambient air level. The fluctuation of CO₂ concentrations in the chambers during steady wind was ± 100 ppm. During the hard wind the variation was higher, but because CO₂ is not a toxic gas, acute injuries due to occasional higher peaks

Table 2. Light intensity (μmol m⁻² s⁻¹) in the chambers and in open air in July 1996.

Treatment	16.7. 1996	24.7. 1996	31.7. 1996
Ambient air	272.5ab	317.5a	135.5a
Filtered air	255.0ab	256.3a	139.3a
No chamber	372.0b	554.0a	147.2a
CO ₂	283.8ab	313.8a	143.3a
O ₃	198.8a	260.0a	145.0a
O ₃ +CO ₂	195.0a	313.8a	121.0a

Means in the same column followed by the same letter are not significantly different (p < 0.05) according to Tukey's multiple range test.

were not expectable. The system was not fully able to maintain twice the ambient CO₂ concentration during daytime and the mean concentrations remained ca. 100 ppm below the target.

Difference between chambers were not remarkable and therefore the repeatability of the CO₂-treatments can be regarded good as well (Fig. 5). Especially in year 1995 the differences in the CO₂ concentrations between chambers were small.

3.4 Temperature, Air Velocity and Light Intensity Measurements

The mean temperature in the chambers was 1.9 degrees higher and the maximum temperature in sunny days was 5–7 degrees higher compared to the open air temperature. At night and during cloudy days difference between ambient air and chambers was much smaller or negligible. The mean temperature between separate chambers varied at most three degrees, the differences between treatments were not statistically significant. The temperature difference between chambers was due to the amount of shading from trees around the chambers. For comparison a difference of 1.3 °C between open air and open-top chamber temperatures was measured by Janous et al. 1996.

The mean air velocity in chambers was 0.5 m/s. In the air velocity measurements no significant differences between treatments were observed. The filtration of ambient air did not significantly lower the air velocity in the chambers.

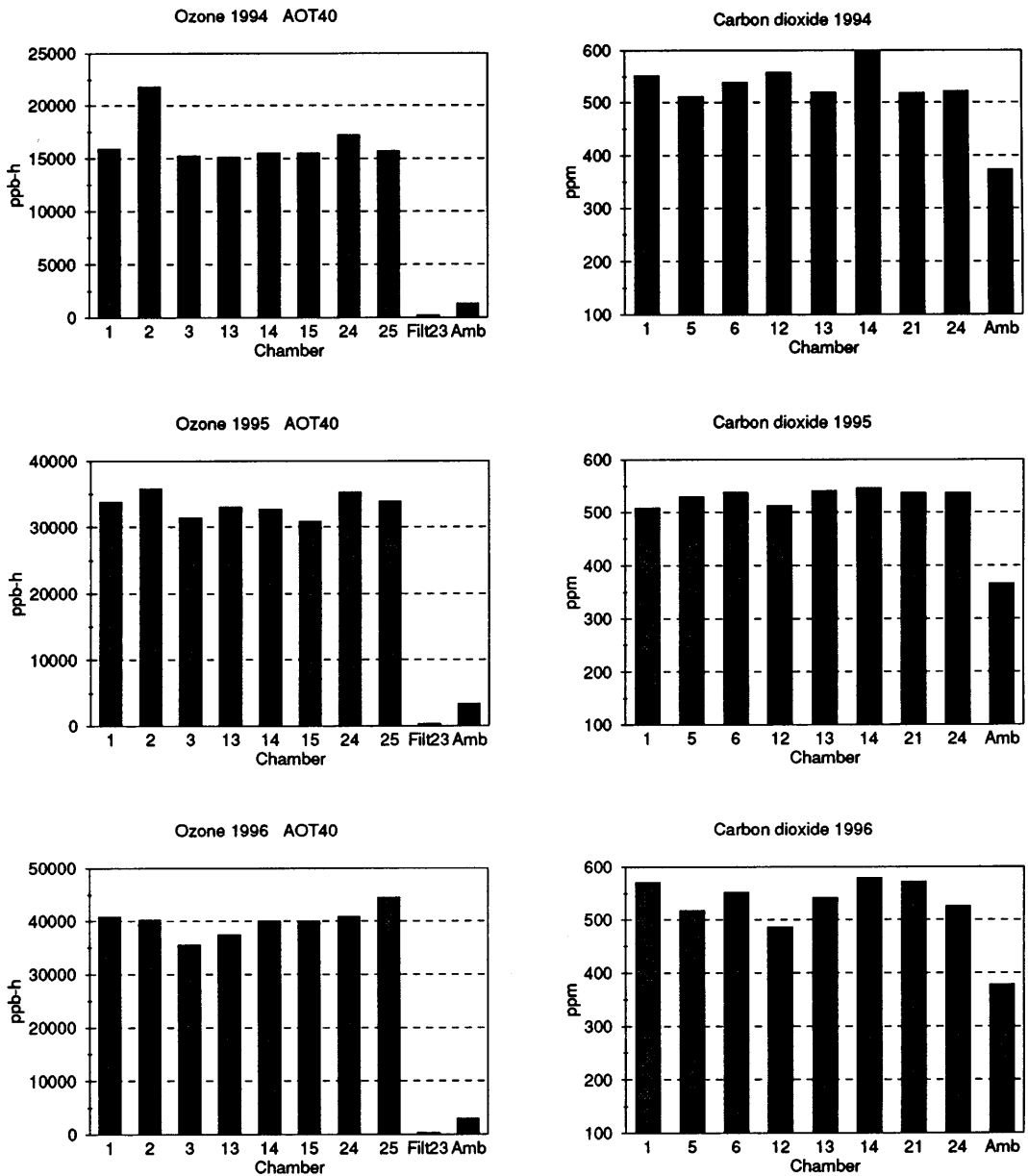


Fig. 5. O₃ dose (AOT40) and CO₂ concentration in chambers and in open air during growing periods 1994–1996.

The light intensity was significantly higher in chamberless control compared to O₃ and O₃+CO₂ fumigation chambers in the measurements made in cloudy afternoon (16 July) but not in other measurements made in cloudy or sunny morning (Table 2). The light intensity between each cham-

ber and also in the open air varied depending on the time of day and the amount and position of trees around the chamber.

Acknowledgements

This study is a subproject of SILMU research programme and was financially supported by the Academy of Finland.

References

- Allen, L.H., Drake, B.G., Rogers, H.H. & Shinn, J.H. 1992. Field techniques for exposure of plants and ecosystems to elevated CO₂ and other trace gases. *Critic. Rev. Plant Sci.* 11: 85–119.
- Ashmore, M.R. & Davison, A.W. 1996. Towards a critical level of ozone for natural vegetation. In: Kärenlampi, L. & Skärby, L. (eds), *Critical levels for ozone in Europe: testing and finalizing the concepts*, UN-ECE Workshop Report., Kuopio. University Printing Office, Kuopio, ISBN 951-780-653-1. p. 58–71.
- Bernier, P.Y., Stewart, J.D. & Hogan, G.D. 1994. Quantifying the uncontrolled CO₂ dynamics of growth chambers. *J. Exp. Bot.* 45: 1143–1146.
- Dahlman, R.C., Strain, B.R. & Rogers H.H. 1985. Research on the response of vegetation to elevated atmospheric carbon dioxide. *J. Environ. Qual.* 14: 1–8.
- 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.
- Heagle, A.S., Body, D.E. & Heck, W.W. 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Quality* 2: 365–368.
- , Philbeck, R.B., Rogers, H.H. & Letchworth, M.B. 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. *Phytopathol.* 69: 15–20.
- Hirvijärvi, E. Huttunen, S. & Rankka N. 1993. Description of the experimental system used in a Northern open-top fumigation project in Oulu. *Aquilo Ser. Bot.* 32: 1–7.
- Janous, D., Dvorak, V., Oplustilova, M. & Kalina J. 1996. Chamber effect and responses of trees in the experiment using open top chambers. *J. Plant Physiol.* 148: 332–338.
- Kellomäki, S. & Wang, K.-Y. 1997. Effects of elevated O₃ and CO₂ concentrations on photosynthesis and stomatal conductance in Scots pine. *Plant Cell Environ.* 20: 995–1006.
- Kickert, R.N., & Krupa, S.V. 1990. Forest responses to tropospheric ozone and global climate change: An analysis. *Env. Pollut.* 68: 29–65.
- Krupa, S.V. & Manning, W.J. 1988. Atmospheric ozone: Formation and effects on vegetation. *Environ. Pollut.* 50: 101–137.
- Laurila, T. & Lättilä, H. 1994. Surface ozone exposures measured in Finland. *Atm. Environ.* 28: 103–114.
- Lewin, K.F., Hendrey, G.R., Nagy, J. & LaMorte, R.L. 1994. Design and application of a free-air carbon dioxide enrichment facility. *Agric. For. Meteorol.* 70: 15–29.
- Lucas, P.W., Cottam, D.A. & Mansfield, T.A. 1987. A large-scale fumigation system for investigating interactions between air pollution and cold stress on plants. *Environ. Pollut.* 43: 15–28.
- Mikkelsen, T.N. & Ro-Poulsen, H. 1994. Exposure of Norway spruce to ozone increases the sensitivity of current year needles to photoinhibition and desiccation. *New Phytol.* 128: 153–163.
- Pääkkönen, E., Metsärinne, S., Holopainen, T. & Kärenlampi, L. 1995. The ozone sensitivity of birch (*Betula pendula*) in relation to the developmental stage of leaves. *New. Phytol.* 132: 145–154.
- Palomäki, V., Laitinen, K., Holopainen, T. & Kellomäki, S. 1996. First-year results on the effects of elevated atmospheric CO₂ and O₃ concentrations on needle ultrastructure and gas exchange responses of Scots pine saplings. *Silva Fennica* 30(2–3): 123–134.
- Rogers, H.H., Runion, G.B. & Krupa, S.V. 1994. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environ. Pollut.* 83: 155–189.
- Runeckles, V.C. & Krupa, S.V. 1994. The impact of UV-B radiation and ozone on terrestrial vegetation. *Environ. Pollut.* 83: 191–213.
- Skärby, L. & Karlsson, P.E. 1996. Critical levels for ozone to protect forest trees – Best available knowledge from the Nordic countries and the rest of Europe In: Kärenlampi, L. & Skärby, L. (eds), *Critical levels for ozone in Europe: testing and finalizing the concepts*, UN-ECE Workshop Report, Kuopio. University Printing Office, Kuopio, ISBN 951-780-653-1. p. 72–85.
- Skelly, J.M. Fredericksen, T.S. Savage, J.E. & Sny-

- der, K.R. 1996. Vertical gradients of ozone and carbon dioxide within a deciduous forest in central Pennsylvania. *Environ. Pollut.* 94: 235–240.
- Wulff, A., Hänninen, O., Tuomainen, A. & Kärelampi, L. 1992. A method for open-air exposure of plants to ozone. *Ann. Bot. Fennici* 29: 253–262.

Total of 23 references