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Interactions of chronic exposure to elevated CO_2 and O_3 levels in the photosynthetic light and dark reactions of European beech (*Fagus sylvatica*)

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SUMMARY

Young trees of European beech (*Fagus sylvatica*) acclimated for one growing season to ambient (*c.* 367 µl l^{-1}) or elevated CO_2 levels (*c.* 660 µl l^{-1}) were exposed during the subsequent year to combinations of the same CO_2 regimes and ambient or twice-ambient ozone (O_3) levels (generated from the database of a rural site). By the end of June, before the development of macroscopic leaf injury, the raised O_3 levels had not affected the light and dark reactions of photosynthesis. However, acclimation to elevated CO_2 had resulted in lowered chlorophyll and nitrogen concentrations, whereas photosynthetic performance, examined over a wide range of parameters from light and dark reactions, remained unchanged or showed only slight reductions (e.g. apparent electron transport rate, ETR; apparent quantum yield of CO_2 gas exchange, Φ_{CO2}; apparent carboxylation efficiency, CE; and photosynthetic capacity at light and CO_2 saturation, PC). In August, after the appearance of leaf necroses, plants grown under ambient CO_2 and twice-ambient O_3 conditions declined in both the photosynthetic light reactions (optimum electron quantum yield, F_v/F_m, non-photochemical energy quenching, NPQ, reduction state of Q_A, apparent electron quantum yield, Φ_{PSI}I, maximum electron transport rates) and the dark reactions as reflected by CE, Φ_{CO2}, as well as the maximum CO_2 uptake rate (i.e. PC). CE, Φ_{CO2} and PC were reduced by *c.* 75, 40 and 75%, respectively, relative to plants exposed to ambient CO_2 and O_3 levels. By contrast, plants exposed to twice-ambient O_3 and elevated CO_2 levels maintained a photosynthetic performance similar to individuals grown either under ambient CO_2 and ambient O_3, or elevated CO_2 and ambient O_3 conditions. The long-term exposure to elevated CO_2 therefore tended to counteract adverse chronic effects of enhanced O_3 levels on photosynthesis. Possible reasons for this compensatory effect in *F. sylvatica* are discussed.

Key words: European beech (*Fagus sylvatica*), elevated CO_2, ozone (O_3), chlorophyll fluorescence, gas exchange, photosynthesis, stomatal conductance, chronic effects.

INTRODUCTION

The increase in the atmospheric carbon dioxide (CO_2) concentration from the pre-industrial 280 µl $l^{-1}$to the present 360 µl l^{-1} (Neftel *et al.*, 1985), arising from extensive burning of fossil fuels and deforestation mainly in the tropics (IPCC, 1996), has progressed since 1980 by *c.* 1.5 µl l^{-1} yr^{-1} (Keeling *et al.*, 1995). CO_2 levels are expected to exceed 700 µl l^{-1} by the end of the next century (King *et al.*, 1992; IPCC, 1996), while tree response to elevated CO_2 is still under debate (Saxe *et al.*, 1998). Various studies have shown that short-term exposure of trees to elevated CO_2 for less than one growing season may increase photosynthesis and biomass production

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(Eamus & Jarvis, 1989; Long, 1991; Ceulemans & Mousseau, 1994), but attention has only recently been directed towards longer-term studies. In European beech (*Fagus sylvatica*), these longer term studies revealed minor down-regulation in photosynthesis (Epron *et al.*, 1996) or even the absence of any effect (El Kohen *et al.*, 1993; Mousseau *et al.*, 1996; Heath & Kerstiens, 1997), while biomass production could be increased by changes in the whole-plant carbon allocation (El Kohen *et al.*, 1993; Rey & Jarvis, 1997; Curtis & Wang, 1998). Remarkably, stomatal conductance was often unaffected or showed only minor reductions (Drake *et al.*, 1996; Heath & Kerstiens, 1997; Curtis & Wang, 1998; Saxe *et al.*, 1998). It is most likely that these findings represent a general response pattern of deciduous trees to extended high CO_2 supply (Curtis & Wang, 1998; Saxe *et al.*, 1998).

Tropospheric ozone (O_3) has also increased, far above pre-industrial levels, during the past century (on average by a factor of two to four, and even higher with respect to peak concentrations; Stockwell *et al.*, 1997). Chronic exposure to enhanced O_3 levels is regarded to be injurious, in particular, to broad-leaf trees including *F. sylvatica*, as a decline in photosynthesis, premature leaf loss and increased dark respiration may limit the biomass production (Matyssek *et al.*, 1991, 1993; Pearson & Mansfield, 1993, 1994; Mikkelsen, 1995; Lippert *et al.*, 1996b; Mikkelsen & Heide-Jörgensen, 1996; Langebartels *et al.*, 1997; Zeuthen *et al.*, 1997; Skärby *et al.*, 1998). Plant sensitivity to ozone has often been found to relate to the stomatal conductance, as high O_3 influx through the stomata may increase the risk of injury (Reich, 1987; Barnes & Wellburn, 1998; Volin *et al.*, 1998). Although both elevated CO_2 and chronic O_3 exposure have the potential of reducing stomatal aperture (Ceulemans & Mousseau, 1994; Matyssek *et al.*, 1995), interactions on and, thus, impact of O_3 uptake, have rarely been investigated with respect to broad-leaf trees.

In herbaceous crop species, elevated CO_2 is known to counteract adverse effects of O_3 on photosynthesis and production (McKee *et al.*, 1995, 1997a; Mulholland *et al.*, 1997; Reid & Fiscus, 1998), which is ascribed, at least in part, to a CO_2-induced decline in stomatal conductance (Allen, 1990; McKee *et al.*, 1995, 1997b; Fiscus *et al.*, 1997; Volin *et al.*, 1998). Neither in young Norway spruce (Barnes *et al.*, 1995; Lippert *et al.*, 1996a, 1997; Pfirrmann *et al.*, 1996; Sehmer *et al.*, 1998) nor in O_3-tolerant and O_3-sensitive aspen clones (Kull *et al.*, 1996), however, could any counteracting effect of elevated CO_2 on O_3-induced damage be detected. In oak and poplar species, CO_2-induced compensation of O_3 impact appeared to be mediated through stomatal narrowing similar to that found in crop plants (Volin & Reich, 1996; Manes *et al.*, 1998; Volin *et al.*, 1998), although the type and extent of the tree response might depend on the species and experimental conditions (Saxe *et al.*, 1998). The issue of CO_2–O_3 interaction has not been addressed, however, for *F. sylvatica*, one of the most important broad-leaf tree species in Central Europe.

The present study aims, therefore, to clarify whether long-term acclimation to elevated CO_2 may counteract the impact of chronic O_3 exposure in the photosynthetic performance of *F. sylvatica*, and if such a compensation might be mediated by stomatal limitation on O_3 uptake. The analysis focuses on the light and dark reactions of photosynthesis, that is chlorophyll fluorescence and leaf gas exchange.

MATERIAL AND METHODS

Plants and treatments

One-yr-old plants of European beech (*F. sylvatica* L.) grown from seeds were planted in 1995 into 10-l pots containing a mixed soil (1/3 quartz sand and 2/3 B-horizon of a dystric cambisol from the Bavarian Forest) and transferred into a shaded glasshouse. Plants were amply provided with deionized water and fertilized regularly during irrigation to ensure non-limiting water and nutrient supply throughout the entire experiment. During the second growing season in 1995, plants were acclimated in the glasshouse to ambient (*c.* 367 $\mu l\ l^{-1}$; Amb. CO_2) or elevated (ambient + 300 $\mu l\ l^{-1}$; +300 CO_2) CO_2 concentrations. During the winter months of 1994–95 and 1995–96, plants were placed outside the glasshouse and exposed to field conditions. In April 1996 (before bud break), the plants were moved back into the glasshouse to their corresponding CO_2 regimes, and, on 2 May, transferred into the four phytotrons of the GSF National Research Center (Payer *et al.*, 1993). Here, the soil moisture of each pot was continuously controlled by tensiometers, triggering irrigation whenever soil water tension had reached 300 hPa. The amounts of water applied to each pot throughout the year to compensate for evapotranspiration were integrated by 1-wk intervals. Whole-tree transpiration rate was related to the actual foliage area present each week and corrected for soil evaporation (estimated 40 ml per pot per d; Jungermann, 1998).

Each phytotron was divided into four subchambers (made of plexiglass and containing six plants each) with independent fumigation control to allow the two CO_2 treatments to be combined with the fluctuating regimes of ambient ($1 \times O_3$) or proportionally increased, twice-ambient O_3 concentrations ($2 \times O_3$). In total, each of the resulting four CO_2/O_3 regimes (i.e. Amb. $CO_2/1 \times O_3$, Amb. $CO_2/2 \times O_3$, $+300\ CO_2/1 \times O_3$ and $+300\ CO_2/2 \times O_3$) was replicated four times across the phytotrons. The $1 \times O_3$ regime was based on the 2-h means

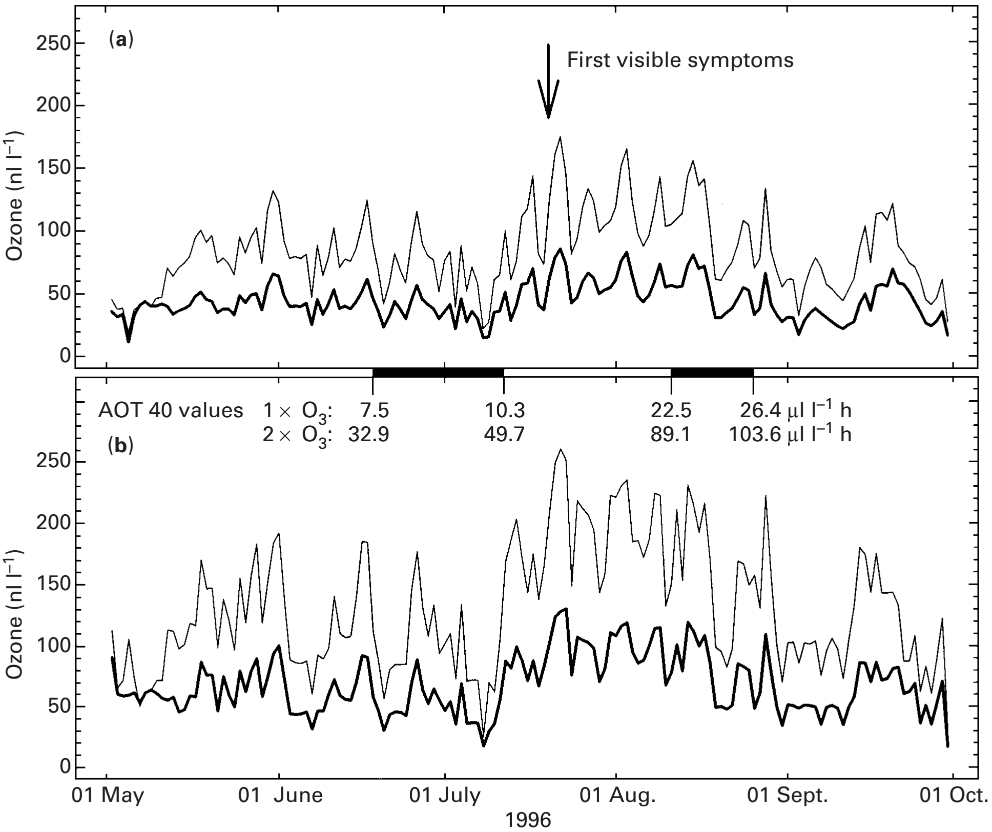

Fig. 1. Time course of (a) daily mean and (b) maximum ozone concentrations as measured inside the fumigation chambers during the growing season. Data are means from eight fumigation chambers. Black bars indicate the measurement periods, with AOT 40 (accumulated O_3 exposure above a threshold of 40 nl l^{-1}) values at the beginning and end of each period.

of the O_3 concentration recorded throughout the growing season of 1990 at the rural site of Schönenbuch near Basel (Switzerland) where O_3 levels were low before July but rather high during midsummer (Fig. 1). Ozone was generated by electric discharge from pure oxygen (Ozone Generator 500, Fischer, Meckenheim, Germany) and continuously controlled by means of a UV-absorption monitor (CSI 3100, Columbia Scientific Industries, Austin, TX, USA); CO_2 was monitored by a photo-acoustic analyser (Siemens, Munich, Germany). Each phytotron was programmed to reproduce the PPFR over the waveband 400–700 nm, air temperature and relative humidity of the Schönenbuch site as they were measured in 1990 in parallel to the O_3 time course. The light spectrum employed in the phytotrons was close to that of sunlight (including UV-B), using four different types of lamps (metal halide, quartz halogen, blue fluorescence and UV-B lamps), and diurnal and seasonal fluctuations in the light spectrum and in PPFR were simulated by switching between different numbers of lamps per lamp type (for details see Thiel *et al.*, 1996). As PPFR was confined inside the sub-chambers to 800 µmol m^{-2} s^{-1} at maximum, low irradiance of overcast days was increased to reach seasonal light sums similar to those at the Schönenbuch site.

Assessment of leaf gas exchange

Measurements were conducted in 1996 on one individual, fully developed, attached and unshaded leaf per tree during early summer (18 June–7 July, before high O_3 levels in mid-July resulted in visible leaf injury) and midsummer (11–26 August, after O_3-caused leaf necroses had established; Fig. 2). Expressing the ‘critical level for ozone’ according to Kärenlampi & Skärby (1996) as an AOT 40 dose (i.e. accumulated O_3 exposure above a threshold of 40 nl l^{-1}), a value of 50 µl l^{-1} h, as found by Lippert *et al.* (1996b) to lower the photosynthetic capacity of *F. sylvatica* by 10%, had not been reached during the first measurement period (Fig. 1). By the beginning of the second measurement period, however, AOT40 of the 2 × O_3 treatment had amounted to 89.1 µl l^{-1} h in the presence of visual leaf injury. The steady-state dependence of the leaf gas exchange (net CO_2 uptake rate and stomatal conductance) on the CO_2 concentration (A/c_i-curves) and PPFR (light response curves) was assessed within the phytotrons, using a mini-cuvette system (H. Walz, Effeltrich, Germany) and a CO_2/H_2O diffusion porometer (HCM-1000, H. Walz). Measurements were performed during the morning hours under standardized conditions (O_3-free air of 20.0°C, dewpoint at 10.0°C), resulting in a relative humidity of *c.* 53% inside the gas exchange

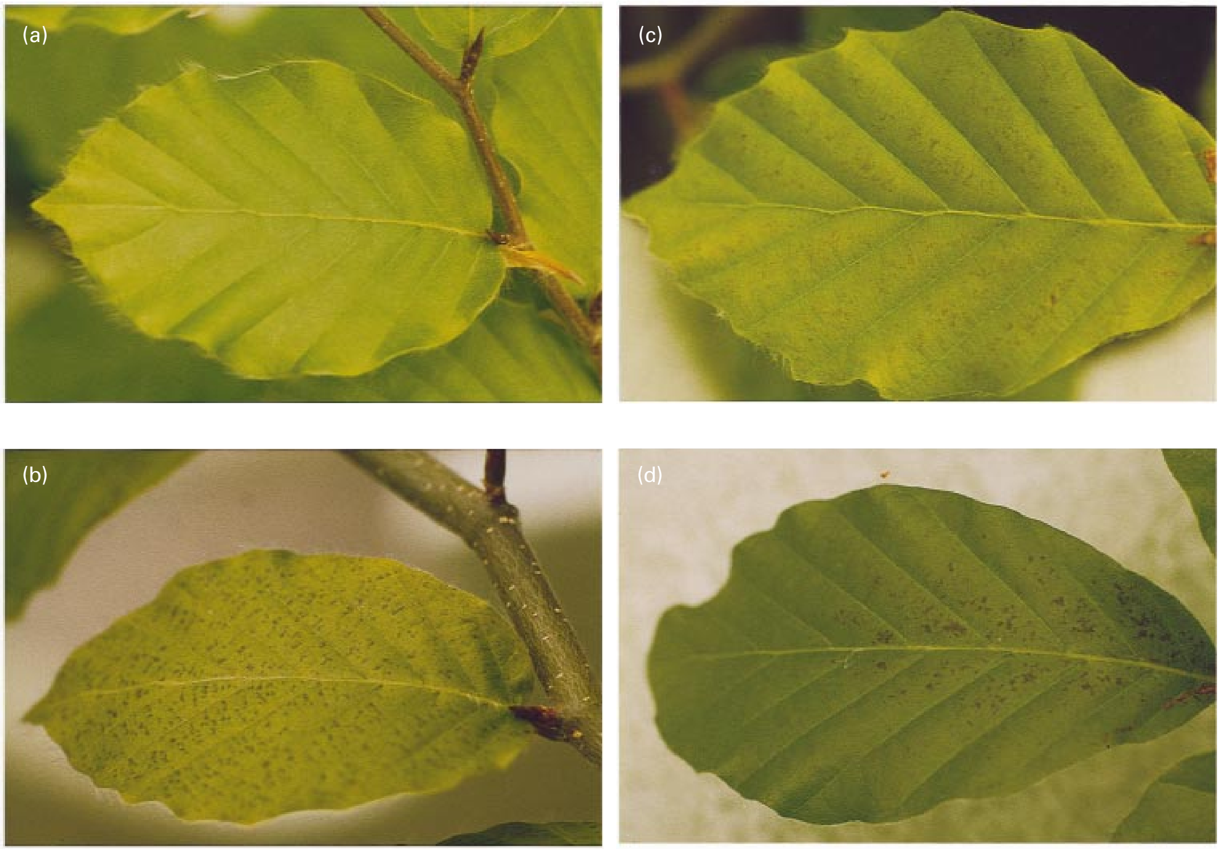

Fig. 2. Characteristic necrotic lesions on four different leaves of *Fagus sylvatica* exposed to the four fumigation regimes: (a) Amb. $CO_2/1 \times O_3$; (b) Amb. $CO_2/2 \times O_3$; (c) $+300$ $CO_2/1 \times O_3$; (d) $+300$ $CO_2/2 \times O_3$. Pictures were taken on August 13 (ambient CO_2 levels) and August 28 ($+300$ CO_2 levels).

cuvette (cf. Lange *et al.*, 1986). Light responses of leaf gas exchange were measured at PPFR of *c.* 0, 5, 40, 80, 150 and 550 μmol m^{-2} s^{-1} and saturating CO_2 supply (1900 μl l^{-1}), whereas responses to CO_2 supply were assessed at *c.* 50, 150, 250, 350 and 2000 μl l^{-1} and saturating PPFR (1800 μmol m^{-2} s^{-1}). Photosynthetic capacity was derived from both saturating PPFR and CO_2 conditions; apparent carboxylation efficiency (CE) and quantum yield (Φ_{CO2}) were calculated as already explained from the linear sections of the photosynthetic CO_2 and light response functions, respectively. A total of 64 response functions were recorded for each of light and CO_2 dependence of the leaf gas exchange. Each measured leaf was harvested to determine its area and concentrations of chlorophyll and nitrogen (see later section). Rates of gas exchange were calculated according to von Caemmerer & Farquhar (1981).

Assessment of chlorophyll a fluorescence

Chlorophyll fluorescence of leaves of the same plants that were subjected to the gas exchange analysis was assessed with a pulse-amplitude modulation fluorometer (PAM 2000, H. Walz), resulting in a total of 64 response functions to PPFR. The leaf was positioned with a clip at a constant distance (approx. 1 cm) and angle (60°) to the fibre optics of the fluorometer (Bilger *et al.*, 1995). PPFR was determined closely above the leaf surface by means of a micro-quantum sensor that had been calibrated with a LI-COR 190 sensor (Li-Cor Inc., Lincoln, NE, USA) as a reference. In all treatments, light responses were measured during the early morning (to prevent photoinhibition by high midday irradiance) and at ambient CO_2, while leaf temperature was 23.2 ± 0.3°C (mean ± SE) and 24.3 ± 0.1°C in June/July and August, respectively. The sequence of readings was started after 30 min of dark adaptation with the determination of minimum and maximum fluorescence, F_0 and F_m, and continued with the assessment of responses to PPFR levels of *c.* 8, 12, 20, 30, 50, 70, 105, 150, 230, 330, 480 and 1500 μmol m^{-2} s^{-1} using the internal LED and halogen lamp of the PAM 2000 as a light source. Each level of PPFR was applied for 6.5 min to ensure steady-state in the photosynthetic response. Parameters of chlorophyll fluorescence were calculated as described by van Kooten & Snel (1990; non-photochemical quenching, NPQ; reduction state of Q_A, $1 - q_P$), Schreiber & Bilger (1993; optimum electron quantum yield, $F_v/F_m{}'$; effective electron quantum yield, $\Delta F/F_m$) and Bilger *et al.* (1995; relative excessive PPFR as a measure for the amount of light absorbed by the leaf that was not used for photosynthesis). The apparent electron transport rate through PSII

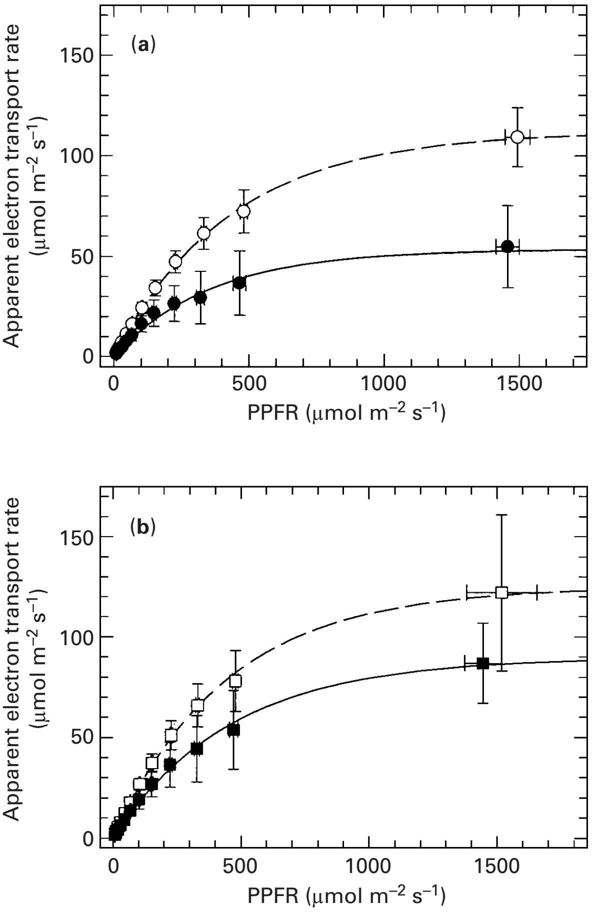

Fig. 3. Light response curves of apparent electron transport rate of *Fagus sylvatica* in August grown under ambient (open symbols) or twice ambient (closed symbols) ozone levels in combination with (a) ambient and (b) +300 CO_2 regimes. Data shown are means (± 1 SE) from eight light response curves of eight different plants per treatment. All measurements were conducted under ambient CO_2 concentrations.

(ETR) was estimated according to Krall & Edwards (1992) as $ETR = \Delta F/F'_m \times PPFR \times a \times f$, assuming the absorptivity of photosynthetic active radiation, a, in the leaves of *F. sylvatica* as 0.84, and setting the light distribution factor between photosystem I and II, *f*, as 0.5 (cf. Krall & Edwards, 1992).

Analysis of leaf chlorophyll and nitrogen

After completion of the gas exchange analysis in the mini-cuvette, each measured leaf was excised and frozen immediately in liquid nitrogen to be stored at −80°C. Chlorophyll concentration was determined according to Porra *et al.* (1989) after chlorophyll extraction in 80% acetone. Nitrogen concentration was assessed by elementary analysis in samples of dried leaf material (Schramel, 1988).

Statistical analysis

Response curves of chlorophyll fluorescence and gas exchange to variable PPFR and CO_2 supply were fitted by means of the Sigma Plot 3.0 fit function (Jandel Scientific, Erkrath, Germany):

$$f(x) = a \times (1 - e^{-bx}) + c$$

According to this equation, the photosynthetic capacity at saturating light and CO_2 supply was calculated as the saturation level $(a+c)$ of the fitted CO_2 response curves. The quantum yield of net CO_2-uptake (Φ_{CO2}) was calculated from the PPFR response function of the net CO_2 uptake rate as the first derivative at 50 µmol photons m^{-2} s^{-1}, and CE was derived accordingly from the CO_2 response function at 100 µl l^{-1} CO_2. For fitting the light response of ETR, *c* was set to zero, while saturation level and initial slope (at 50 µmol photons m^{-2} s^{-1}) were derived according to the analysis of the gas exchange data (cf. Fig. 3). Means of each fumigation treatment ($n = 8$) were compared through two-factorial (CO_2 and O_3) analysis of variance (ANOVA), using the Statistical Analysis Software (SAS version 6.12, SAS Institute Inc., NC, USA). Subsequently, the Tukey Studentized Range Test was applied whenever the null hypothesis was rejected (Dufner *et al.*, 1992).

RESULTS

The optimum electron quantum yield (F_v/F_m) did not show significant differences in June/July between the treatments (Table 1/1), whereas acclimation to elevated CO_2 had raised, to some extent, the relative excessive PPFR (Table 1/2). This latter effect may relate to the significantly raised reduction state of Q_A at light saturation (Table 1/4). Reduced electron transport was also indicated by the lowered saturation level of ETR after acclimation to +300 CO_2 (Table 1/6). However, non-photochemical quenching (NPQ) was not increased at elevated relative to ambient CO_2 (Table 1/3). Likewise, the apparent quantum yield of ETR (Φ_{PSII}) and CO_2 uptake (Φ_{CO2}) remained unchanged (Table 1/6,7). Regarding the photosynthetic dark reactions, acclimation to +300 CO_2 induced minor reductions in CE and PC (Table 1/8,9). Overall, effects by elevated CO_2 were rather small and not significant in June/July, while O_3 impact was not detectable in leaf photosynthesis. Furthermore, leaves did not display macroscopic injury in June/July (not shown).

During both measurement periods (June/July and August), the fresh-weight : area ratio of leaves (f. wt : a) were slightly but significantly increased in +300 CO_2 relative to Amb. CO_2 plants (Tables 2, 3), in the absence of any apparent ozone effect. The fresh-weight related chlorophyll (a and b) concentration displayed an overall decline from June/July to August, with the lowest levels occurring in leaves of +300 CO_2 plants at each sampling date. However, chlorophyll a : b-ratios remained unchanged during the annual course with slightly

Table 1. *Photosynthetic parameters of* Fagus sylvatica *leaves under four different fumigation regimes in June/July*

			Fumigation treatment			
No.	Photosynthetic parameter	Evaluated part of the curve	Amb. CO_2 $1 \times O_3$	Amb. CO_2 $2 \times O_3$	+300 CO_2 $1 \times 0_3$	+300 CO_2 $2 \times O_3$
1	Optimum electron quantum yield (F_v/F_m)	–	0.69 ± 0.02	0.73 ± 0.02	0.70 ± 0.02	0.70 ± 0.05
2	Relative excessive PPFR	Initial slope	0.0026 ± 0.0001	0.0024 ± 0.0001	0.0034 ± 0.0006	0.0032 ± 0.0006
		Saturation level	0.74 ± 0.08	0.83 ± 0.06	0.85 ± 0.02	0.81 ± 0.03
3	Non-photochemical-quenching (NPQ)	Initial slope	0.0048 ± 0.0009	0.0039 ± 0.0006	0.0045 ± 0.0006	0.0051 ± 0.0005
		Maximum	2.00 ± 0.26	2.23 ± 0.17	1.86 ± 0.20	1.84 ± 0.23
4	Reduction state of Q_A $(1-q_P)$	Initial slope	0.0019 ± 0.0002	0.0020 ± 0.0002	0.0021 ± 0.0003	0.0019 ± 0.0003
		Saturation level	0.32 ± 0.04	0.32 ± 0.04	0.60 ± 0.07	0.71 ± 0.08
5	Apparent electron quantum yield (Φ_{PSII}) (mole electrons mol^{-1} $photons^{-1}$)	Initial slope	0.48 ± 0.03	0.52 ± 0.02	0.45 ± 0.06	0.48 ± 0.05
6	Apparent electron transport rate (ETR) ($\mu mol\ m^{-2}\ s^{-1}$)	Saturation level	124 ± 15	123 ± 13	93 ± 20	100 ± 16
7	Apparent quantum yield of CO_2 gas exchange (Φ_{CO2}) (mol CO_2 mol^{-1} photons)	Initial slope	0.042 ± 0.004	0.041 ± 0.003	0.038 ± 0.003	0.039 ± 0.002
8	Carboxylation efficiency (CE) ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$ $\mu l^{-1}\ CO_2$)	Initial slope	0.041 ± 0.005	0.041 ± 0.002	0.032 ± 0.003	0.037 ± 0.003
9	Photosynthetic capacity of CO_2 gas exchange (PC) ($\mu mol\ m^{-2}\ s^{-1}$)	Saturation level	12.9 ± 1.0	14.9 ± 1.4	11.8 ± 0.8	13.7 ± 1.2

F_v/F_m values were derived from measurements under growth conditions. Initial slopes and saturation levels of light response curves of chlorophyll fluorescence were measured under ambient CO_2-concentrations (relative excessive PPFR, NPQ, reduction state of Q_A, apparent electron quantum yield, ETR), light response curves of CO_2-exchange were recorded under CO_2-saturating conditions (apparent quantum yield of CO_2 gas exchange and CO_2-response curves were conducted under light saturating conditions (PC and CE). Data are expressed as means ± 1 SE, $n = 8$.

Table 2. *Fresh weight to area ratio (f. wt:a), fresh weight related chlorophyll content (Chl a+b), chlorophyll a:b ratio and dry weight related nitrogen concentration of leaves of* Fagus sylvatica *used in gas exchange measurements during June/July and August*

	Treatments			
Parameter	Amb. CO_2 1 × O_3	Amb. CO_2 2 × O_3	+300 ppm CO_2 1 × O_3	+300 ppm CO_2 2 × O_3
June/July				
f. wt:a (g m^{-2})	100.04 ± 2.41	102.99 ± 2.54	111.28 ± 2.78	117.15 ± 4.83
Chl a+b (μg g f. wt)	1939.22 ± 138.56	1839.12 ± 123.18	1364.34 ± 44.43	1329.80 ± 62.83
Chl a:b ratio	4.33 ± 0.15	3.84 ± 0.13	4.19 ± 0.21	4.46 ± 0.17
N (mg g^{-1} d. wt)	17.3 ± 0.8	18.1 ± 0.8	14.7 ± 0.6	14.8 ± 0.7
August				
f. wt:a (g m^{-2})	106.18 ± 4.79	102.34 ± 2.54	111.11 ± 3.05	122.63 ± 4.20
Chl a+b (μg g^{-1} f. wt)	1436.14 ± 101.82	1218.97 ± 47.08	926.2 ± 74.45	1022.38 ± 53.67
Chl a:b ratio	3.84 ± 0.16	3.35 ± 0.32	4.36 ± 0.25	4.31 ± 0.11
N (mg g^{-1} d. wt)	16.6 ± 0.8	16.2 ± 0.4	13.2 ± 0.5	14.9 ± 0.6

Data are expressed as means ± 1 SE, $n = 16$.

Table 3. *Main effects of CO_2, O_3 and CO_2–O_3 interactions ($CO_2 \times O_3$) on leaf characteristics and photosynthetic paramaters of* Fagus sylvatica *in June/July and August*

		June/Jule			August		
Parameter	Evaluated part of the curve	CO_2	O_3	$CO_2 \times O_3$	CO_2	O_3	$CO_2 \times O_3$
Fresh wt:area ratio (g m^{-2})	–	***	–	–	**	–	*
Chlorophyll a+b (μg g^{-1} f. wt)	–	***	–	–	***	–	*
Chlorophyll a:b ratio	–	–	–	*	***	–	–
N (mg g^{-1} d. wt)	–	***	–	–	***	–	–
Optimal electron quantum yield (F_v/F_m)	–	–	–	–	–	**	–
Relative excessive PPFR	Initial slope	–	–	–	–	**	–
	Saturation level	–	–	–	–	–	–
Non-photochemical-quenching (NPQ)	Initial slope	–	–	–	**	**	–
	Maximum	–	–	–	–	–	–
Reduction state of Q_A $(1-q_P)$	Initial slope	–	–	–	–	***	–
	Saturation level	***	–	–	–	–	*
Apparent electron quantum yield (Φ_{PSII}) (mol electrons mol^{-1} photons)	Initial slope	–	–	–	*	***	–
Maximum apparent electron transport rate (ETR) (μmol m^{-2} s^{-1})	Saturation level	–	–	–	**	***	–
Apparent quantum yield of CO_2 gas exchange (Φ_{CO2}) (mol CO_2 mol^{-1} photons)	Initial slope	–	–	–	–	–	***
Carboxylation efficiency (CE) (μmol CO_2 m^{-2} s^{1-}μl^{-1} CO_2)	Initial slope	–	–	–	*	***	–
Photosynthetic capacity of CO_2 gas exchange (PC) (μmol m^{-2} s^{-1})	Saturation level	–	–	–	–	*	*

Significant levels of main effects are classified as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

reduced values at Amb. $CO_2/2 \times O_3$. At each date, the leaf nitrogen concentration (expressed on a dry-weight basis) was also significantly reduced by +300 CO_2, while no effect of ozone was detectable (Tables 2, 3).

By August, spot-like brownish necroses had mainly developed at the Amb. $CO_2/2 \times O_3$ treatment, whereas such symptoms were less pronounced at +300 $CO_2/2 \times O_3$ and +300 $CO_2/1 \times O_3$, and injury was hardly visible at Amb. $CO_2/1 \times O_3$. Characteristic ozone-induced necroses in each treatment are shown in Fig. 2. Plants grown at Amb. $CO_2/2 \times O_3$ had, in August, distinctly reduced values of ETR (i.e. lower initial slope and saturation level of the light dependence) compared with plants of the Amb. $CO_2/1 \times O_3$ regime (Fig. 3a). At +300 $CO_2/1 \times O_3$, initial slope and saturation level of the light dependence of ETR were slightly increased relative to those of the Amb. $CO_2/1 \times O_3$ plants (Figs 3a,b). In addition, the reduction in ETR as induced by 2 × O_3 was less pronounced in the presence of elevated CO_2 than at ambient CO_2 supply. Light and CO_2 response curves of other photosynthetic parameters, analysed for their initial slopes and saturation levels, proved to be highly affected by ozone (Tables 3, 4). The optimum electron quantum yield (F_v/F_m) was only *c.*

Table 4. *Photosynthetic parameters of* Fagus sylvatica *leaves under four different fumigation regimes in August*, *otherwise as Table 1*

No.	Photosynthetic parameter	Evaluated part of the curve	Fumigation treatment Amb. CO_2 $1\times O_3$	Amb. CO_2 $2\times O_3$	+300 CO_2 $1\times O_3$	+300 CO_2 $2\times O_3$
1	Optimum electron quantum yield (F_v/F_m)	–	0.70 ± 0.01	0.58 ± 0.05	0.69 ± 0.02	0.62 ± 0.03
2	Relative excessive PPFR	Initial slope	0.00020 ± 0.0002	0.0031 ± 0.0003	0.0022 ± 0.0002	0.0025 ± 0.0002
		Saturation level	0.88 ± 0.04	0.91 ± 0.03	0.86 ± 0.02	0.87 ± 0.06
3	Non-photochemical-quenching (NPQ)	Initial slope	0.0053 ± 0.0009	0.0138 ± 0.0031	0.0032 ± 0.0006	0.0057 ± 0.0013
		Maximum	1.87 ± 0.14	2.59 ± 0.46	1.69 ± 0.24	1.54 ± 0.26
4	Reduction state of Q_A $(1-q_P)$	Initial slope	0.0022 ± 0.0002	0.0035 ± 0.0004	0.0017 ± 0.0002	0.0029 ± 0.0003
		Saturation level	0.59 ± 0.04	0.71 ± 0.05	0.66 ± 0.06	0.59 ± 0.04
5	Apparent electron quantum yield (Φ_{PSII}) (mole electrons mol^{-1} $photons^{-1}$)	Initial slope	0.47 ± 0.02	0.26 ± 0.03	0.51 ± 0.02	0.36 ± 0.04
6	Apparent electron transport rate (ETR) (μmol m^{-2} s^{-1})	Saturation level	113 ± 6	50 ± 8	130 ± 15	96 ± 9
7	Apparent quantum yield of CO_2 gas exchange (Φ_{CO2}) (mol CO_2 mol^{-1} photons)	Initial slope	0.37 ± 0.002	0.022 ± 0.001	0.022 ± 0.003	0.032 ± 0.003
8	Carboxylation efficiency (CE) (μmol CO_2 m^{-2} s^{-1} μl^{-1} CO_2)	Initial slope	0.024 ± 0.002	0.008 ± 0.002	0.026 ± 0.003	0.021 ± 0.003
9	Photosynthetic capacity of CO_2 gas exchange (PC) (μmol m^{-2} s^{-1})	Saturation level	5.9 ± 0.5	1.5 ± 0.5	4.8 ± 0.6	5.2 ± 1.5

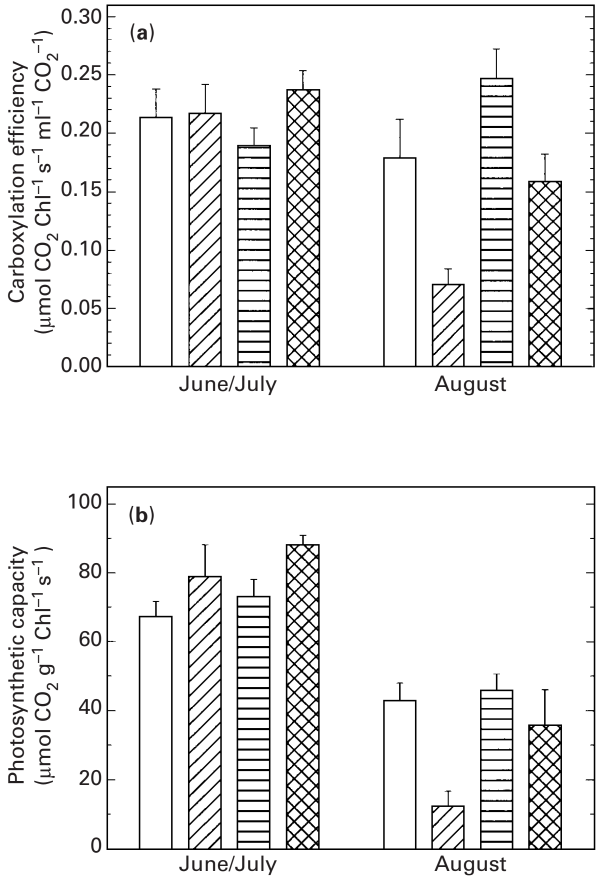

Fig. 4. Carboxylation efficiency (a) and photosynthetic capacity (b) as leaf chlorophyll in June/July and August. Each bar represents a mean (±1 SE) of eight measurements of eight different plants. Treatments: Amb, $CO_2/1 \times O_3$, open bars; Amb. $CO_2/2 \times O_3$, diagonally-hatched bars; +300 $CO_2/1 \times O_3$, bars with horizontal lines; +300 $CO_2/2 \times O_3$ cross-hatched bars.

0.6 at $2 \times O_3$ and *c.* 0.7 at $1 \times O_3$, regardless of the CO_2 supply (Table 4/1). Subsequently, plants exposed to $2 \times O_3$ displayed, at low irradiance, increases in the relative excessive PPFR (Table 4/2), whereas the saturation level remained unchanged in all treatments. Significant increases were also found in the light dependence of the reduction state of Q_A (Table 4/4), with the saturation level being highest at Amb. $CO_2/2 \times O_3$. The +300 $CO_2/2 \times O_3$ exposure did not result in substantial changes of NPQ (Table 4/3) relative to the Amb. $CO_2/1 \times O_3$ treatment. However, NPQ was reduced at +300 $CO_2/1 \times O_3$ and low irradiance. By contrast, the Amb. $CO_2/2 \times O_3$ regime raised NPQ under low-light conditions by *c.* 135%, and the saturation level was increased by *c.* 40% compared with Amb. $CO_2/1 \times O_3$. Doubling of the O_3 concentration significantly lowered both the quantum yield and the light saturation level of ETR by a factor of two at ambient CO_2 concentration (Table 4/5 and 6), and these O_3 effects were paralleled in the CO_2 uptake rate by a marked decline in Φ_{CO2} under ambient rather than elevated CO_2 conditions (Table 4/7). CE and PC were affected most by the enhanced O_3 regime (significant reductions of *c.* 75% each, Table 4/8,9) but the O_3 effects were strongly counterbalanced by elevated CO_2 supply. When the O_3 impact was low, elevated CO_2 supply slightly increased ETR at both low and high-light conditions (Table 4/5,6), although Φ_{CO2} dropped by *c.* 40% relative to the Amb. $CO_2/1 \times O_3$ regime (Table 4/7). The statistical analysis indicated, in June/July, CO_2–O_3 interaction in the chlorophyll a : b ratio and, in August, in the leaf differentiation and reduction state of Q_A, PC and Φ_{CO2} (Table 3). In addition to the main effects on leaf differentiation, elevated CO_2 in August also caused NPQ, Φ_{PSII}, maximum ETR and CE to respond significantly. The enhanced O_3 regime displayed main effects on most of the investigated photosynthetic parameters only in August, but, as already seen in June/July, did not influence the leaf differentiation (Table 3).

To exclude bias by necroses, CE and PC, which appeared to be strongly affected by the CO_2/O_3 regimes when expressed on a leaf area basis, were also related to the chlorophyll concentration of the living leaf tissue (Fig. 4). This recalculation confirmed the conclusions drawn before: in June/July, neither elevated CO_2 nor O_3 levels significantly affected CE or PC, which was distinctly reduced, however, by ozone in August (Fig. 4a). Elevated CO_2 prevented reduction of both, CE and PC, by the $2 \times O_3$ regime, and low O_3 stress enabled the high CO_2 supply to slightly increase CE.

The transpiration rate of all plants was highest in July (Fig. 5). During the first half of the growing season, plants exposed to ambient CO_2 tended to show, regardless of the ozone regime, a significantly higher transpiration rate than plants at +300 CO_2, whereas in August and early September the influence of CO_2 supply appeared to diminish. Throughout the whole period depicted in Fig. 5, trees grown at +300 CO_2 transpired *c.* 25% less water (73.0 and 75.1 l m^{-2} at $1 \times O_3$ and $2 \times O_3$, respectively) than did plants at Amb. CO_2 (98.8 and 101.3 l m^{-2} at $1 \times O_3$ and $2 \times O_3$, respectively). In June/July the stomatal conductance (g_{H2O}) ranged between 90 and 180 mmol m^{-2} s^{-1} in all treatments, when the apparent CO_2 concentration in the intercellular space of the mesophyll (c_i) was < 300 µl l^{-1} (Fig. 6a,b). At high c_i, however, g_{H2O} was lowered to 50 mmol m^{-2} s^{-1} in plants grown at $2 \times O_3$ regardless of the CO_2 regime as well as in plants grown at +300 $CO_2/1 \times O_3$. The stomatal conductance stayed as high as 100 mmol m^{-2} s^{-1} in plants exposed to the Amb. $CO_2/1 \times O_3$ regime. In August, plants exposed to $2 \times O_3$ displayed, at high c_i, levels of g_{H2O} being doubled relative to the findings in June/July. At $1 \times O_3$ and at $c_i < 300$ µl l^{-1} for plants exposed to $2 \times O_3$, seasonal changes in g_{H2O} were less pronounced (Fig. 6d). However, plants grown at Amb. $CO_2/1 \times O_3$ had reached the highest g_{H2O} levels between 140–170 mmol m^{-2} s^{-1} in August (Fig. 6c).

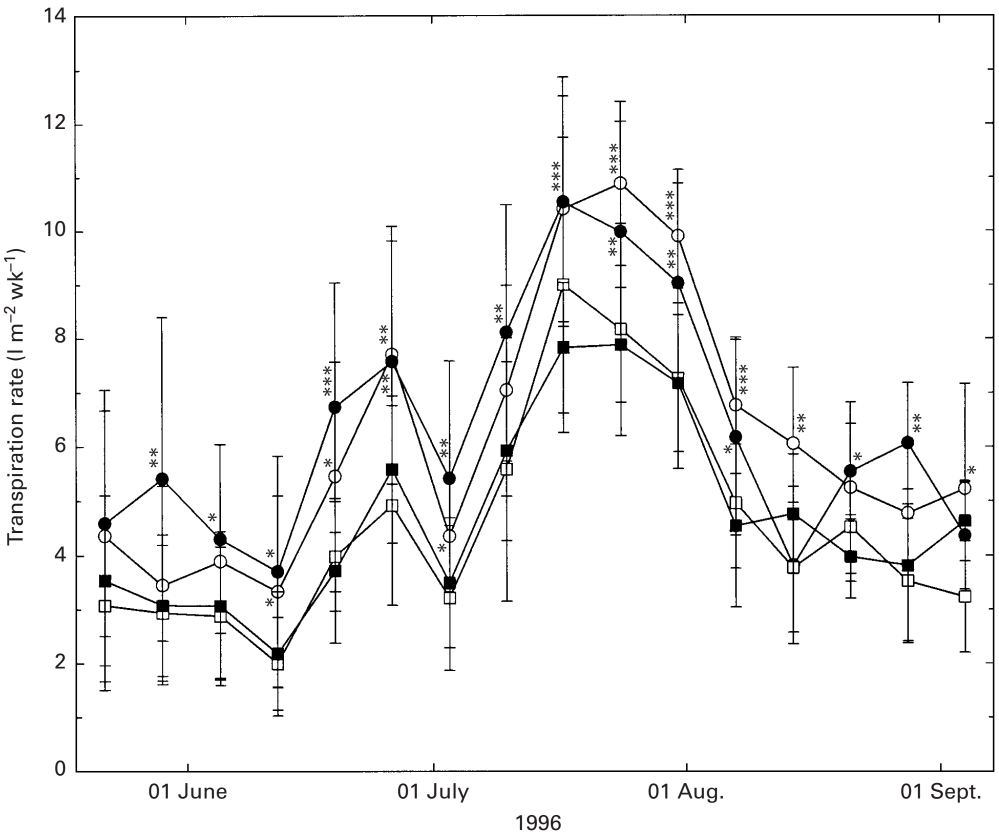

Fig. 5. Transpiration rates of *Fagus sylvatica* from 22 May to 4 September 1996 as derived from the amounts of irrigation water supplied each week. Data are expressed as means ± 1 SE, $6 \leqslant n \leqslant 16$. Significance levels between treatments (same O_3 levels, Amb. CO_2 vs. +300 CO_2) according to Student's *t*-test: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Ambient $CO_2/1\times O_3$ (open circles); +300 $CO_2/2\times O_3$ (closed squares); ambient $CO_2/2\times O_3$ (closed circles); +300 $CO_2/1\times O_3$ (open squares).

DISCUSSION

While the nitrogen concentration of the beech leaves was stable throughout the year under the ambient CO_2 regime, chlorophyll levels showed some decrease in August compared with June/July. However, long-term acclimation to elevated CO_2 resulted in a significant decrease in both the chlorophyll and N levels (cf. Tables 2, 3), as previously shown in other studies on trees (Eamus *et al.*, 1995; Epron *et al.*, 1996; Curtis & Wang, 1998), and by August, the chlorophyll a : b ratio was increased at elevated CO_2 supply (cf. Table 2). Nevertheless, most parameters of electron transport (cf. Table 1) remained unchanged, with the exception, however, of the reduction state of Q_A which was increased at photosynthetic light saturation. This increase probably resulted from analysing the light dependence of chlorophyll fluorescence under ambient CO_2 supply, which caused an approx. 20% decrease in ETR between the photosystems and may have led to an electron overflow at PSII. However, Φ_{CO2}, CE and PC were hardly reduced at elevated CO_2 supply, which has been confirmed by C. Lütz (pers. comm.) who also found, in the same experimental plants, a rather stable PC when assessed as photosynthetic oxygen evolution. Slight reductions in Φ_{CO2}, CE and PC under elevated CO_2 supply are in agreement with earlier reports on *F. sylvatica* (El Kohen *et al.*, 1993; Epron *et al.*, 1996; Mousseau *et al.*, 1996; Grams & Matyssek, 1999) and with a recent meta-analysis by Curtis & Wang (1998) of >500 reports on CO_2 effects in trees.

In August, O_3 effects became apparent in the photosynthetic performance of the beech plants (cf. Fig. 2, Tables 3, 4) and were most probably the consequence of high episodic O_3 levels which had occurred since mid-July (cf. Fig. 1). Macroscopic O_3 injury of leaves (i.e. bronze discoloration and spot-like necroses) became visible towards the end of July, and premature leaf loss set in during early August (S. Anegg & C. Langebartels, pers. comm.). Lippert *et al.* (1996b) observed a similar progression of O_3 injury when beech was exposed to similar growth conditions at ambient CO_2 and four different O_3 regimes between 0.2 and 2.0 × ambient O_3. Also the O_3-induced decline in CE, PC, Φ_{CO2} was confirmed by Lippert *et al.* (1996b) and was consistent with several other studies on *F. sylvatica* (Mikkelsen, 1995; Zeuthen *et al.*, 1997; Dixon *et al.*, 1998) and other tree species (Matyssek *et al.*, 1991, 1993; Samuelson, 1994; Kull *et al.*, 1996; Lippert *et al.*,

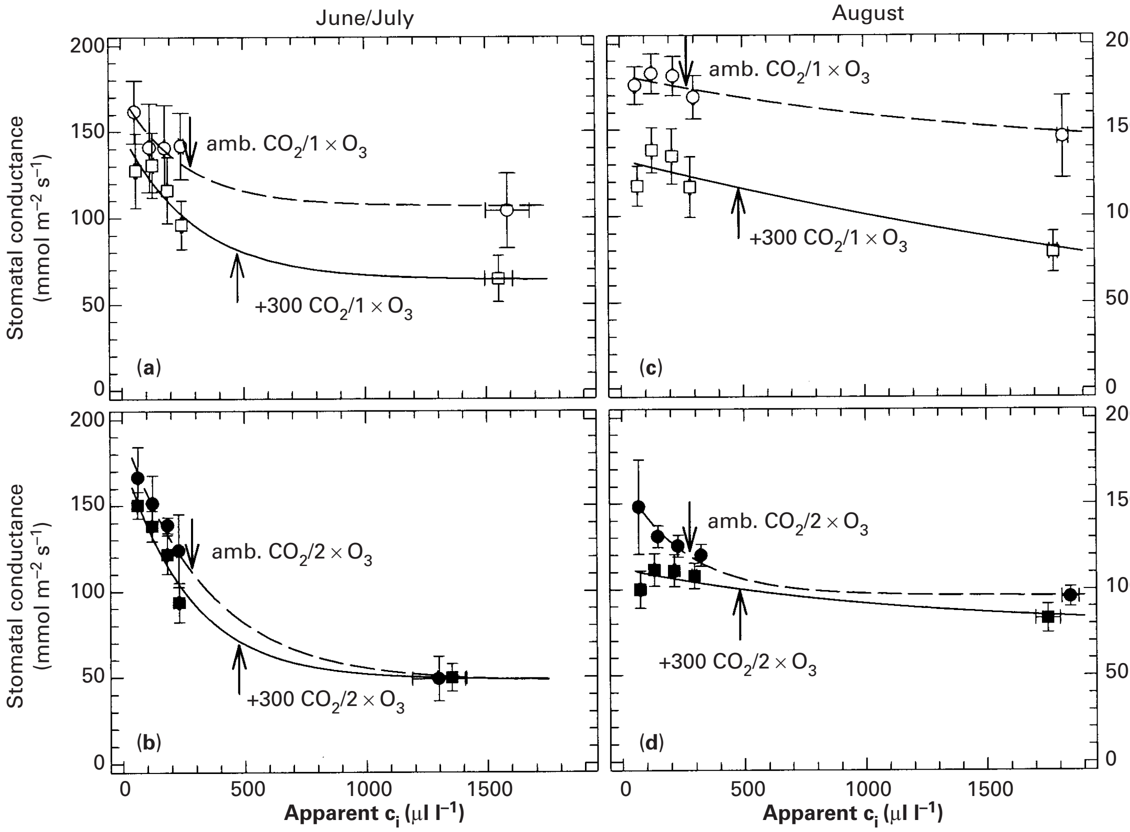

Fig. 6. CO_2 response curves of stomatal conductance of *Fagus sylvatica* in June/July (a, b) and August (c, d) grown under ambient (open symbols) and twice-ambient (closed symbols) ozone exposure and in combination with ambient (circles) or +300 CO_2 supply (squares). The arrows mark stomatal conductance of plants at growth conditions. Data shown are means (±1 SE) from eight CO_2 response curves of eight different plants per treatment. All measurements were conducted under saturating light conditions.

1996a; Wieser, 1997). The observed decline in the area-based CO_2 uptake rate was not only due to the spreading of necrotic lesions in the leaf area, but relating CE and PC to the leaf chlorophyll content (cf. Fig. 4) also indicated lowered photosynthesis in the remaining, still living leaf tissue. Thus, reductions in CE (cf. Table 4) as also found in other studies (Lehnherr *et al.*, 1987; Matyssek *et al.*, 1991; Lippert *et al.*, 1996b; Reichenauer *et al.*, 1997) may be explained, at least in part, by an inhibited Rubisco activity in the living cells. In fact, such an inhibition was found by C. Lütz (pers. comm.) in the same experimental plants by directly assessing the activity of Rubisco. The loss of Rubisco activity is generally associated with decreasing protein levels as observed in herbaceous plants under O_3 stress (Sane *et al.*, 1996; Pell *et al.*, 1997). However, the signal transduction from the apoplast as the primary site of the O_3 impact to the chloroplasts with their photosynthetic processes still needs to be clarified (Sandermann *et al.*, 1998).

While elevated CO_2 did not cause a significant down-regulation in photosynthesis (cf. Tables 1, 4), it counteracted the adverse effects of the $2 \times O_3$ regime on the investigated photosynthetic parameters (cf. Fig. 3, Tables 3, 4). Thus, the photosynthetic dark and light reactions were hardly affected under co-occurring elevated CO_2 and O_3 levels relative to the Amb. $CO_2/1 \times O_3$ regime. Several other studies on broad-leaf trees report on a similar buffering capacity of elevated CO_2 supply towards O_3 impact (Volin & Reich, 1996; Manes *et al.*, 1998; Volin *et al.*, 1998), although such a compensation did not occur in aspen clones (Kull *et al.*, 1996) and Norway spruce (Barnes *et al.*, 1995; Lippert *et al.*, 1997). The compensation of O_3-induced reductions in the area-based CO_2 uptake rate cannot only be explained by a diminished proportion of the necrotic leaf area at elevated CO_2 supply, as indicated by expressing CE and PC on a leaf chlorophyll basis (cf. Fig. 4 and discussion above). Most studies on broad-leaf trees and herbaceous plants claim a lowered O_3 uptake rate, mediated through stomatal narrowing in response to elevated CO_2, as the cause of reduced O_3 injury (McKee *et al.*, 1995, 1997b; Volin & Reich, 1996; Fiscus *et al.*, 1997; Manes *et al.*, 1998, Volin *et al.*, 1998). However, in broad-leaf trees long-term exposure to elevated CO_2 often appears to reduce stomatal conductance only in the range of 10–20% (Heath & Kerstiens, 1997; Curtis & Wang, 1998; Saxe *et al.*, 1998). In this present study, g_{H2O} of plants grown at +300 CO_2 tended to be reduced in June/July (Figs 5, 6). At the end of the growing season, the influence of CO_2 supply appeared to diminish (Figs 5, 6; K.-H. Häberle, pers. comm.). Thus, the compensatory effect of high CO_2 supply on the photosynthetic decline caused by ozone, as found in this study, appears to be only in part mediated through stomatal behaviour. Moreover, one has to keep in mind that episodes of high O_3 levels most likely coincide with high leaf-to-air water vapour pressure deficit and soil drought. Because of the reduced responsiveness of stomata to elevated CO_2 under low water availability, the protective effect of elevated CO_2 against O_3 injury may be much less than is often assumed (Heath, 1998). Rather, metabolic compensation in the leaf mesophyll needs to be considered as concluded by S. Anegg & C. Langebartels (pers. comm.) for same experimental beech plants. Overall, long-term acclimation to elevated CO_2 supply does counteract the O_3-induced decline of photosynthetic light and dark reactions in *F. sylvatica*.

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REFERENCES

Allen Jr LH. 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. *Journal of Environmental Quality* **19**: 15–34.

Barnes JD, 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.

Barnes JD, Wellburn AR. 1998. Air pollutant combinations. In: De Kok LJ, Stulen I, eds. *Responses of plant metabolism to air pollution and global change*. Leiden, The Netherlands: Backhuys, 147–164.

Bilger W, Schreiber U, Bock M. 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* **102**: 425–432.

Ceulemans R, Mousseau M. 1994. Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist* **17**: 425–446.

Curtis PS, Wang X. 1998. A meta-analysis of elevated CO_2 effects on wood plant mass, form, and physiology. *Oecologia* **113**: 299–313.

Dixon M, Le Thiec D, Garrec JP. 1998. Reactions of Norway spruce and beech trees to two years of ozone exposure and episodic drought. *Environmental and Experimental Botany* **40**: 77–91.

Drake BG, Gonzàlez-Meler MA, Long SP. 1996. More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609–639.

Dufner J, Jensen U, Schumacher E. 1992. *Statistik mit SAS*. Stuttgart, Germany: Teubner-Verlag.

Eamus D, Duff GA, Berryman CA. 1995. Photosynthetic responses to temperature, light flux-density, CO_2 concentration and vapour pressure deficit in *Eucalyptus tetrodonta* grown under CO_2 enrichment. *Environmental Pollution* **90**: 41–49.

Eamus D, Jarvis PG. 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Advances in Ecological Research* **19**: 1–55.

El Kohen A, Venet L, Mousseau M. 1993. Growth and photosynthesis of two deciduous forest species at elevated carbon dioxide. *Functional Ecology* **7**: 480–486.

Epron D, Liozon R, Mousseau M. 1996. Effects of elevated CO_2 concentration on leaf characteristics and photosynthetic capacity of beech (*Fagus sylvatica*) during the growing season. *Tree Physiology* **16**: 425–432.

Fiscus EL, Reid CD, Miller JE, Heagle AS. 1997. Elevated CO_2 reduces O_3 flux and O_3-induced yield losses in soybeans: possible implications for elevated CO_2 studies. *Journal of Experimental Botany* **48**: 307–313.

Grams TEE, Matyssek R. 1999. Elevated CO_2 counteracts the limitation by chronic ozone exposure on photosynthesis in *Fagus sylvatica* L.: comparison between chlorophyll fluorescence and leaf gas exchange. *Phyton* 39. (In press.)

Heath J. 1998. Stomata of trees growing in CO_2-enriched air show reduced sensitivity to vapour pressure deficit and drought. *Plant, Cell and Environment* **21**: 1077–1088.

Heath J, Kerstiens G. 1997. Effects of elevated CO_2 on gas exchange in beech and oak at two levels of nutrient supply: consequences for sensitivity to drought in beech. *Plant, Cell and Environment* **20**: 57–67.

Intergovernmental Panel on Climate Change (IPCC). 1996. *Climate change* 1995: *the science of climate change*. In: Houghton JT, Meira Filho LG, Callander BA, Harris N, Kattenberg A, Maskell K, eds.. Cambridge, UK: Cambridge University Press.

Jungermann A. 1998. *Photosynthese, Wasserbedarf und Allokation in Buche* (Fagus sylvatica *L.*) *unter kombinierten* CO_2/O_3*-Regimen*. Diploma-thesis, LMU München, Germany.

Kärenlampi L, Skärby L. 1996. *Critical levels for ozone in Europe: testing and finalizing the concepts*. UN-ECE Workshop Report. Kuopio, Finland: University of Kuopio.

Keeling CD, Whorf TP, Whalen M, van der Plicht J. 1995. Interannual extremes in the rate of atmospheric carbon dioxide since 1980. *Nature* **375**: 666–670.

King AW, Emanuel WR, Post WM. 1992. Projecting future concentrations of atmospheric CO_2 with global carbon cycle models: the importance of simulating historical changes. *Environmental Management* **16**: 91–108.

Krall JP, Edwards GE. 1992. Relationship between photosystem II activity and CO_2 fixation. *Physiologia Plantarum* **86**: 180–187.

Kull O, Sober A, Coleman MD, Dickson RE, Isebrands JG, Gagnon Z, Karnosky DF. 1996. Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO_2. *Canadian Journal of Forest Research* **26**: 639–648.

Lange OL, Gebel J, Schulze E-D, Walz H. 1986. Eine Methode zur raschen Charakterisierung der photosynthetischen Leistungsfähigkeit von Bäumen unter Freilandbedingungen – Anwendung zur Analyse 'neuartiger Waldschäden' bei der Fichte. *Forstwissenschaftliches Centralblatt* **104**: 186–198.

Langebartels C, Ernst D, Heller W, Lütz C, Payer H-D, Sandermann H Jr. 1997. Ozone responses of trees: results from controlled chamber exposures at the GSF phytotron. In: Sandermann H Jr, Wellburn AR, Heath RL, eds. *Forest decline and ozone*. Berlin, Germany: Springer-Verlag, 163–200.

Lehnherr B, Grandjean A, Mächler F, Fuhrer J. 1987. The effect of ozone in ambient air on ribulose-bisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. *Journal of Plant Physiology* **130**: 189–200.

Lippert M, Häberle K-H, Steiner K, Payer H-D, Rehfuss K-E. 1996a. Interactive effects of elevated CO_2 and O_3 on photosynthesis and biomass production of clonal 5-year-old Norway spruce [*Picea abies* (L.) Karst.] under different nitrogen nutrition and irrigation treatments. *Trees* **10**: 382–392.

Lippert M, Steiner K, Payer H-D, Simons S, Langebartels C, Sandermann H Jr. 1996b. Assessing the impact of ozone on photosynthesis of European beech (*Fagus sylvatica* L.) in environmental chambers. *Trees* **10**: 268–275.

Lippert M, Steiner K, Pfirrmann T, Payer H-D. 1997. Assessing the impact of elevated O_3 and CO_2 on gas exchange characteristics of differently K supplied clonal Norway spruce trees during exposure and the following season. *Trees* **11**: 306–315.

Long SP. 1991. Modification of responses of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? *Plant, Cell and Environment* **14**: 729–739.

Manes F, Vitale M, Donato E, Paoletti E. 1998. O_3 and O_3+CO_2 effects on a Mediterranean evergreen broadleaf tree, Holm Oak (*Quercus ilex* L.). *Chemosphere* **36**: 801–806.

Matyssek R, Günthardt-Goerg MS, Keller T, Schneidegger C. 1991. Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations. *Trees* **5**: 5–13.

Matyssek R, Günthardt-Goerg MS, Maurer S, Keller T. 1995. Night-time exposure to ozone reduces whole-plant production in *Betula pendula*. *Tree Physiology* 15: 159–165.

Matyssek R, Keller T, Koike T. 1993. Branch growth and leaf gas exchange of *Populus tremula* exposed to low ozone concentrations throughout two growing seasons. *Environmental Pollution* **79**: 1–7.

McKee IF, Bullimore JF, Long SP. 1997a. Will elevated CO_2 concentrations protect the yield of wheat from O_3 damage? *Plant, Cell and Environment* **20**: 77–84.

McKee IF, Eiblmeier M, Polle A. 1997b. Enhanced ozone-tolerance in wheat grown at an elevated CO_2 concentration: ozone exclusion and detoxification. *New Phytologist* **137**: 275–284.

McKee IF, Farage PK, Long SP. 1995. The interactive effects of elevated CO_2 and O_3 concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45**: 111–119.

Mikkelsen TN. 1995. Physiological responses of *Fagus sylvatica* L. exposed to low levels of ozone in open-top chambers. *Trees* **9**: 355–361.

Mikkelsen TN, Heide-Jörgensen HS. 1996. Acceleration of leaf senescence in *Fagus sylvatica* L. by low levels of tropospheric ozone demonstrated by leaf colour, chlorophyll fluorescence and chloroplast ultrasturcture. *Trees* **10**: 145–156.

Mousseau M, Dufrêne E, El Kohen A, Epron D, Godard D, Liozon R, Pontailler JY, Saugier B. 1996. Growth strategy and tree responses to elevated CO_2: a comparison of beech (*Fagus sylvatica* L.) and sweet chestnut (*Castanea sativa* Mill.). In: Koch GW, Mooney HA, eds. *Carbon dioxide and terrestrial ecosystens*. London, UK: Academic Press, 71–86.

Mulholland BJ, Craigon J, Black CR, Colls JJ, Atherton J, Landon G. 1997. Impact of elevated atmospheric CO_2 and O_3 on gas exchange and chlorophyll content in spring wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **48**: 1853–1863.

Neftel A, Moor E, Oeschger H, Stauffer B. 1985. Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries. *Nature* **315**: 45–47.

Payer H-D, Blodow P, Köfferlein M, Lippert M, Schmolke W, Seckmeyer G, Seidlitz H, Strube D, Thiel S. 1993. Controlled environment chambers for experimental studies on plant responses to CO_2 and interactions with pollutants. In: Schulze E-D, Mooney HA, eds. *Design and execution of experiments on CO_2 enrichment*. Brussels, Belgium: Commission European Communities, 127–145.

Pearson M, Mansfield TA. 1993. Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). *New Phytologist* **123**: 351–358.

Pearson M, Mansfield TA. 1994. Effects of exposure to ozone and water stress on the following season's growth of beech (*Fagus sylvatica* L.). *New Phytologist* **126**: 511–515.

Pell EJ, Schlagnhaufer CD, Arteca RN. 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. *Phyiologium Plantarum* **100**: 264–273.

Pfirrmann T, Barnes JD, Steiner K, Schrammel P, Busch U, Küchenhoff, Payer H-D. 1996. Effects of elevated CO_2, O_3 and K deficiency on Norway spruce (*Picea abies*): nutrient supply, content and leaching. *New Phytologist* **134**: 267–278.

Porra RJ, Thompson WA, Kriedemann PE. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochemica et Biophysica Acta* **975**: 384–394.

Reich PB. 1987. Quantifying plant response to O_3: a unifying theory. *Tree Physiology* **3**: 63–91.

Reichenauer T, Bolhàr-Nordenkampf HR, Soja G. 1997. Chronology of changes within the photosynthetic apparatus of *Populus nigra* under ozone stress. *Phyton* **37**: 345–250.

Reid CD, Fiscus EL. 1998. Effects of elevated $[CO_2]$ and/or ozone on limitations to CO_2 assimilation in soybean (*Glycine max*). *Journal of Experimental Botany* **49**: 885–895.

Rey A, Jarvis PG. 1997. Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO_2 exposure. *Annals of Botany* **80**: 809–816.

Samuelson LJ. 1994. Ozone exposure responses of black cherry and red maple seedlings. *Environmental and Experimental Botany* **34**: 355–362.

Sandermann H Jr, Ernst D, Heller W, Langebartels C. 1998. Ozone: an abiotic elicitor of plant defence reactions. *Trends in Plant Science* **3**: 47–50.

Sane PV, Yunus M, Tripathi RD. 1996. Impact of ozone on carbon metabolism in plants. In: Yunus M, Iqbal M, eds. *Plant response to air pollution*. Chichester, UK: John Wiley and Sons, 295–318.

Saxe H, Ellsworth DS, Heath J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist* **139**: 395–436.

Schramel P. 1988. ICP and DCP emission spectrometry for trace element analysis in biomedical and environmental samples. A review. *Spectrochimica Acta* **34B**: 881–896.

Schreiber U, Bilger W. 1993. Progress in chlorophyll fluorescence research: major developments during the past years in retrospect. *Progress in Botany* **54**: 151–173.

Sehmer L, Fontaine V, Antoni F, Dizengremel P. 1998. Effects of ozone and elevated atmospheric carbon dioxide on carbohydrate metabolism of spruce needles. Catabolic and detoxification pathways. *Physiologia Plantarum* **102**: 605–611.

Skärby L, Ro-Poulsen H, Wellburn FAM, Sheppard LJ. 1998. Impacts of ozone on forests: a European perspective. *New Phytologist* **139**: 109–122.

Stockwell WR, Kramm G, Schell H-E, Mohnen VA, Seiler W. 1997. Ozone formation, destruction and exposure in Europe and the United States. In: Sandermann H Jr, Wellburn AR, Heath RL, eds. *Forest decline and ozone*. Berlin, Germany: Springer-Verlag, 1–38.

Thiel S, Döhring T, Köfferlein M, Kosak A, Martin P, Seidlitz HK. 1996. A phytotron for plant stress research: how far can artificial lighting compare to natural sunlight? *Journal of Plant Physiology* 148: 456–463.

van Kooten O, Snel JFH. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* **25**: 147–150.

Volin JC, Reich PB. 1996. Interaction of elevated CO_2 and O_3 on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiologia Plantarum* **97**: 674–684.

Volin JC, Reich PB, Givnish TJ. 1998. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. *New Phytologist* **138**: 315–325.

von Caemmerer S, Farquhar GH. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376–387.

Wieser G. 1997. Ozone impact on photosynthetic capacity of mature and young Norway Spruce (*Picea abies* (L.) Karst.): external versus internal exposure. *Phyton* **37**: 297–302.

Zeuthen J, Mikkelsen TN, Paludan-Müller G, Ro-Poulsen H. 1997. Effects of increased UV-B radiation and elevated levels of trophospheric ozone on physiological processes in European beech (*Fagus sylvatica*). *Physiologia Plantarum* **100**: 281–290.