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In situ Autumn Ozone Fumigation of Mature Norway Spruce – Effects on Net Photosynthesis

By

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Summary

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Twelve cuvettes were installed on current year's twigs in the top of the canopy of a 35 years old Norway spruce stand in Denmark. From 10 to 16 hours, six of the cuvettes received 5-60 nl l⁻¹ ozone in addition to ambient air and six cuvettes received ambient air with a 40% reduced ozone concentration. The experiment was conducted during 70 days during the autumn. Our system could not detect any ozone effects on dark respiration, but eventually effects on dark respiration could be masked in signal noise. An inhibition of daily net photosynthesis in ozone treated shoots was apparent, and it was found that a mean increase in ozone concentration of 10 nl l⁻¹ reduced net photosynthesis with 7.4 %. This effect should be related to a pre-exposure during the season of AOT40 12.5 µl l⁻¹ h.

Introduction

Tropospheric ozone can affect plants negatively by inhibiting photosynthesis and growth (e.g. DIZENGREMEL 2001). This effect is dependent on numerous factors e.g. ozone dose and microclimate (predisposing and during measurements), species, provenance, crown structure, age of plants, time of year and type of fumigation. Ozone effects on Norway spruce have been studied intensively during the last decades and during the last 10 years more than 100 peer-reviewed papers have been published (ISI web of science®). Norway spruce is considered a relatively ozone resistant species which under certain conditions can tolerate enhanced ozone concentrations over several growing seasons (e.g. WULFF & al. 1996, LUCAS &

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DIGGLE 1997), but fumigation experiments lasting several growing seasons have showed decline in photosynthesis or growth (e.g. WALLIN & al. 1990). The apparent contradictory results in the literature are probably a consequence of different combinations of the above-mentioned factors and methods for detecting the effects.

Overall, the quantitative risk assessment of ozone impacts on mature trees and forests is vague at the European scale, as most knowledge is derived from controlled ozone fumigations of young trees, grown individually in exposure chambers (MATYSSEK & INNES 1999). To improve and validate data it is necessary to study effects at stand conditions. In this study, we exposed twigs from mature trees in situ to enhanced ozone levels compared to reduced ambient levels.

Methods chosen to detect ozone effects are also an important factor. Depending on method, parameters are measured before, during and after ozone exposure. Physiological effects of ozone can be short-term and do often interact with other dynamic parameters such as microclimate e.g. photoinhibition (MIKKELSEN & RO-POULSEN 1994, FARAGE 1996) therefore, it is important to monitor key parameters continuously. Here, we monitored gas exchange and microclimate parameters continuously over the fumigation period in the autumn.

Material and Methods

The gas exchange system was computer controlled and designed to control and monitor 14 temperature regulated PTFE/aluminum chambers enclosing spruce twigs for several months with minimum ozone absorption. 12 cuvettes were installed on sun-exposed shoots in the top of three 35 years old Norway spruce trees in a stand in western Jutland, Denmark, growing on a poor, sandy loam soil. On each tree, four cuvettes were installed on current year twigs. Two cuvettes were facing SW and two cuvettes were facing NW (Fig. 1). Two cuvettes were used as control without plants.

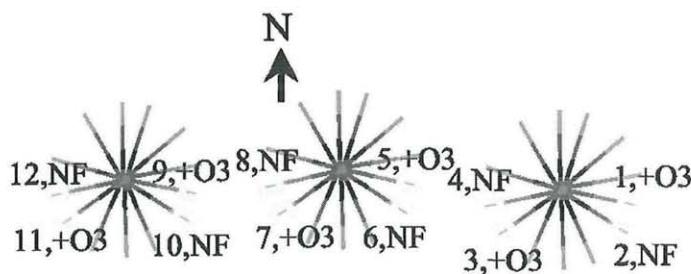


Fig. 1. Set-up of cuvettes on the three trees. On each tree two cuvettes were facing E and SE and two cuvettes were facing W and SW. The angles in the horizontal plane are varying $\pm 20^\circ$. NF = cuvettes receiving non-filtered ambient air. O₃+ = cuvettes receiving non-filtered air plus ozone.

Six cuvettes received ambient air (NF). From 10 to 16 hours, six of the cuvettes received 5-60 nl l⁻¹ ozone in addition to ambient air (O₃+). The cuvettes were installed in the beginning of September, fumigations were initiated on September 11th and continued until November 23rd 1995.

Each cuvette was equipped with a PAR calibrated diode positioned parallel in relation to the upper side of the twig, and a shaded temperature sensor. The temperatures inside the cuvettes were maintained close to ambient level by a computer controlled peltiers element enclosed by a water-cooled jacket. Ambient air was continuously pumped into a glass/Teflon manifold and distributed in Teflon tubes to the 12 cuvettes on the trees and the two control cuvettes without a twig. Ozone, generated by a UV-B tube, was distributed via a manifold to six cuvettes. Air was sampled sequentially from all 12 cuvettes and distributed to a LI-COR CO₂/H₂O monitor and an ozone monitor from Monitor labs. Air was sampled 3 min. from each cuvette sequentially. During the last 20 sec. data were collected from the gas monitors and during the last 30-10 sec., data were collected from the cuvette sensors.

The control cuvettes were measured in between all the 12 cuvettes. See MIKKELSEN & RO-POULSEN 1995 for further details on the system and site.

For data analysis a separation of data into day and night were based on the incoming light. Light levels < 3 μmol m⁻² s⁻¹ was defined as night. During data handling days with less than 80 % of a complete dataset available were excluded from further data analysis. All gas exchange parameters were calculated according to VON CAEMMERER & FARQUHAR 1981 and related to the projected needle area. The ozone flux (FO₃) were calculated according to WIESER & al. 2000.

Results

In the period before fumigation (from bud break mid May until cuvette installation in the beginning of September) the current years shoots used in this experiment were exposed to ambient ozone corresponding to an AOT40 of 12.5 μl l⁻¹ h calculated for daylight hours only. During the fumigation from September 11th to November 23rd 1995 the NF and O₃+ twigs were exposed to 3.08 and 0.15 μl l⁻¹ h AOT40, respectively, calculated for daylight hours. The cuvette system reduced the ambient ozone concentration with approximately 40%. Because ozone addition were manually adjusted and due to technical failures there was a large variation in the ozone concentration in the O₃+ cuvettes (Fig. 2). However, one-hour peak levels in O₃+ were not higher than 85 nl l⁻¹.

The temperatures in the cuvettes were controlled in relation to the ambient temperature within the range -2 to +3 °C during 95% of the period. Maximum deviations from ambient were 6 °C during sudden sun exposures. During the whole period the day/night average temperatures in NF and O₃+ were 16.7/12.9 and 17.2/12.7 °C, respectively.

The average light levels during daylight hours in NF and O₃+ were 226 and 279 μmol m⁻² s⁻¹, respectively. The difference was largest during direct sunlight and non-existing during periods with diffuse light (Fig. 3). The light sensors registered only incoming light to the upper side of the twigs, but the 3-D structure of the twigs, the angles of the branches and the cuvette design ensured light absorption from all angles. The twigs were not forced into equal angles and this might account for the difference between NF and O₃+. It is a question whether this difference is for real or an artifact related to the light measuring principle.

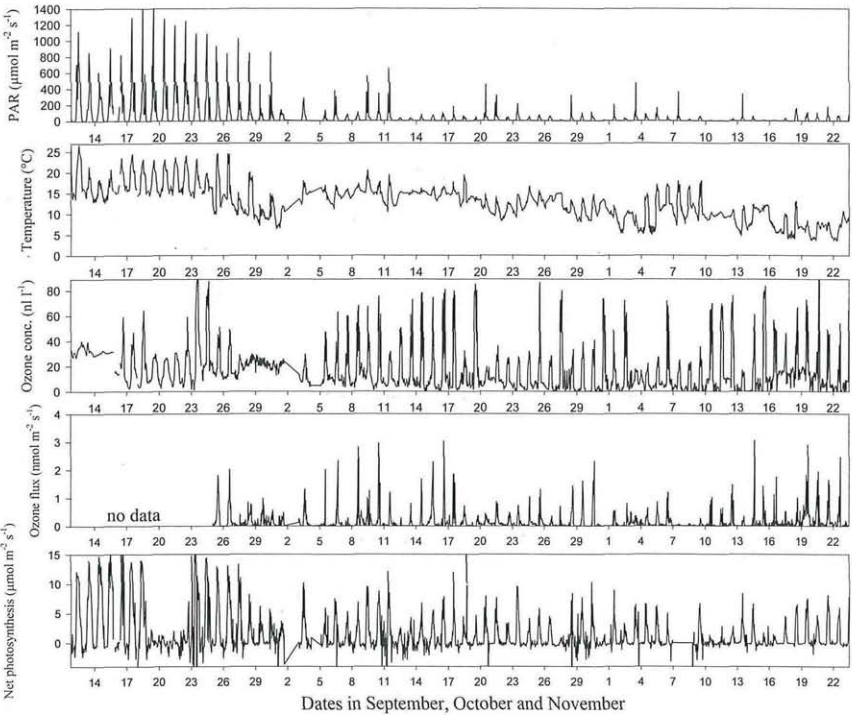


Fig. 2. Complete data set (PAR, temperature, ozone concentration and calculated ozone flux into the leaves and net photosynthesis) from an O_3+ cuvette during the whole fumigation period.

Fig. 2 shows that the fumigation period can be separated into three consecutive groups according to the light levels and temperature: September, October and November. Cuvettes in September, October and November received on average 487, 124 and 89 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, during daylight hours and the average temperature was 16.2, 13.2 and 7.4 °C. In the following, we will investigate if this could affect the respiration responses to ozone. For dark respiration (R_n), no significant differences were seen between treatments during the three months. R_n decreased with night temperature (°C/ $\mu\text{mol m}^{-2} \text{s}^{-1}$): Sep.: NF: 13.0/0.8; O_3+ : 12.8/1.1. Oct.: NF: 12.2/0.4; O_3+ : 12.1/0.5. Nov.: NF: 6.4/0.2; O_3+ : 6.4/0.2.

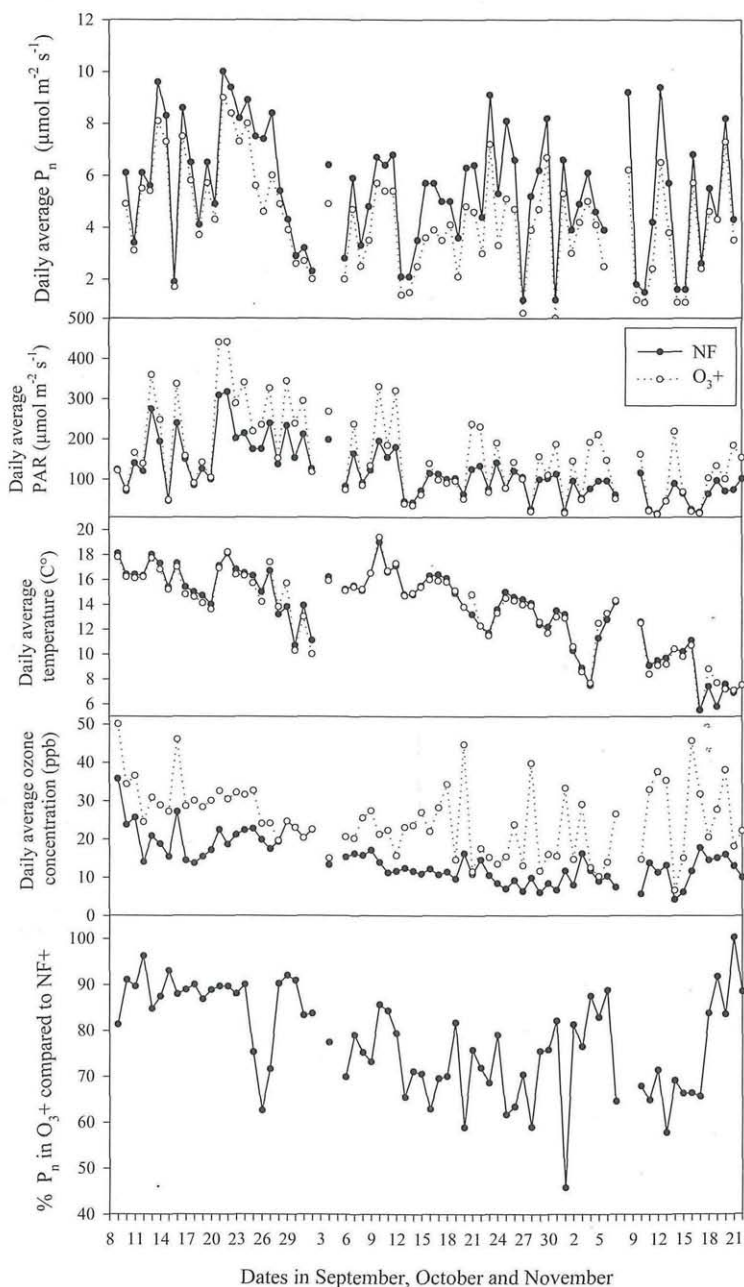


Fig. 3. Daily means from O_3+ and NF cuvettes during the full fumigation period. P_n , PAR, temperature, ozone concentration and P_n in O_3+ shown as percentage of the NF treatment.

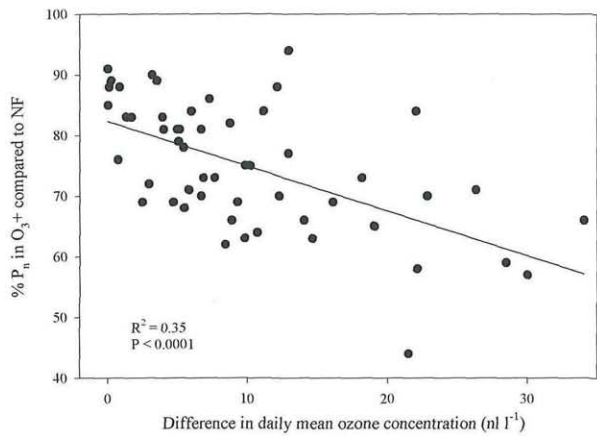


Fig. 4. Correlation analysis. P_n in O₃+ shown as percentage of the NF treatment in relation to the difference in daily mean ozone concentration between NF and O₃+

It is suggested that ozone increases R_n due to an induced increased repair rate and increased detoxifying processes (DIZENGREMEL 2001). However, a correlation analysis between daily mean ozone concentrations versus R_n the following night demonstrates no correlation.

Mean daily photosynthesis rate (P_n) was reduced in O₃+ throughout the fumigation period (Fig. 3). Due to a large variation the difference are not significant. It is not possible to explain effects due to changes in stomatal conductance or FO₃ since stomatal conductance could not be estimated because of emergence of condensing water inside the cuvette produced by the cooling system. In Fig. 2 FO₃ is calculated for one cuvette, but the condensing water in some cuvettes prevents further analysis.

A correlation between the daily average P_n in O₃+ compared to NF versus differences in daily mean ozone concentrations between the two treatments reveals a negative correlation. A few days with very low P_n have been removed from the analysis to avoid extreme variations on the Y-axis (Fig. 4).

Discussion

The microclimatic conditions inside the cuvettes were similar concerning temperature. The PAR is higher in O₃+ than NF maybe due to different branch angles. The light levels were relatively high during September and the additional light cannot be ruled out as an extra stressor in addition to ozone. We have previously shown inhibition of P_n in O₃+ at high radiation input during midday (MIKKELSEN & RO-POULSEN 1995), but in average over the whole day, days with high radiation

levels were having the lowest ozone induced inhibition of P_n compared to days with diffuse radiation.

Only a few ozone experiments are conducted on older trees in the forest like this study (e.g. TJOELKER & al. 1995, WIESER & al. 2000). One remark is that only a small part of the tree is exposed to the manipulated ozone concentration and that allocation patterns in the tree might interfere with the physiological responses. On the other hand, several differences exist between a juvenile tree commonly used in ozone effects experiments and the mature tree growing in an old forest. WIESER 1997 found that photosynthetic capacity of mature trees was less affected by ozone exposure than that of seedlings and that differences in O_3 uptake may explain observed differences in sensitivity. KOLB & MATYSSEK 2001 propose several hypotheses derived from multi-factor studies relevant to scaling O_3 impacts from juvenile to mature forest trees e.g. that supply of labile carbon available for defense, compensation, and repair responses to O_3 stress will be lower in mature than juvenile trees because of higher respiratory costs for maintaining living tissues and lower photosynthetic rate of mature trees. These counteracting results/hypotheses stress the needs for studies conducted in the forest, particularly when factors such as slow and rapid changes of ozone concentrations also affects the physiological behaviors of current-year needles of Norway spruce as shown by WIESER & al. 1998.

The numbers of cuvettes (two times six) are relatively few in relation to a dynamic biological system, and this might explain why we cannot show daily statistical significant effects on R_n and P_n . It is also difficult with our gas exchange system to demonstrate effects on R_n because differences in CO_2 concentrations are very small and this results in a large variation in R_n . Consequently, eventual effects on R_n can be concealed in signal noise. The inhibition of P_n in O_3+ day after day is evident, and in Fig. 4 it is shown that an increases in ozone concentration of 10 nl l^{-1} reduces P_n with 7.4 %. This effect should be related to the ozone exposure during the season of AOT40 $12.5 \text{ } \mu\text{l l}^{-1} \text{ h}$. This concentration is above the considered threshold ($10 \text{ } \mu\text{l l}^{-1} \text{ h}$) for a 10% decrease in growth for ozone sensitive tree species (SKÄRBY & al. 1998).

Several authors have proven that Norway spruce is relatively insensitive to ozone (e.g. WIESER & HAVRANEK 1996, UTRIAINEN & HOLOPAINEN 2001), especially current years needles due to the short exposure time compared to older needles (WALLIN & al. 1990). Short-term physiological effects on current needles documented by continuous measurements like this one will probably not reduce growth significantly. The documented effects should, however, be considered as an indicator for a certain stress load on the current needles and taken into account in combination with other natural and anthropogenic stressors.

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