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Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*)

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Abstract The extraordinary drought during the summer of 2003 in Central Europe allowed to examine responses of adult beech trees (*Fagus sylvatica*) to co-occurring stress by soil moisture deficit and elevated O_3 levels under forest conditions in southern Germany. The study comprised tree exposure to the ambient O_3 regime at the site and to a twice-ambient O_3 regime as released into the canopy through a free-air O_3 fumigation system. Annual courses of photosynthesis (A_{max}), stomatal conductance (g_s), electron transport rate (ETR) and chlorophyll levels were compared between 2003 and 2004, the latter year representing the humid long-term climate at the site. ETR, A_{max} and g_s were lowered during 2003 by drought rather than ozone, whereas chlorophyll levels did not differ between the years. Radial stem increment was reduced in 2003 by drought but fully recovered during the subsequent, humid year. Comparison of AOT40, an O_3 exposure-based risk index of O_3 stress, and cumulative ozone uptake (COU) yielded a linear relationship throughout humid growth conditions, but a changing slope during 2003. Our findings support the hypothesis that drought protects plants from O_3 injury by stomatal closure, which restricts O_3 influx into leaves and decouples COU from high external ozone levels. High AOT40 erroneously suggested high O_3 risk under drought. Enhanced ozone levels did not aggravate drought effects in leaves and stem.

Keywords Drought · Ozone exposure and uptake · Stomatal conductance · Photosynthesis · Photosynthetic electron transport · Stem growth

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Introduction

"Global Change" scenarios are basically driven by the anthropogenic release of CO_2 and other "climate-effective" gases into the atmosphere (IPCC 2001). Predictions include rise in temperature coupled with altered precipitation, which regionally increase the probability of drought (Scarascia-Mugnozza et al. 2001). Compared to the long-term record, the summer of 2003 showed extraordinarily high air temperature and low precipitation regimes in Central Europe (DWD 2003; Luterbacher et al. 2004; Schär and Jendritzky 2004; Ciais et al. 2005). As seasonal drought is typically associated with high insolation, as was the case in 2003, conditions are conducive to the formation of high tropospheric ozone (O_3) levels (Stockwell et al. 1997; Fabian 2002). Hence, exposure-based indices of O_3 stress (e.g. the AOT40 concept of "Critical Levels for Ozone", Fuhrer and Achermann 1994) indicate enhanced risks of O_3 injury to forest trees (Matyssek and Innes 1999). In view of current Global Change scenarios, O_3 exposure might become exacerbated during the upcoming

Table 1 Weather conditions and ozone regimes at the site "Kranzberger Forst" near Freising/Germany, during the years 2002, 2003 and 2004

	2002	2003	2004
Annual sum of global radiation above canopy (GJ m^{-2})	4.1	4.6	4.4
Mean annual air temperature at canopy height, 24 m above ground (°C)	8.8	9.1	9.4
Mean daily air temperature during growing season (May to October) at canopy height, 24 m above ground (°C)	14.1	16.2	14.9
Annual sum of precipitation[a] (mm)	1015	557	786
Precipitation during growing season (May to October)[a] (mm)	626	359	448
SUM0 1 × O_3 (µl O_3 l^{-1} h)	126.0	193.6	142.7
SUM0 2 × O_3 (µl O_3 l^{-1} h)	234.0	357.0	232.6
AOT40 1 × O_3 (µl O_3 l^{-1} h)	16.0	33.0	17.3
AOT40 2 × O_3 (µl O_3 l^{-1} h)	67.0	117.0	63.0
COU 1 × O_3 (mmol m^{-2})	22.5	18.9	24.3
COU 2 × O_3 (mmol m^{-2})	28.2	32.2	28.0

Note. Precipitation was measured in a forest clearing at 1 km distance from the site. SUM0 is the "Sum of all O_3 concentrations", AOT40 is the "accumulated exposure over a threshold of 40 nl O_3 l^{-1}", COU is "cumulative ozone uptake"

[a]Data by courtesy of LWF, G. Gietl

decades (Fowler et al. 1999; Ashmore 2005). However, it is the actual dose of O_3 uptake through leaf stomata rather than exposure that determines the O_3 stress and drives the stress response in plants (Matyssek and Sandermann 2003; Wieser et al. 2003; Matyssek et al. 2004). Drought usually leads to a decrease in stomatal conductance (Schulze 1994) and limits the O_3 flux into plants (Pääkkönen et al. 1998a,b). In fact, drought might "protect" against O_3 stress, although findings conflict about interacting drought-ozone effects in trees (Dobson et al. 1990; Chappelka and Freer-Smith 1995; Maier-Maercker 1998; Nali et al. 2004). The uncertainty on such interactions is substantial, in particular in adult forest trees, because the large majority of studies have focused on O_3 stress in juvenile woody plants under chamber conditions (Kolb and Matyssek 2001). As chronic O_3 stress bears the risk of predisposing trees to other stresses like drought (or frosts or pests), severe episodes of such stressors can eventually cause break-down of trees and forests (cf. Miller and McBride 1999).

The present study was conducted on adult beech trees (*Fagus sylvatica*) growing at a forest site in Central Europe, where they are exposed to ambient or an experimentally enhanced O_3 regime imposed by free-air canopy fumigation (Nunn et al. 2002; Werner and Fabian 2002). Trees were examined for their responsiveness to O_3 across the tree-internal scaling levels of cells, leaves, branches and the whole tree (Nunn et al. 2005a,b). We assessed stomatal conductance, photosynthetic characteristics, chlorophyll levels and stem growth during the exceptional drought of 2003 and the humid year of 2004 which represented long-term climatic average conditions. Potential O_3-induced reduction in radial stem growth was regarded as an indication of economic damage (sensu CLRTAP 2004). The following hypotheses were tested: (1) Cumulative ozone uptake (COU) rather than exposure-based O_3 indices like AOT40 reflects risk of O_3-induced damage (e.g. in stem production). (2) The effect of drought on adult *F. sylvatica* trees is aggravated by enhanced ozone levels.

Materials and methods

Study site and experimental design

The study site is located within a mixed beech/spruce forest ("Kranzberger Forst") in southern Germany near Munich (48°25′08″ N, 11°39′41″ E, elevation 485 m a.s.l.). The soil at the site is a luvisol derived from loess over tertiary sediments. Beech trees (*Fagus sylvatica* L.) used in this study were about 60 years old and up to 28 m high (Pretzsch et al. 1998). Scaffolding and a research crane provided access to the crowns of ten representative *F. sylvatica* trees (Reiter et al. 2005). A free-air ozone fumigation system (Nunn et al. 2002; Werner and Fabian 2002) was employed since the year 2000 to expose the joint canopies of five of the ten study trees to a twice-ambient ozone regime (2 × O_3). To prevent acute O_3 injury, the O_3 levels of this regime were restricted to a maximum of 150 nl O_3 l^{-1}. Five adjacent *F. sylvatica* trees under the unchanged ambient regime (1 × O_3) prevailing at the site served as control (Nunn et al. 2002). Long-term regional annual air temperature is 7.5°C, and annual precipitation amounts to 788 mm (monitored by DWD at climate station "Weihenstephan", at 4 km distance from the research site; DWD Offenbach, Germany). The weather conditions at the research site of the years 2002 through 2004 which were covered in this study are given in Table 1. Ecophysiological assessments during the humid year 2004 served as the reference of those in the dry year 2003. Regarding weather conditions and O_3 regimes, 2002 was included additionally for comparison.

Assessment of micro-climate, ozone and radial stem growth

Global radiation above the canopy was measured with a pyranometer (type CM 11; Kipp & Zonen, Delft, The Netherlands), and air temperature along with air humidity

at 24 m height within the canopy with an aspiration psychrometer (model Assmann; Theiss, Göttingen, Germany). Rainfall was recorded by a rain gauge (model Pluvio; Ott Messtechnik, Kempten, Germany) in a forest clearing at 1 km distance from the site. O_3 levels were monitored throughout growing seasons by O_3 analysers (TML 8811; Teledyne Monitor Labs, Englewood, USA) within the canopy, both under the $1 \times O_3$ and $2 \times O_3$ regime. O_3 regimes were expressed as "Sum of all ozone concentrations" (SUM0) and "Accumulated exposure over a threshold of 40 nl O_3 l^{-1}" (AOT40, Fuhrer and Achermann 1994), as currently adopted by UNECE. All O_3 indices have been calculated for the time period between leaf expansion in spring and senescence in autumn, which is the time span relevant for ozone uptake. Phenological data have been assessed separately in each O_3 regime. Cumulative ozone uptake (COU) during the growing season was calculated according to Emberson et al. (2000), accounting for drought effects according to Nunn et al. (2005a). COU is a function of maximum stomatal conductance for O_3 and external O_3 concentration (Eqs. (1) and (2)):

$$F_{O_3} = g_{O_3} [O_3] \quad (1)$$

$$\mathrm{COU} = \sum_{\mathrm{SGS}}^{\mathrm{EGS}} F_{O_3} \quad (2)$$

with F_{O_3} being the stomatal O_3 flux, g_{O_3} the stomatal conductance for O_3, and SGS and EGS the beginning and end of the growing season, respectively. g_{O_3} is a function of maximum and minimum stomatal conductance, light, phenology, temperature, VPD, soil moisture, day and night time (see Nunn et al. 2005a for further details about the employed model and its parameterisation).

Annual increment of radial stem growth was monitored in each study tree at breast height with permanent girth measurement tapes (model D1, UMS, München, Germany) across the years 2001 through 2004 (2001 as reference for the subsequent years).

Assessment of leaf gas exchange

Gas exchange and chlorophyll fluorescence parameters were assessed using a Licor 6400 CO_2/H_2O diffusion porometer equipped with a "Leaf Chamber Fluorometer 6400-40" (Li-Cor, Lincoln, USA). Measurements were made at 40–60% relative air humidity, 360 µmol mol^{-1} CO_2 of the ambient air and saturating light conditions (1500 µmol m^{-2} s^{-1} photosynthetic photon flux density, PPFD) within the chamber. PPFD of light-saturated photosynthesis (A_{max}) had been previously determined by separate assessments (data not shown). Contrasting with 2004, the leaf temperature of 25°C could not always be maintained in 2003 because of high ambient air temperatures; nevertheless, leaf temperature never exceeded 30°C in 2003 during measurements. Leaves acclimated to the chamber conditions for at least 2 min until gas exchange readings became stable. Measurements were recorded then every 10 s for at least 3 min. A saturating light flash (duration 0.8 s, PPFD > 7000 µmol m^{-2} s^{-1}) concluded each measurement to determine the electron transport rate (ETR). ETR was calculated after Krall and Edwards (1992):

$$\mathrm{ETR} = \Delta F \big/ F m' \times \mathrm{PPFD} \times a \times f \quad (3)$$

with the absorptivity (a) set to 0.84 and light distribution between Photosystem I and II (f) to 0.5.

Assessment of water potential

Pre-dawn twig water potential was measured in June, August and October 2003 and in July 2004 with a Scholander pressure bomb (Model 3000, Soilmoisture Equipment Corporation, Santa Barbara, USA) on detached foliated twigs of 30 cm length, preventing water loss through enclosure in plastic bags prior to measurement.

Biochemical analysis

Leaf samples taken for biochemical analysis were frozen at once in liquid nitrogen and kept at –70°C until lyophilisation. Lyophilised leaves were ground in a dismembrator (Retsch, Haan, Germany), and stored in humidity-proof plastic vials at –25°C prior to HPLC analysis. Pigments were analysed in acetone extracts using the HPLC gradient method according to Pfeifhofer (1989) and Tausz et al. (2003). About 60 mg lyophilised plant powder and some calcium carbonate were extracted in 1 ml acetone in dark 1.5 ml reaction tubes. The extract was shaken with the Vortex-shaker (Heidolph, Reax 2000) for half a minute and centrifuged (Beckman, Avanti 30 Centrifuge, 14,000 rpm) for 10 min at 4°C. The supernatant was filled in calibrated tubes and kept closed in the dark at 4°C. The pellet was re-extracted in 1 ml acetone, shaken and centrifuged. The supernatants were merged and the resulting volumes of the acetone extract were noted. Aliquots of the extracts were centrifuged for 30 min at 4°C and subjected to the HPLC analysis consisting of SunFlow 100 pump with gradient former GF (SunChrom) and online degasser (Knauer), LKB2151 UV/VIS detector (440 nm), integrator PC software ChromStar, Midas Spark Holland autosampler cooled at 4°C, Chrom Spherisorb S5 ODS-2 250 mm × 4.6 mm column with Chrom Spherisorb S5 ODS-2 10 mm × 4.6 mm precolumn; gradient setting, solvent A: acetonitril:methanol:water = 100:10:5 (v/v/v); solvent B: acetone:ethylacetate = 2:1 (v/v); linear gradient from 90% (v) A to 20% (v) A in 18 min, 5 min at 20%, back to 90% A in 3 min; total run time of 30 min, at flow rate of 1 ml min^{-1}. Calibration was done with pure pigment standards.

Sampling protocol

Leaf gas exchange and chlorophyll fluorescence of sun-exposed *F. sylvatica* leaves were assessed four times in 2003 (June, July, September and October) and five times in 2004 (May, June, July, September and October). To ensure comparable light and temperature conditions, sampling of leaves took place around solar noon on sunny days of similar insolation.

Statistics

Statistical analysis was performed by means of the "general linear model" (GLM) of the SPSS 13 software (SPSS Inc., Chicago, USA). For each sampling date, univariate analysis was employed for comparison of responses to O_3 regimes. The overall effect of ozone, integrating all sampling dates, was analysed through the "repeated measurements" subcategory of GLM. To meet prerequisites for GLM, data had been tested for homogeneity using "Levene Test for Equality of Variances." Given the fact that the study trees at "Kranzberger Forst" are of same age but not same size, and that size might influence water and carbon relations of trees (Ryan et al. 2000), stem diameter at breast height was used as covariate in all statistical tests.

Analysis of linear and hyperbolic regressions was performed using the SigmaPlot 9 software package (Systat Software, Erkrath, Germany) in combination with SPSS 13 (SPSS Inc., Chicago, USA) and Graphpad Prism 4 (GraphPad Software Inc., San Diego, USA).

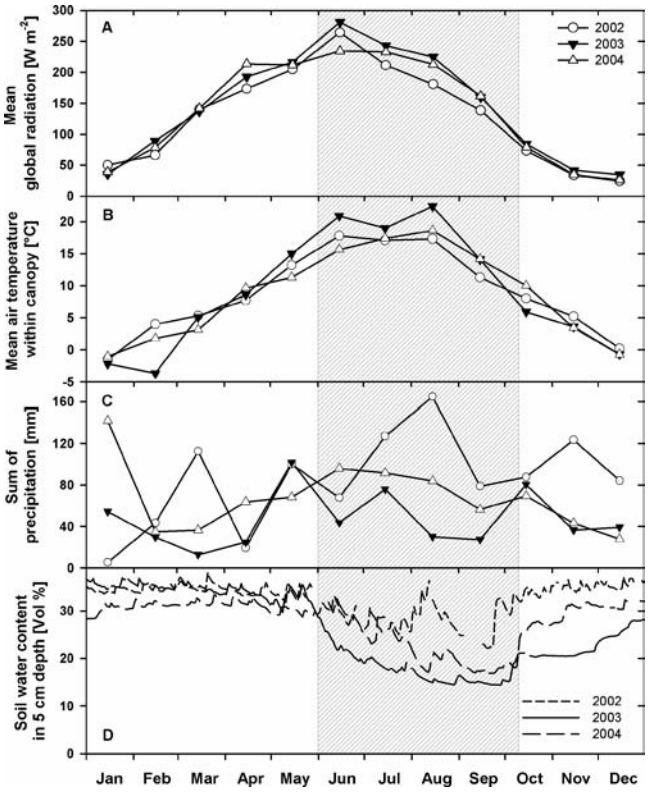

Fig. 1 Monthly sum of global radiation (**A**), air temperature at canopy height, i.e. 24 m above ground (**B**), precipitation (**C**) and soil water content (**D**) during the years 2002, 2003 and 2004 at the research site "Kranzberger Forst" in southern Germany. *Symbols* given in (**A**) also apply for (**B**) and (**C**). *Hatched area* indicates drought period in 2003. Data in (**C**) and (**D**) by courtesy of LWF (G. Gietl and W. Grimmeisen)

Results

The years 2002 through 2004 slightly varied in the cumulative annual insolation and mean air temperature as recorded inside the canopy at the study site (Table 1), with the annual maxima within this 3-year period occurring 2003 in global radiation and 2004 in air temperature. The mean daily air temperature at the canopy position determining tree transpiration, i.e. the sun-exposed foliage, was conspicuously higher during the growing season of 2003 as compared to the respective time period in the previous or succeeding year. The minor annual variation was reflected in rather similar seasonal courses of global radiation (as affected by changing cloudiness) and mean monthly air temperature (Fig. 1A and B), although the latter reached maxima of above 20°C during the summer of 2003 (these levels not being reached during the respective periods in 2002 and 2004). Precipitation varied between years, both during the growing season and on an annual basis, as the cumulative rainfall in 2002 exceeded that in 2003 by a factor of almost two (Table 1, Fig. 1C), in addition to high precipitation in March and November of 2002, abundant rainfall occurred throughout the summer (maximum in August), whereas precipitation was remarkably low across the entire year of 2003 (in particular during summer). The year 2004 was intermediate in precipitation between 2002 and 2003, both during summer and on an annual basis (Table 1; Fig. 1C). Volumetric soil water content hardly fell below 30% in 2002, but decreased to about 15% in August of 2003 and stayed that low until early October of the same year (Fig. 1D). At 14% no water is left available to plant uptake in the given soil (Raspe et al. 2004). The soil water content did not fully recover during winter after the drought of 2003 so that a water deficit was occurring again during summer 2004 compared to 2002; nevertheless, effects on the trees were much less pronounced in 2004. As a consequence of the persisting soil drought in 2003, the predawn water potential (Ψ_{pre}) of *F. sylvatica* branches dropped to minima of about –1.3 to –1.4 MPa in August, regardless of the O_3 regime (Fig. 2). Such levels are extraordinarily low for Central European forest conditions (cf. Larcher 2001). The daily minimum water potential (Ψ_{min}), as assessed during early afternoon, never dropped below –2 MPa (means $\pm$ SD of Ψ_{min} during 2003 were -1.9 ± 0.38 MPa in $1 \times O_3$ and -2.0 ± 0.37 MPa in $2 \times O_3$) – these minimum levels are in consistency with the findings on deciduous forest trees of the temperate climate zone (cf. Larcher 2001). In contrast, Ψ_{pre} was high at ample rainfall (around –0.1 MPa), as exemplified in July 2004 (Fig. 2).

The sunny and dry weather conditions during the summer of 2003 favoured the formation of ozone so that

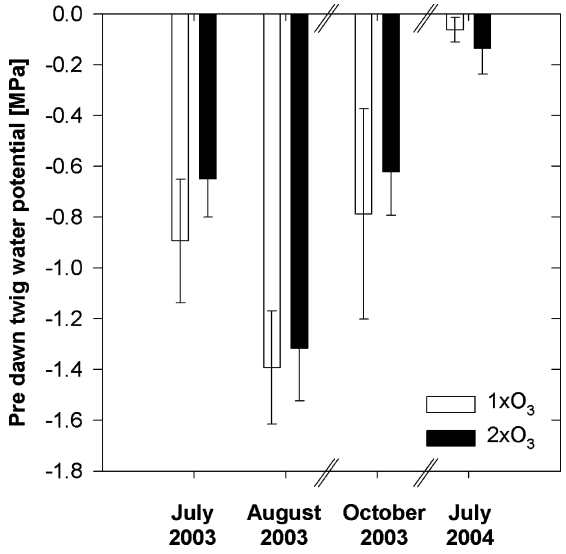

Fig. 2 Pre-dawn twig water potential in July, August, October 2003 and July 2004. Columns represent mean values ± standard deviation ($n = 5$ trees in each bar)

SUM0 of $1 \times O_3$ during the growing season amounted to 194 µl O_3 l^{-1} h (Table 1), exceeding the respective levels of both 2002 and 2004 by a factor of 1.5. Across the 3-year period, SUM0 of the $2 \times O_3$ regime was not enhanced by a factor of 2 relative to $1 \times O_3$ (i.e. about 1.6-fold in 2004 and 1.8-fold in 2003 and 2002), because the experimentally imposed O_3 regime was restricted to maximum O_3 levels of 150 nl O_3 l^{-1} (see Materials and methods section). This relative increase of SUM0 at $2 \times O_3$ was similar across the three study years (Table 1). AOT40 of $2 \times O_3$ during the growing season 2003 did reach, however, about twice the levels of 2002 and 2004 (Table 1). During each of the three study years, AOT40 exceeded the "Critical Level of Ozone" of 10 µl O_3 l^{-1} h which is proposed by UNECE (Fuhrer et al. 1997 – or 5 µl O_3 l^{-1} h as suggested recently by Karlsson et al. 2004) even at $1 \times O_3$. Across the 3 years, AOT40 under $2 \times O_3$ was between 3.5 and 4.2 times the levels that were reached annually at $1 \times O_3$. Remarkably, COU under $1 \times O_3$ was lower in 2003 (by a factor of 0.8) than during the two more humid years, although annual SUM0 and AOT40 were highest in 2003 (Table 1). Regarding $2 \times O_3$, COU in 2003 only slightly exceeded the levels that amounted during each of the humid years (by a factor of 1.1).

In 2003, the relationship between COU and AOT40 stayed approximately linear until July 31 at $1 \times O_3$ and August 2 at $2 \times O_3$ (Fig. 3B, see arrows), when a breakpoint was reached as the soil water content approached its minimum (Fig. 1). The slope of the linear regressions ($r^2 > 0.9$, $p < 0.001$ each) before and after these dates differed significantly ($p < 0.001$) from each other under both O_3 regimes. The slope declined, as COU per unit decreased under water limitation (Fig. 3B). The relationship between COU and AOT40 during the years 2002 and 2004 did not display such a breakpoint (Fig. 3A and C), as COU nearly linearly increased with AOT40 ($r^2 > 0.99$, $p < 0.001$) in both

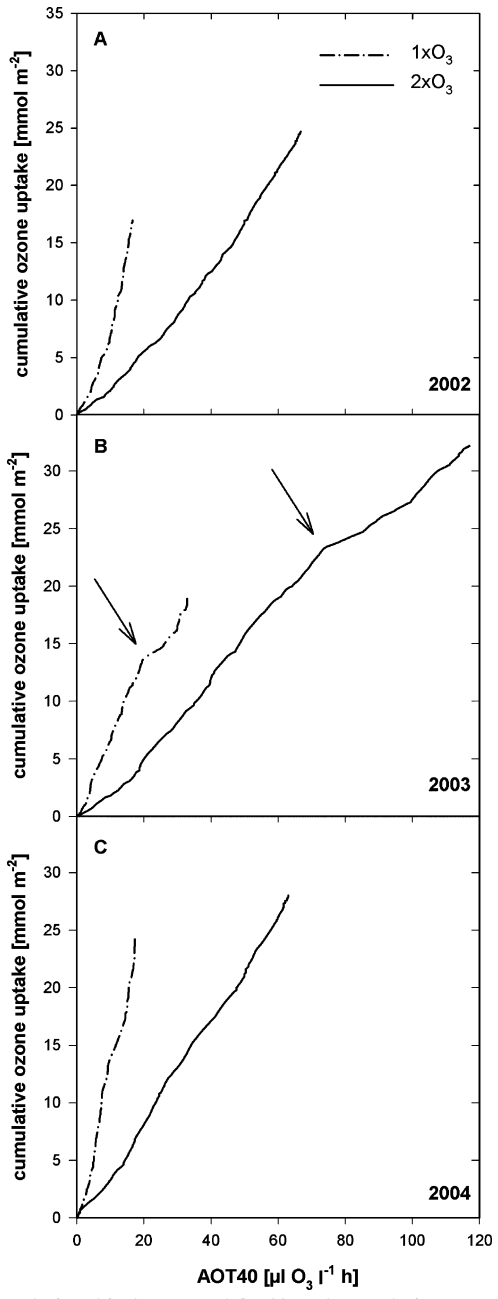

Fig. 3 Relationship between AOT40 and cumulative ozone uptake during 2002 (**A**), 2003 (**B**) and 2004 (**C**). *Arrows* in (**B**) indicate July 31, 2003 in $1 \times O_3$ and August 2, 2003 in $2 \times O_3$, when soil moisture became limiting. (**B**) Re-evaluated after Matyssek et al. (2006)

years and O_3 regimes. All three study years differed significantly in these slopes under $1 \times O_3$ and $2 \times O_3$ ($p < 0.001$, in 2003 only data points used prior to the breakpoint).

The cause of the change in slope between COU and AOT40 between dry and humid years was stomatal regulation, as g_s was strongly reduced during drought to less than one-third of the levels that were reached under the humid conditions of 2004 (Fig. 4B). During the period of lowest Ψ_{pre} (Fig. 2), g_s did not differ anymore between the two O_3 regimes, although in June 2003, g_s was reduced under $2 \times O_3$ already prior to the onset of drought (Fig. 4B).

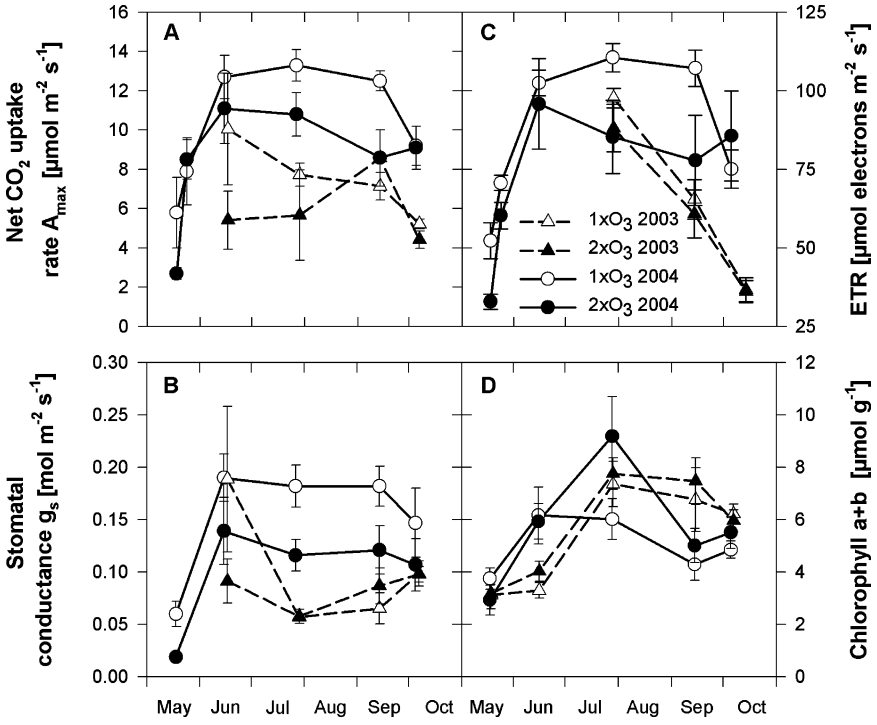

Fig. 4 Net CO_2 uptake rate (A_{max}) (**A**), stomatal conductance to water vapour (g_s) (**B**), electron transport rate (ETR) (**C**) (all measured at saturating light conditions) and dry mass related chlorophyll levels (**D**) during 2003 and 2004 (means ± one standard error of the mean of $n = 3$–5 trees in each pair of values). *Symbols* given in (**C**) are valid also for (**A**), (**B**) and (**D**)

In addition, g_s under $2 \times O_3$ appeared to be less sensitive to drought than under $1 \times O_3$. In contrast, the seasonal course of g_s was fairly constant under humid conditions, as exemplified during 2004, although levels of g_s were lower and less stable under the $2 \times O_3$ than $1 \times O_3$ regime (Fig. 4B). g_s was significantly reduced under $2 \times O_3$ conditions in July 2004 ($p = 0.047$) as compared to $1 \times O_3$. Analysis of the annual course (see Materials and methods section) showed that g_s was significantly reduced during 2004 in $2 \times O_3$ ($p = 0.039$). The course of photosynthetic performance resembled that of g_s (Fig. 4A) in each year, however, A_{max} did not recover in October 2003. A_{max} was reduced significantly under the $2 \times O_3$ regime in September 2004 ($p = 0.048$). The annual course of A_{max} in 2003 was significantly different from that in 2004 in both O_3 regimes ($p < 0.001$ at $1 \times O_3$ and $p < 0.02$ at $2 \times O_3$).

Beginning in July 2003, ETR declined gradually until October, irrespective of the O_3 regime (Fig. 4C), as opposed to 2004, when under $1 \times O_3$ ETR was significantly higher in May ($p = 0.04$) and slightly enhanced in July and September as compared to $2 \times O_3$. Due to reduced ETR levels under $2 \times O_3$, differences between the 2 years under this O_3 regime were smaller than under $1 \times O_3$, although ETR was significantly lower in October 2003 than in October 2004 ($p = 0.04$). The relationship between ETR and stomatal conductance (Fig. 5) distinctly differed between 2003 and 2004, as correlation was apparent during the humid year ($r^2 = 0.63$, $p < 0.001$), but not so under the drought of 2003 ($r^2 < 0.01$). Correlation coefficients for hyperbolic rectangular regressions were significantly different between both years ($p = 0.0016$). Even when the regression was applied only to the data range covered by both years ($g_s < 0.15$ mol m^{-2} s^{-1}), the hyperbolic regression rendered a similar result in 2004 ($r^2 = 0.60$, $p < 0.01$) so that still both years could not be described ($p = 0.018$) by one regression. Levels of dry mass-related chlorophyll a and b (Fig. 4D) neither differed with respect to the annual water availability nor to the O_3 regimes.

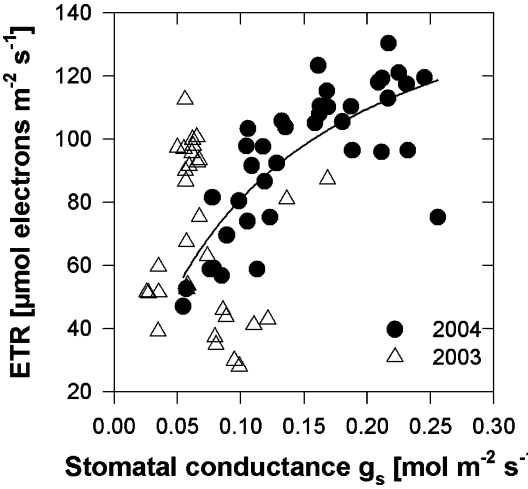

Fig. 5 Relationship between electron transport rate (ETR) and stomatal conductance for water vapour (g_s) during 2003 and 2004. Hyperbolic regression (of single rectangular type) at $r^2 = 0.63$ only in 2004; $r^2 < 0.01$ in 2003 (regression line not shown)

Radial stem growth at breast height (Fig. 6) was limited under the drought of 2003. Nine out of the ten examined trees displayed reduced annual increment as compared with that in 2001. Given the high variability between the individual *F. sylvatica* trees in this stem response, the mean radial stem increment did not significantly differ between the two O_3 regimes.

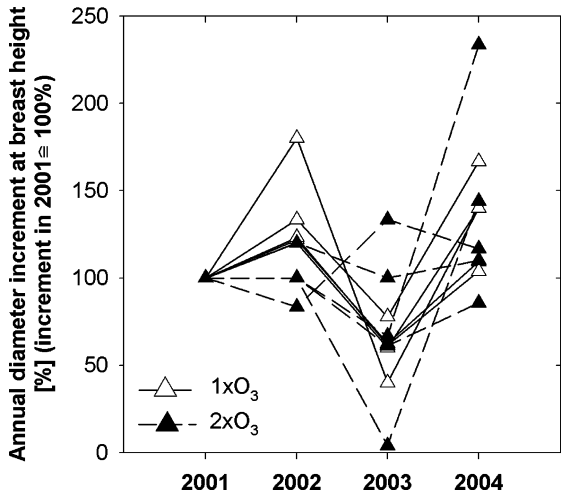

Fig. 6 Relative annual stem diameter increment at breast height of the ten adult *F. sylvatica* trees examined in this study during the years 2001, 2002, 2003 and 2004 (increment in 2001 ≙ 100%)

Discussion

In general, drought is viewed to counteract adverse O_3 effects through, at least, partial closure of the stomata (Schulze 1994) which determine the pathway of O_3 uptake into plants (Matyssek and Sandermann 2003). Stomatal responses to moisture deficits can limit the O_3 uptake to the extent that the influx of O_3 may be less during sunny, dry days with high O_3 levels than under the lower O_3 regimes of overcast, humid days (Wieser and Havranek 1993). Moisture-related contrasts in O_3 influx can also be observed at the annual time scale, as shown by the reduced O_3 uptake of adult *F. sylvatica* under the high O_3 exposure during the sunny and dry growing season of 2003 relative to the high uptake under the humid summer conditions and lower O_3 levels of 2002 and 2004. Although sunny and dry weather favours O_3 formation (Stockwell et al. 1997; Fabian 2002), the higher external O_3 exposure, as expressed through SUM0 or AOT40, was not translated into increased uptake into the trees, which leads us to accept hypothesis (1) in 2003. In our study, g_s and O_3 uptake declined under both O_3 regimes at "Kranzberger Forst" as Ψ_{pre} of twigs decreased, along with soil moisture (Panek 2004), to levels that were exceptionally low for trees of the Central European climate (Larcher 2001). However, only severe drought – as encountered in the present field study – tends to protect from O_3 impact (Retzlaff et al. 2000). In our study, this was supported by the absence of major macroscopic leaf injury in adult *F. sylvatica* in 2003. Mild soil moisture deficit, on the contrary, can exacerbate O_3 injury (Grulke et al. 2003) and accelerate leaf loss (cf. Pääkkönen et al. 1998b).

O_3 impact may change sensitivity in stomatal regulation (Matyssek and Sandermann 2003), as indicated by sluggishness in stomatal movements or inefficient control of transpiration (Keller and Häsler 1984; Barnes et al. 1990a, b; Pearson and Mansfield 1993; Karlsson et al. 2004; Paoletti and Grulke 2005) so that trees may be predisposed to drought injury. Remarkably, g_s under $2 \times O_3$ conditions already was lower before the onset of drought and seemed to be less sensitive to drought (cf. Paoletti and Grulke 2005). Such O_3-induced changes may be mediated through altered "mechanics" in the stomatal apparatus upon reduced cell wall lignification (Maier-Maercker 1998; cf. Kivimäenpää et al. 2003). Another explanation is the disturbed osmotic control of the guard cells upon membrane impairment (Heath and Taylor 1997; cf. Nali et al. 2004), although stomatal responsiveness to ABA has not yet been clarified under O_3 stress (Torsethaugen et al. 1999). In adult *F. sylvatica*, the relative decrease in g_s in response to drought was smaller under $2 \times O_3$ than $1 \times O_3$; however, it is remarkable that during the remainder of the drought period g_s stayed at similarly low levels in both O_3 regimes. This may relate to Ψ_{min} which was maintained at around -2 MPa regardless of the O_3 regime, although Ψ_{pre} had dropped to unusually low levels as a consequence of soil drought. It appears that stomatal regulation stayed intact under O_3 stress to the extent that the daily maximum in water tension, represented through Ψ_{min}, was stabilized, which may prevent catastrophic cavitation in the xylem vessels under persisting drought (cf. Tyree and Zimmermann 2002). O_3 stress per se did not significantly affect water potentials.

Stomatal closure during the drought of 2003 underlined the shortcoming of exposure-based indices like SUM0 and AOT40 in assessing the O_3 risk to trees (Panek 2004). Maximum O_3 uptake does not necessarily correlate with periods of peak external O_3 levels. SUM0 and AOT40 may adequately correlate with O_3 uptake only when soil moisture is not limiting, which can lead to erroneous conclusions about risks of O_3 injury in dry years (Panek et al. 2002). Until the end of July 2003, the relationship between cumulative ozone uptake and AOT40 was linear, although such correlations may be rather variable, and linearity cannot be generalized (Karlsson et al. 2004; Matyssek et al. 2004). However, the relationship changed its slope around the end of July 2003, when g_s decreased as a consequence of the continuing drought. This effect was more pronounced under the $2 \times O_3$ than the $1 \times O_3$ regime. Panek et al. (2002) suggested to weight O_3 levels by g_s or soil moisture. One approach, which was adopted in the present study, is to extend g_s-based models of O_3 uptake (Emberson et al. 2000) by adequate algorithms, such as the model extension by Nunn et al. (2005a). The extension given there significantly increased modelling precision of O_3 uptake.

However, stomatal exclusion of O_3 is accompanied by restricted CO_2 fixation (Panek and Goldstein 2001), and the latter effect may even be exacerbated through synergism between O_3 and drought (Grulke et al. 2002). In adult *F. sylvatica* of our study, drought-driven decline in A_{max} in parallel to decreasing g_s was observed under $1 \times O_3$ during the year 2003, but not so under $2 \times O_3$, where A_{max} and g_s had been low since early summer. Nevertheless, it is reported that O_3 may even seasonally increase g_s and A_{max}. This may be a consequence of sink induction for carbon in relation to O_3 defence (Kolb and Matyssek 2001; cf. Körner 2003) or resource retranslocation from old injured into newly formed leaves (Beyers et al. 1992; Maurer et al. 1997).

ETR showed a distinct drought effect in 2003 (cf. Fig. 4C) in the *F. sylvatica* trees at "Kranzberger Forst". Other studies reported only slight drought effects on ETR (Ogaya and Penuelas 2003, Flexas et al. 1999). Furthermore, ETR was only poorly related to g_s at "Kranzberger Forst" in the dry year 2003 ($r^2 < 0.01$; Fig. 5). This relationship was well established under the humid conditions of 2004 ($r^2 = 0.63$). The correlation was similar to that reported by Flexas et al. (2002) from *Vitis vinifera* which was grown both under irrigated and non-irrigated conditions in the Mediterranean region, characterized by regular summer drought. In addition, this kind of relationship between ETR and g_s has been reported by Medrano et al. (2002) for several plant species from different climatic zones. The absence of such a correlation in *F. sylvatica* during 2003 apparently underlines, in Central Europe, the anomaly of the severe drought in that year (Luterbacher et al. 2004). Nevertheless, high levels of ETR at low g_s in 2003 are consistent with the conceptual scheme by Medrano et al. (2002, Fig. 3 given there) to the extent that this scheme may be applicable to *F. sylvatica* at "Kranzberger Forst". High ETR at low g_s during drought may indicate ongoing photorespiration as counteracting photoinhibition (Guan et al. 2004, Medrano et al. 2002). Despite the variation in ETR, chlorophyll levels in *F. sylvatica* were neither affected by drought nor ozone, suggesting – in accordance with findings by Herbinger et al. (2002) in *Triticum durum* and *T. aestivum* – intactness of the photosynthetic apparatus.

Given the trade-off between protection against O_3 injury at high O_3 exposure and reduced A_{max} through the drought-driven closure of stomata, limitations in biomass production, resource allocation and structural differentiation may be the "price" of the exclusion of ozone (Matyssek and Sandermann 2003). On mesic sites, O_3 impact resulted, in *Pinus jeffreyi*, in thinner branches but earlier needle loss than on xeric sites (Grulke et al. 2003). In *F. sylvatica*, Stribley and Ashmore (2002) reported decrease in shoot growth to be mainly related to drought and concluded that, depending on the site, also O_3 can contribute to the decline of *F. sylvatica*. Nutrition was affected in *Picea abies* through drought rather than O_3 (Wallin et al. 2002; Kivimäenpää et al. 2003), and even though O_3 limited stem growth, the pollutant was regarded less important than drought in this latter respect. Also in the adult *F. sylvatica* trees of "Kranzberger Forst", drought rather than O_3 limited radial stem growth in 2003. The absence of an O_3 effect on stem growth under $2 \times O_3$ relative to $1 \times O_3$ was consistent with the observation during humid years. Hence, no O_3-induced damage on stem production was substantiated (Wipfler et al. 2005). Although Hypothesis 1 was corroborated in that COU was uncoupled from AOT40, the low O_3 sensitivity of stem growth prevented, in the given case of beech at "Kranzberger Forst", the damage to materialize. Apparently, stem growth of tall trees is highly buffered against O_3 stress, which appears to be in contrast to juvenile trees (cf. Kolb and Matyssek 2001). This difference may be due to a high reserve storage capacity (backing processes of detoxification and repair) or stress compensation already within the crown of tall trees. The metabolic responsiveness at the crown level leads to conclude that risks of chronic O_3 stress still cannot be ruled out (Nunn et al. 2005a, b). Suggestions that chronic O_3 stress may enhance the susceptibility of trees to drought (cf. Matyssek et al. 2006), resulting in reduced stem growth during subsequent years (Dittmar and Elling 1999; Dittmar et al. 2003) or even decline of trees and forests (Miller and McBride 1999), were not confirmed in our study. Hence, irrespective of the analysed tree parameter, hypotheses (2) was to be rejected, the more so as drought and ozone led to differential effects on g_s, A_{max}, ETR and stem growth of the study trees.

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References

Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. Plant Cell Environ 28:949–964

Barnes JD, Eamus D, Brown KA (1990a) The influence of ozone, acid mist and soil nutrient status on Norway spruce [*Picea abies* (L.) Karst]: 1. Plant–water relations. New Phytol 114:713–720

Barnes JD, Eamus D, Brown KA (1990b) The influence of ozone, acid mist and soil nutrient status on Norway spruce [*Picea abies* (L.) Karst]: 2. Photosynthesis, dark respiration and soluble carbohydrates of trees during Late Autumn. New Phytol 115:149–156

Beyers JL, Riechers GH, Temple PJ (1992) Effects of long-term ozone exposure and drought on the photosynthetic capacity of Ponderosa Pine (*Pinus ponderosa* Laws.). New Phytol 122:81–90

Chappelka AH, Freer-Smith PH (1995) Predisposition of trees by air-pollutants to low-temperatures and moisture stress. Environ Pollut 87:105–117

Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grunwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533

CLRTAP (ed) (2004) Mapping manual. Convention on long-range transboundary air pollution, http://www.oekodata.com/icpmapping/

Dittmar C, Elling W (1999) Radial growth of Norway spruce and European beech in relation to weather and altitude. Forstwissenschaftliches Centralblatt 118:251–270

Dittmar C, Zech W, Elling W (2003) Growth variations of Common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in Europe—a dendroecological study. Forest Ecol Manage 173:63–78

Dobson MC, Taylor G, Freersmith PH (1990) The control of ozone uptake by *Picea abies* (L.) Karst and *P. sitchensis* (Bong) Carr during drought and interacting effects on shoot water relations. New Phytol 116:465–474

DWD (2003) Rekordsommer 2003. Available online at http://www.dwd.de/de/FundE/Klima/KLIS/prod/spezial/temp/Rekordsommer_2003.pdf

Emberson L, Ashmore M, Cambridge H, Simpson D, Tuovinen J (2000) Modelling stomatal ozone flux across Europe. Environ Pollut 109:404–413

Fabian P (2002) Leben im Treibhaus—Unser Klimasystem und was wir daraus machen. Springer, Berlin

Flexas J, Badger M, Chow WS, Medrano H, Osmond CB (1999) Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. Plant Physiol 121:675–684

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct Plant Biol 29:461–471

Fowler D, Cape JN, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999) The global exposure of forests to air pollutants. Water Air Soil Pollut 116:5–32

Fuhrer J, Achermann B (1994) Critical levels for ozone—a UN-ECE Workshop report, vol. 16. Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene, Bern

Fuhrer J, Skärby L, Ashmore MR (1997) Critical levels for ozone effects on vegetation in Europe. Environ Pollut 97:91–106

Grulke NE, Johnson R, Esperanza A, Jones D, Nguyen T, Posch S, Tausz M (2003) Canopy transpiration of Jeffrey pine in mesic and xeric microsites: O_3 uptake and injury response. Trees 17:292–298

Grulke NE, Preisler HK, Rose C, Kirsch J, Balduman L (2002) O_3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. New Phytol 154:621–631

Guan XQ, Zhao SJ, Li DQ, Shu HR (2004) Photoprotective function of photorespiration in several grapevine cultivars under drought stress. Photosynthetica 42:31–36

Heath RL, Taylor GE (1997) Physiological processes affecting plant responses to ozone exposure. In: Sandermann H, Wellburn AR, Heath RL (eds) Forest decline and ozone: a comparison of controlled chamber and field experiments. Springer, Berlin, pp 317–368

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A, Grill D (2002) Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiol Biochem 40:691–696

IPCC (2001) Climate change 2001: the scientific basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK

Karlsson PE, Uddling J, Braun S, Broadmeadow M, Elvira S, Gimeno BS, Le Thiec D, Oksanen E, Vandermeiren K, Wilkinson M, Emberson L (2004) New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. Atmos Environ 38:2283–2294

Keller T, Häsler R (1984) The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia 64:284–286

Kivimäenpää M, Sutinen S, Karlsson PE, Sellden G (2003) Cell structural changes in the needles of Norway spruce exposed to long-term ozone and drought. Ann Bot 92:779–793

Kolb TE, Matyssek R (2001) Limitations and perspectives about scaling ozone impacts in trees. Environ Pollut 115:373–393

Körner C (2003) Carbon limitation in trees. J Ecol 91:4–17

Krall JP, Edwards GE (1992) Relationship between Photosystem-II activity and CO_2 fixation in leaves. Physiol Plant 86:180–187

Larcher W (2001) Ökophysiologie der Pflanzen. UTB, Stuttgart

Luterbacher J, Dietrich D, Xoplaki E, Grosjean M, Wanner H (2004) European seasonal and annual temperature variability, trends, and extremes since 1500. Science 303:1499–1503

Maier-Maercker U (1998) Dynamics of change in stomatal response and water status of Picea abies during a persistent drought period: a contribution to the traditional view of plant water relations. Tree Physiol 18:211–222

Matyssek R, Innes JL (1999) Ozone—a risk factor for trees and forest in Europe? Water Air Soil Pollut 116:199–226

Matyssek R, Sandermann H (2003) Impact of ozone on trees: an ecophysiological perspective. Prog Bot 64:349–404

Matyssek R, Le Thiec D, Löw M, Dizengremel P, Nunn AJ, Häberle KH (2006) Drought stress in the presence of O_3 impact on forest trees. Plant Biol 8:11–17

Matyssek R, Wieser G, Nunn AJ, Kozovits AR, Reiter IM, Heerdt C, Winkler JB, Baumgarten M, Häberle KH, Grams TEE (2004) Comparison between AOT40 and ozone uptake in forest trees of different species, age and site conditions. Atmos Environ 38:2271–2281

Maurer S, Matyssek R, Günthard-Goerg MS, Landolt W, Einig W (1997) Nutrition and the ozone sensitivity of birch (*Betula pendula*) I. Responses at the leaf level. Trees 12:1–10

Medrano H, Escalona JM, Bota J, Gulias J, Flexas J (2002) Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. Ann Bot 89:895–905

Miller P, McBride J (1999) Oxidant air pollution impacts in the Montane Forests of Southern California: the San Bernardino Mountain Case Study. Springer, New York

Nali C, Paoletti E, Marabottini R, Della Rocca G, Lorenzini G, Paolacci AR, Ciaffi M, Badiani M (2004) Ecophysiological and biochemical, strategies of response to ozone in Mediterranean evergreen broadleaf species. Atmos Environ 38:2247–2257

Nunn AJ, Kozovits AR, Reiter IM, Heerdt C, Leuchner M, Lutz C, Liu X, Löw M, Winkler JB, Grams TEE (2005a) Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*). Environ Pollut 137:494–506

Nunn AJ, Reiter IM, Häberle KH, Langebartels C, Bahnweg G, Pretzsch H, Sandermann H, Matyssek R (2005b) Response patterns in adult forest trees to chronic ozone stress: identification of variations and consistencies. Environ Pollut 136:365–369

Nunn AJ, Reiter IM, Häberle KH, Werner H, Langebartels C, Sandermann H, Heerdt C, Fabian P, Matyssek R (2002) "Free Air" ozone canopy fumigation in an old-growth mixed forest: concept and observations in beech. Phyton 42:105–119

Ogaya R, Penuelas J (2003) Comparative field study of *Quercus ilex* and *Phillyrea latifolia*: photosynthetic response to experimental drought conditions. Environ Exp Bot 50:137–148

Pääkkönen E, Günthardt-Goerg MS, Holopainen T (1998a) Responses of leaf processes in a sensitive birch (*Betula pendula* Roth) clone to ozone combined with drought. Ann Bot 82:49–59

Pääkkönen E, Vahala J, Pohjolai M, Holopainen T, Kärenlampi L (1998b) Physiological, stomatal and ultrastructural ozone responses in birch (*Betula pendula* Roth.) are modified by water stress. Plant Cell Environ 21:671–684

Panek JA (2004) Ozone uptake, water loss and carbon exchange dynamics in annually drought-stressed *Pinus ponderosa* forests: measured trends and parameters for uptake modeling. Tree Physiol 24:277–290

Panek JA, Goldstein AH (2001) Response of stomatal conductance to drought in ponderosa pine: implications for carbon and ozone uptake. Tree Physiol 21:337–344

Panek JA, Kurpius MR, Goldstein AH (2002) An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem. Environ Pollut 117:93–100

Paoletti E, Grulke NE (2005) Does living in elevated CO_2 ameliorate tree response to ozone? A review on stomatal responses. Environ Pollut 137:483–493

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

Pfeifhofer HW (1989) Evidence for chlorophyll b and lack of lutein in *Neottia nidus* Avis plastids. Biochemie und Physiologie der Pflanzen 184:55–61

Pretzsch H, Kahn M, Grote R (1998) Die Fichten-Buchen-Mischbestände des Sonderforschungsbereiches "Wachstum oder Parasitenabwehr?" im Kranzberger Forst. Forstwissenschaftliches Centralblatt 117:241–257

Raspe S, Grimmeisen W, Schulte B (2004) Der Sommer 2003 grub dem Wald das Wasser ab. LWF aktuell 43:4–6

Reiter IM, Häberle KH, Nunn AJ, Heerdt C, Reitmayer H, Grote R, Matyssek R (2005) Competitive strategies in adult beech and spruce: space-related foliar carbon investment verus carbon gain. Oecologia 146:337–349

Retzlaff WA, Arthur MA, Grulke NE, Weinstein DA, Gollands B (2000) Use of a single-tree simulation model to predict effects of ozone and drought on growth of a white fir tree. Tree Physiol 20:195–202

Ryan MG, Bond BJ, Law BE, Hubbard RM, Woodruff D, Cienciala E, Kucera J (2000) Transpiration and whole-tree conductance in ponderosa pine trees of different heights. Oecologia 124:553–560

Scarascia-Mugnozza G, Karnosky DF, Ceulemans R, Innes J (2001) The impact of CO_2 and other greenhouse gases on forest ecosystems: an introduction. In: Karnosky DF, Scarascia-Mugnozza G, Ceulemans R, Innes J (eds) The impacts of carbon dioxide and other greenhouse gases on forest ecosystems. CABI Press, Wallingford, UK, pp 1–16

Schär C, Jendritzky G (2004) Climate change: Hot news from summer 2003. Nature 432:559–560

Schulze E (1994) Flux control in biological systems. Academic Press, San Diego

Stockwell W, Kramm G, Scheel H-E, Mohnen V, Seiler W (1997) Ozone formation, destruction and exposure in Europe and the United States. In: Sandermann H, Wellburn A, Heath R (eds) Forest decline and ozone, a comparison of controlled chamber and field experiments, vol 127. Springer, Berlin Heidelberg New York, p 400

Stribley GH, Ashmore MR (2002) Quantitative changes in twig growth pattern of young woodland beech (*Fagus sylvatica* L.) in relation to climate and ozone pollution over 10 years. For Ecol Manage 157:191–204

Tausz M, Wonisch A, Grill D, Morales D, Jimenez MS (2003) Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. J Exp Bot 54:1505–1510

Torsethaugen G, Pell EJ, Assmann SM (1999) Ozone inhibits guard cell K^+ channels implicated in stomatal opening. Proc Natl Acad Sci USA 96:13577–13582

Tyree M, Zimmermann M (2002) Xylem structure and the ascent of sap. Springer, Berlin, Germany

Wallin G, Karlsson PE, Sellden G, Ottosson S, Medin EL, Pleijel H, Skarby L (2002) Impact of four years exposure to different levels of ozone, phosphorus and drought on chlorophyll, mineral nutrients, and stem volume of Norway spruce, *Picea abies*. Physiol Plant 114:192–206

Werner H, Fabian P (2002) Free-air fumigation of mature trees: a novel system for controlled ozone enrichment in grown-up beech and spruce canopies. Environ Sci Pollut Res Int 9:117–121

Wieser G, Havranek W-M (1993) Ozone uptake in the sun and shade crown of spruce: Quantifying the physiological effects of ozone exposure. Trees 7:227–232

Wieser G, Matyssek R, Kostner B, Oberhuber W (2003) Quantifying ozone uptake at the canopy level of spruce, pine and larch trees at the alpine timberline: an approach based on sap flow measurement. Environ Pollut 126:5–8

Wipfler P, Seifert T, Heerdt C, Werner H, Pretzsch H (2005) Growth of adult Norway spruce (*Picea abies* [L.] Karst.) and European beech (*Fagus sylvatica* L.) under free-air ozone fumigation. Plant Biol 7:611–618