Elevated CO$_2$ Counteracts the Limitation by Chronic Ozone Exposure on Photosynthesis in *Fagus sylvatica* L.: Comparison between Chlorophyll Fluorescence and Leaf Gas Exchange

By

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**Key words:** Chlorophyll fluorescence, elevated carbon dioxide (CO$_2$), *Fagus sylvatica* (European beech), leaf gas exchange, ozone (O$_3$).

**Summary**


The interaction of elevated CO$_2$ and enhanced chronic ozone (O$_3$) impact was analysed throughout the growing season in the photosynthetic response (chlorophyll fluorescence and leaf gas exchange) of beech saplings (*Fagus sylvatica*) which had been acclimated to CO$_2$ supply during the year prior to the experiment. Both light and dark reactions (i.e. electron transport rate and photosynthetic capacity) of plants grown at ambient CO$_2$ and twice-ambient O$_3$ concentrations were distinctly reduced by August. The O$_3$-induced decline was counteracted by elevated CO$_2$ supply (i.e. ambient +300 ppm). Plants grown at high CO$_2$ supply and ambient or twice-ambient O$_3$ concentrations displayed a photosynthetic performance similar to plants exposed to ambient CO$_2$ and O$_3$ conditions. Responses in chlorophyll fluorescence were found to be consistent with those in leaf gas exchange.

**Introduction**

In parallel with the increase in the carbon dioxide (CO$_2$) concentration of the atmosphere (KEELING & al. 1995, HOUGHTON & al. 1995), also tropospheric ozone
(O3) has reached levels far above pre-industrial scenarios (STOCKWELL & al. 1997). While elevated CO2 may increase photosynthesis and biomass production in trees (CEULEMANS & MOUSSEAU 1994, CURTIS & WANG 1998, SAXE & al. 1998) including Fagus sylvatica (EL KÖHEN & al. 1993, EPRON & al. 1996, HEATH & KERSTIENS 1997), ozone is regarded as one of the most phytotoxic air pollutants presently encountered in the environment (SANDERMANN & al. 1997). Enhanced O3 regimes were shown, in Fagus sylvatica, to reduce photosynthesis, stomatal conductance and biomass production, to increase dark respiration and to accelerate leaf loss (PEARSON & MANSFIELD 1994, MIKKELSEN 1995, LIPPERT & al. 1996, MIKKELSEN & HEIDE-JÖRGENSEN 1996, ZEUTHEN & al. 1997). However, given the increases in both CO2 and O3 levels, interactive effects of these gases on broad-leaf trees have rarely been investigated and are not yet accounted for Fagus sylvatica, one of the most important tree species in Central Europe. It is still unclear, whether CO2 may buffer the O3 impact on broad-leaf trees as has been observed in crops (MCECH & al. 1995, 1997, FISCUS & al. 1997). Some evidence for a buffering effect of elevated CO2 was derived from studies on oak and poplar species which seem to support the view that elevated CO2 counteracts limitations by ozone via reductions in stomatal conductance and, thus, decrease O3 uptake (VOLIN & REICH 1996, MANES & al. 1998, VOLIN & al. 1998), while this effects was not seen in a long-term study on O3-tolerant and O3-sensitive aspen clones (KULL & al. 1996). However, the relevance of genetics and factorial scenarios in tree response to CO2/O3 interactions remain an issue of debate (SAXE & al. 1998).

The study presented on Fagus sylvatica deals with the seasonal effects of elevated CO2 and enhanced O3 levels on the photosynthesis of plants which had been acclimated to the CO2 supply throughout the year preceding the experiment. The aim was to clarify if CO2/O3 interactions do confirm in the photosynthetic light and dark reactions as assessed by the analysis of chlorophyll fluorescence and leaf gas exchange.

Material and Methods

Plant culture and experimental set-up

Plants of European beech (Fagus sylvatica L.) were grown from seeds in the glasshouse in 1994 and planted into 10 l pots containing forest soil. Fertilisation as applied during irrigation aimed at providing non-limiting nutrient supply to the plants during the entire experiment. Throughout the second growing season in 1995, plants were acclimated to ambient or elevated (i.e. ambient + 300 ppm) CO2 supply. In May 1996, at the beginning of the third growing season, plants were transferred to four phytotrons (at the GSF-National Research Center for Environment and Health, see PAYER & al. 1993). Each phytotron contained four sub-chambers with independent fumigation control. The two CO2 exposures (“ambient CO2” and “+300 CO2”) were combined with the diurnal and seasonal O3 regimes of fluctuating ambient (1xO3) or proportionally increased twice-ambient O3 concentrations (2xO3), resulting in four different fumigation treatments (i.e. amb. CO2/1xO3, amb. CO2/2xO3, +300 CO2/1xO3 and +300 CO2/2xO3) with 24 plants each. Ozone was generated from pure oxygen and controlled as described by PAYER & al. 1993. Each treatment was reproduced four times across the four phytotrons. The time course of the 1xO3 regime had been recorded, on an two-hourly basis, in Schönenuhur near Basel/Switzerland along with the air temperature, relative humidity and irradiance throughout the entire growing season of 1990.
Assessment of leaf gas exchange

Measurements of gas exchange were conducted before noon using ozone-free air at 20.0 °C air temperature and a dewpoint of 10.0 °C, resulting in a relative humidity of about 53% inside the gas exchange cuvette. The photosynthetic capacity of single non-shaded leaves attached to well-watered trees was assessed as the net CO$_2$ uptake rate under saturating light and CO$_2$ conditions (approx. 1850 µmol photons m$^{-2}$ s$^{-1}$ and 1900 ppm CO$_2$, respectively), using a mini-cuvette system (H. Walz, Effeltrich, Germany). Measurements were done around the end of June/early July after completion of leaf growth (when AOT40 had reached about 8.9 and 41.3 µl l$^{-1}$ h for 1xO$_3$ and 2xO$_3$, respectively) and in late August, after leaf necroses caused by O$_3$ exposure had become apparent (AOT40 amounting to about 24.5 and 96.4 µl l$^{-1}$ h for 1xO$_3$ and 2xO$_3$, respectively). Gas exchange measurements under limiting CO$_2$ supply were performed using a porometer (HCM-1000, H. Walz, Effeltrich, Germany) at concentrations of about 50, 150, 250 and 350 ppm CO$_2$ under saturating light conditions (1800 µmol photons m$^{-2}$ s$^{-1}$) for eight plants of each fumigation treatment. Rates of gas exchange and apparent internal CO$_2$ concentration, $c_i$, were calculated according to von Caemmerer & Farquhar 1981 and related to the one-sided leaf area.

Assessment of chlorophyll a fluorescence

Measurements of chlorophyll a fluorescence were performed in August with a pulse-amplitude modulation fluorometer (Mini-PAM, H. Walz, Effeltrich, Germany) during the gas exchange analysis as described above, using the same leaves. The fibre optics (0.2 cm in diameter) of the fluorometer was mounted to the upper lid of the gas exchange cuvette at a constant distance (approx. 0.5 cm) and angle (60°) to the leaf. Chlorophyll fluorescence parameters were calculated as described by Genty & al. 1989. Apparent electron transport rates through photosystem II (ETR) were estimated according to Krall & Edwards 1992 as $\Delta F/F_m - PPFD \cdot a \cdot f$ assuming an absorptivity $a$ of the leaves of Fagus sylvatica in the photosynthetic active radiation of 0.84 and a light distribution factor between photosystem I and II, $f$, of 0.5 (Krall & Edwards 1992).

Statistical analysis

Mean values of each treatment were compared by means of a two-factor (CO$_2$ and O$_3$) analysis of variance (ANOVA), using the Statistical Analysis Software (SAS version 6.12, SAS Institute Inc., North Carolina, USA). Subsequently, the Tukey Studentised Range Test was applied whenever the null hypothesis was rejected.

Results

Long-term acclimation of two growing seasons to elevated CO$_2$ resulted in a slight but not significant reduction of the photosynthetic capacity (PC) in June/July. Likewise, no significant effect of the O$_3$ exposure was apparent at that time of the year (Fig. 1). However, later in the season, in August, the PC of plants grown at amb. CO$_2$/2xO$_3$ was only one third of that at the amb. CO$_2$/1xO$_3$ treatment. This O$_3$ effect proved to be significant (p<0.05). The O$_3$-induced decrease in PC was buffered by elevated CO$_2$, as the decline was less pronounced at the +300 CO$_2$/2xO$_3$ relative to the amb. CO$_2$/2xO$_3$ treatment and the ANOVA revealed a significant O$_3$xCO$_2$ interaction (p<0.01). As already indicated in June, plants grown at elevated CO$_2$ displayed slightly lower PC in August than those at the amb. CO$_2$/1xO$_3$ treatment, but again this CO$_2$ effect was not statistically relevant.
photosynthetic capacity

Fig. 1. Photosynthetic capacity of the net CO$_2$ exchange of *Fagus sylvatica* as derived from plants grown under the four fumigation treatments in June/July and August. Each column represents a mean (± SE) of eight measurements on different plants.

The overall response of the maximum apparent electron transport rate through photosystem II (ETR) to the CO$_2$/O$_3$ treatments resembled, in August, the pattern observed in PC (Fig. 2). Plants grown at +300 CO$_2$ reached, regardless of the O$_3$ regime, a maximum ETR similar to that in plants from the amb. CO$_2$/1xO$_3$ treatment, where the electron transport capacity was about twice as high as at amb. CO$_2$/2xO$_3$. Given the high standard errors, the O$_3$ and CO$_2$ effects on maximum ETR were not significant, but the O$_3$xCO$_2$ interaction proved to be statistically relevant (p<0.05).

Fig. 2. Maximum apparent electron transport rate through photosystem II (ETR) of *Fagus sylvatica* exposed to the four fumigation treatments in August. Data were calculated from measurements of chlorophyll fluorescence under light and CO$_2$ saturation. Each column represents a mean (± SE) of eight measurements on different plants.
The relationship between ETR and (limited) CO$_2$ supply showed, in August, the highest slope, m, of the regression line in plants at amb. CO$_2$/1xO$_3$ (m = 0.22, Fig. 3). In contrast, trees exposed to ambient CO$_2$ supply but 2xO$_3$ displayed the lowest slope (m = 0.07). Plants acclimated to +300 CO$_2$ did not show reductions by 2xO$_3$ in the ETR/c$_i$-relationship, while the slope (m = 0.15) was similar to the amb. CO$_2$/1xO$_3$ and to the +300 CO$_2$/1xO$_3$ treatment (m = 0.20).

![Graph showing CO$_2$ response curves of the apparent electron transport rate through photosystem II (ETR) of Fagus sylvatica in August grown under the four fumigation treatments. The slope (m) given for each fumigation treatment was derived from a linear regression. Each data point represents a mean (± SE) of eight measurements on different plants.](image)

Fig. 3. CO$_2$ response curves of the apparent electron transport rate through photosystem II (ETR) of Fagus sylvatica in August grown under the four fumigation treatments. The slope (m) given for each fumigation treatment was derived from a linear regression. Each data point represents a mean (± SE) of eight measurements on different plants.

To examine if reductions in the photosynthetic light reactions are reflected in the dark reactions of photosynthesis, each determination of ETR is compared (pooling data from Figs. 2 and 3) with its corresponding net CO$_2$ uptake rate as measured at the same time (Fig. 4). Despite reductions in photosynthetic performance to varying extents at the different exposure treatments the comparison provides an overall linear relationship between ETR (as derived from chlorophyll fluorescence) and net CO$_2$ gas exchange ($r^2 = 0.82$).
Discussion

Acclimation of *Fagus sylvatica* to elevated CO$_2$ (ambient + 300 ppm) for two growing seasons resulted in a slight but not significant reduction in photosynthetic capacity (PC) of about 10% (cf. June/July in Fig. 1), similar to reports by other studies (EL Kohen & al. 1993, Epron & al. 1996, Grams & al. 1999). Slight or even no decline in photosynthetic performance in response to elevated CO$_2$ seems to characterise trees as concluded from a recent meta-analysis of more than 500 reports on this subject (see Curtis & Wang 1998).

While in June/July no significant effects of ozone on the photosynthetic capacity of *Fagus sylvatica* were found, distinct responses became apparent by August (Figs. 2, 3 and 4). Lippert & al. 1996 and Grams & al. 1999 observed similar effects of twice-ambient ozone concentrations on photosynthetic light (e. g. F$_{v}$/F$_{m}$, Φ$_{PSII}$, ETR) and dark reactions (PC, carboxylation efficiency) in *Fagus sylvatica*, as measured at ambient CO$_2$ supply and under the same climate and ozone.

Remarkably, PC and ETR were ameliorated at +300 CO₂/2xO₃ as compared with the amb. CO₂/2xO₃ treatment (cf. Figs. 1, 2 and 3), which is consistent with findings about the photosynthetic behaviour and biomass production in broad-leaf trees by other studies (VOLIN & REICH 1996, MANES & al. 1998, VOLIN & al. 1998, GRAMS & al. 1999). However, such compensatory effects by elevated CO₂ on O₃ stress were not found for aspen clones (KULL & al. 1996) and young trees of Norway spruce (BARNES & al. 1995, LIPPERT & al. 1997). Although a buffering capacity of elevated CO₂ relative to O₃-induced limitations in the photosynthetic light and dark reactions did exist in the young beech trees (cf. Figs. 1, 2 and 3), the assessment of chlorophyll fluorescence was less indicative than the analysis of leaf gas exchange. This is probably due to the rather small area (about 0.3 cm²) probed in the leaf when applying the fibre optics of the fluorometer. In contrast, gas exchange measurements integrate the entire leaf area and, by this, results are less affected by local inhomogeneities in the leaf surface as caused by O₃-induced necrotic lesions (ANEGG unpublished, GRAMS & al. 1999) or stomatal patchiness (BEYSCHLAG & ECKSTEIN 1998). Nevertheless, the comparison of ETR with the apparent CO₂ concentration in the intercellular spaces of the mesophyll, cᵢ, revealed differences between the photosynthetic performance of leaves from the different fumigation treatments (cf. Fig. 3). These differences are consistent with treatment-specific effects on the dependence of CO₂ uptake on apparent cᵢ (GRAMS & al. 1999).

One has to keep in mind, though, that the dark reactions of photosynthesis are probably impaired by ozone before effects substantiate in the light reactions (LEINHERR & al. 1988, FARAGE & al. 1991, GUPTA & al. 1991, REICHENAUER & al. 1997). Thus, the linear relationship between ETR and net CO₂ uptake rate (cf. Fig. 4) may be observed only if O₃-induced effects are well established - as apparently was the case and is shown for the present experiment by ANEGG unpublished for the development of bronzing and necrotic spots on the leaves.

Taking into account that similar stomatal conductance was observed in the beech plants irrespective of the CO₂/O₃ regime (ANEGG unpublished, HABERLE unpublished, Grams & al. 1999), compensation of adverse O₃ effects on photosynthesis must be assumed to be also mediated by mechanisms other than reduced O₃ uptake (cf. VOLIN & REICH 1996, MANES & al. 1998, VOLIN & al. 1998) in the presence of elevated CO₂ supply.

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