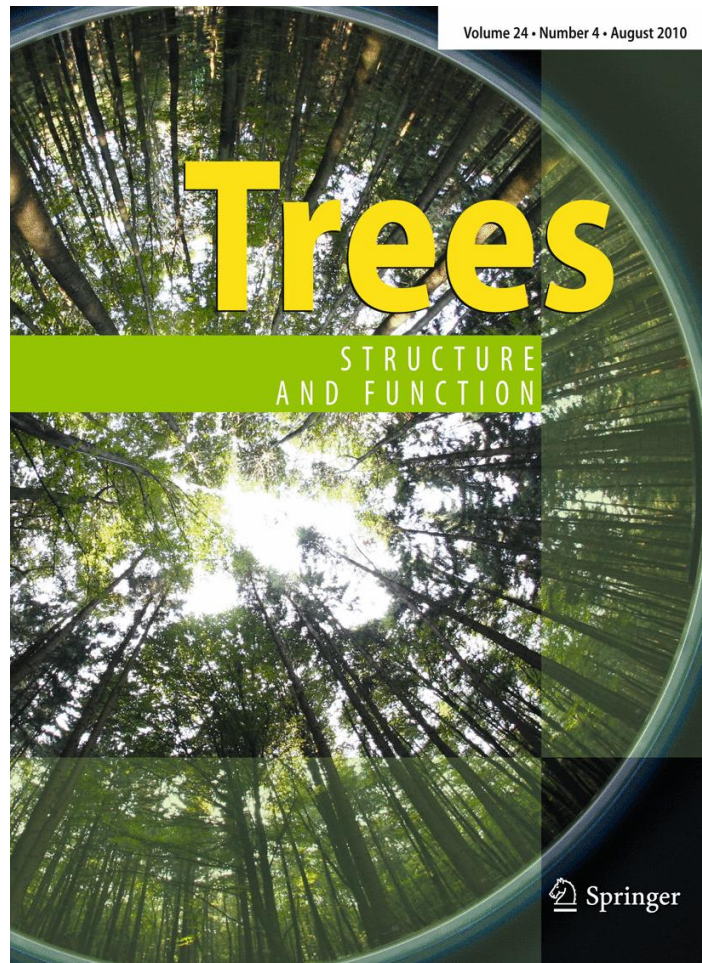


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Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements

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Abstract An open-top chamber experiment was carried out in Curno (Northern Italy) in 2004 and 2005 on seedlings of *Fagus sylvatica* (FS), *Quercus robur* (QR), and an ozone-sensitive *Populus* (POP) clone, to investigate the role of two stress factors: tropospheric ozone and water shortage. Treatments were filtered air to achieve a 50% reduction in the environmental ozone concentrations (charcoal filtered, CF); and non-filtered air, with a 5% reduction in the environmental ozone concentrations (non-filtered, NF). Overall ozone exposure (AOT40) in open air (April–September) was 26,995 ppb h in 2004 and 25,166 ppb h in 2005. The plants were either watered (W) or not watered (dry, D). We investigated the above-ground biomass, tree-ring growth, stable carbon isotopes ratio, i.e.

$\delta^{13}\text{C}$ of tree rings, and the photosynthetic parameter Driving forces (DF_{ABS}), derived from chlorophyll *a* fluorescence analysis. Ozone-induced growth reduction (in terms of biomass) in POP, and that reduction was more pronounced in D plots. A synergistic effect of ozone and drought stress was evidenced by DF_{ABS} in POP and QR, but not in FS. The water availability was revealed as the main factor influencing the isotopic ratio $\delta^{13}\text{C}$. In drought-stressed seedlings, the increase in $\delta^{13}\text{C}$ value was accompanied by the reduction in stomatal conductance and increased DF_{ABS} . Fast-growing plant species with high water requirements are more susceptible to ozone and drought stress.

Keywords Biomass · Carbon isotope discrimination · Chlorophyll *a* fluorescence · *Fagus sylvatica* · *Populus* clone · *Quercus robur* · Driving forces · Ring width

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Introduction

Variations in environmental conditions influence the development of annual rings and trees' diameter growth (Fritts 1976). Several authors have studied the relationship between ring width, ozone and/or drought (Bartholomay et al. 1997; Somers et al. 1998; Dittmar et al. 2003; Novak et al. 2007). Novak et al. (2007), in an experiment carried out at the Lattecaldo facility in southern Switzerland found significant reductions in diameter growth only in a fast-growing *Populus* clone, thus confirming the findings of Bortier et al. (2000), i.e. the growth response to ozone is more marked in fast growing when compared with slow-growing species. Chemical features of tree rings can be used to investigate the history of trees (Ferretti et al. 2002).

Amongst these, the analysis of stable carbon isotope signatures ($\delta^{13}\text{C}$) has proven to be useful in assessing plant responses to several environmental stress factors, including drought and air pollution (Martin and Sutherland 1990; Saurer et al. 1995; Dawson et al. 2002; Wagner and Wagner 2006). During photosynthesis, plants discriminate between $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. In atmospheric CO_2 , ^{13}C accounts for about 1.11% of the overall carbon. $\delta^{13}\text{C}$ expresses the relative abundance of ^{13}C in relation to ^{12}C in ‰. Francey and Farquhar (1982) demonstrated that carbon isotope variations are subject to strong physiological control through gas exchange in leaves. Farquhar et al. (1989) developed a model that suggests that water-use efficiency (WUE), i.e. the ratio of photosynthetic activity to leaf transpiration can be estimated from the parameter $\delta^{13}\text{C}$. Less negative $\delta^{13}\text{C}$ values indicate that the trees are acclimating to water shortage, using atmospheric CO_2 more completely when stomata are closed and carbon uptake is limited. Other studies, however, highlight the role of the enzyme phosphoenol pyruvate carboxylase (PEPc), which has a greater affinity for ^{13}C than ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). The production and the activity of these enzymes are stimulated, both in C3 and C4 plants, by a variety of stressors, including drought stress and ozone (Freyer 1979; Martin et al. 1988; Matyssek et al. 1992; Singh-Sangwan et al. 1994; Saurer et al. 1995; Lütz et al. 2000; Fontaine et al. 2003; Gaucher et al. 2004; Inclan et al. 2005; Novak et al. 2007).

A tree's growth is directly related to its net photosynthesis (P_N), but the relationship between growth and chlorophyll *a* fluorescence is more difficult to ascertain. It is commonly assumed that there is a linear relationship between the rate of electron transport (ETR) and CO_2 assimilation (Genty et al. 1989). Parameters derived from the fluorescence transient analysis (as performance index on absorption basis, PI_{ABS} , and driving forces DF_{ABS} , see Strasser et al. 2000, 2004) were also suggested as predictors for growth in *Fagus sylvatica* trees subjected to ozone fumigations (Clark et al. 2000). According to Adams and Demming-Adams (2004), photosystem II (PSII) efficiency provides no direct information on P_N : electron flux is not necessarily used in carbon metabolism, because it may also be re-routed to other biochemical pathways, such as reduction in O_2 , or nitrite, or sulphate, or thioredoxin or photorespiration (see also Sánchez-Rodríguez et al. 1997; Cornic and Fresnau 2002). However, moderate conditions of drought stress may improve the efficiency of PSII, in terms of both quantum yield of primary photochemistry and electron transport capacity (Bukhov and Carpentier 2004; Ohashi et al. 2006). Ozone, too, may increase temporarily the values of PI_{ABS} (Bussotti et al. 2007, 2008), indicating the activation of processes of physiological adjustment to stress.

According to a recent review (Bussotti 2008), plants adapted against drought stress are resistant also to ozone. The combined effects of O_3 and drought may be either antagonistic (by inducing stomatal closure, Emberson et al. 2000) or synergistic (by stimulating an excessive production of reactive oxygen species, Tausz et al. 2007). Plant species typical of stressful environments (for example, in dry sites) are less sensitive to ozone than species with opposite ecological requirements. This statement is considered true when we consider well-defined ecosystems (for example, Mediterranean vs. alpine and continental vegetation, see Bussotti and Gerosa 2002; Bussotti 2008). Within the same ecological region, differences in ozone sensitivity are assumed to be related to the autoecological characteristics of each plant species and/or population. In this paper, we analyse the responses to ozone and drought stress of three different mesophytic tree species from North Italy. The hypothesis to be tested is that the individual effects of ozone and drought stress, as well their possible additive effects are stronger ("synergistic") in species with higher water requirements.

Materials and methods

Experimental set-up

The experiment was conducted at the open-top chambers (OTCs) facility at Curno (C.R.IN.E.S., Centre of Research on Effects of Pollutants on Ecosystems, Lombardy, North Italy, $45^\circ 41' \text{ N}$, $9^\circ 37' \text{ E}$, elevation 245 m a.s.l.), where the ozone levels are well above the UN-ECE thresholds (Bacci et al. 1990; Staffelbach et al. 1997; Gerosa and Ballarin-Denti 2003). The soil type is typic Paleudalf fine-silty, mixed, mesic according to the USDA (United States Department of Agriculture) classification, and was prepared carefully to assure homogeneity of conditions across the chambers. The experimental set-up consisted of eight OTCs, four of which were charcoal filtered (CF) and four with ambient, non-filtered air (NF). The OTCs were constructed according to Heagle et al. (1973), with diameter = 3 m and height = 2.5 m. Each CF chamber was equipped with 12 filters ($1 \times 1 \text{ m}$), containing 2.5 kg of powdered activated carbon. Ozone concentrations within each chamber, and outside, were continuously monitored with a Dasibi 1108 RS automatic analyser (Dasibi Italia s.r.l., Milano, Italy), via a solenoid valve switching system, which draws air from sampling points in the centre of each plot at a height of 90 cm. The experiment was carried out from 1 April to 30 September in 2004 and 2005. Plants in the CF chambers were exposed to 50% of the ozone concentration of the ambient air, and those grown in the NF chambers were exposed to 95% of the ambient air ozone.

The wind speed in the chambers was on an average 1 m s^{-1} .

Two plots of each ozone treatment (CF and NF) were regularly watered to field capacity (W plots) with an automatic sprayer. The other two plots per ozone treatment received only emergency watering, i.e. when leaf wilting was evident (D plots, dry). Overall, over the period from April to September, W plots were given 928 mm of water in 2004 and 1,063 mm in 2005, whilst D plots were given 487 mm of water in 2004 and 582 mm in 2005. The volumetric soil water content ($\text{Vol}_{\text{H}_2\text{O}}/\text{Vol}_{\text{SOIL}}$) was constantly measured with automatic TDR CS616 sensors (Campbell Scientific Inc., Logan, UT, USA). Table 1 reports mean monthly water contents in the W and D plots, expressed as a percentage of the field capacity. In W plots, the water content remained constantly around 30%, which was about 95% of field capacity (FC). In D plots, the water content decreased over the course of the summer months to about 15% (i.e. 50% of FC). In addition, Table 1 shows meteorological data, including the ozone pollution features. This work is part of a set of papers dealing with the same experiment (Bussotti et al. 2007; Gerosa et al. 2008, 2009; Marzuoli et al. 2009). The findings which have already been published will be specified within the text, and will be used here as “supporting data”.

Plant arrangement in the OTCs and sampling

The species considered were *Fagus sylvatica* L. (FS), a typical plant of mesophytic pre-alpine forests, *Quercus robur* L. (QR), a plain land woods species, and the

sensitive poplar clone *Populus maximowiczii* Henry \times *P. \times berlinensis* Dippel (Oxford clone, according to Schreiner and Stout (1934), called *Populus*—POP—in this text). The latter is a fast growing tree employed in short rotation forestry. Foliar characteristics were reported in Bussotti et al. (2007): leaf mass per area and leaf density were respectively: 13.7 mg cm^{-2} and 487.3 mg cm^{-3} in POP; 10.8 mg cm^{-2} and 656.8 mg cm^{-3} in QR; 7.1 mg cm^{-2} and 568.6 mg cm^{-3} in FS.

In each chamber (both CF and NF) 4 1-year-old seedlings of each tree species were planted in the ground in spring 2003 (in the case of POP, cuttings were used). Each seedling was planted with a space of $70 \times 70 \text{ cm}$, and arranged randomly to avoid competition. The OTC experiment began in April 2004, after a period of acclimation. Thus, observations were carried out on 2- and 3-year-old seedlings, except for the POP plants, which grew so rapidly that they were coppiced at the end of winter, in early 2004 and again in early 2005. Two plants per each chamber, placed in the central part of the chamber to avoid an “edge” effect, were selected for all measurements and samplings.

Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence assessment was carried out on the individuals of POP, FS and QR selected for sampling, at the following dates: 30 May, 28 June, 26 July, 30 August, 26 September 2005. Measurements of chlorophyll fluorescence were repeated on four leaves on each plant, on each of the sampling dates. Chl *a* fluorescence transients of

Table 1 Meteorological parameters, ozone exposure and soil water content in the two growing seasons 2004–2005

	Temperature		O_3 max (ppb)	AOT40 (ppb h)	Water received		Soil water content	
	Max °C	Min °C			Dry plots (mm)	Wet plots (mm)	Dry plots (% FC)	Wet plots (% FC)
2004								
Apr	25.9	3.7	88	1,938	117	117	75	70
May	27.7	6.1	101	3,100	85	99.5	72	74
Jun	33.4	13.9	153	7,225	44	96.9	47	61
Jul	34.1	12.3	144	8,636	81	164	49	76
Aug	31.9	15.1	122	4,731	124	300	59	80
Sep	31.3	8.9	130	3,453	35	148	43	83
2005								
Apr	24.5	118.2	118.2	1,502	118	118	76	76
May	31.8	60.2	138	4,793	60	138	59	68
Jun	35	52.7	181	8,174	52	181	43	73
Jul	34.2	91.1	172.3	6,426	91	172	56	88
Aug	36.4	114.4	232.1	3,551	114	232	46	89
Sep	31	145.3	221.6	1,640	145	221	73	97

O_3 max maximum hourly ozone concentration recorded, AOT40 accumulated ozone over a threshold of 40 ppb (calculated from the outside values), FC field capacity

intact leaves were measured with a HandyPEA fluorimeter (Hansatech Instruments, Pentney—Norfolk, UK) on dark adapted leaves (20 min). The rising fluorescence transients were induced by red light (peak at 650 nm) of 600 W m^{-2} provided by an array of six light-emitting diodes; they were recorded for 1 s, starting from 20 μs after the onset of illumination, with 12-bit resolution. The analysis of the transient is called the JIP test and has been elaborated both in terms of theory and application (Strasser et al. 2000, 2004). Amongst the JIP test parameters, in this paper, we have considered the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry ($\phi_{\text{P}_0} = T_{\text{R}_0}/\text{ABS}$), the efficiency ($\Psi_{\text{E}_0} = E_{\text{T}_0}/T_{\text{R}_0}$) with which a trapped photon can move an electron into the electron transport chain further than Q_{A}^- , and the concentration of active PSII reaction centres per excited cross section (RC/CS). Strasser et al. (2000) introduced a multiparametric expression of these three independent steps contributing to photosynthesis, the so-called performance index (PI_{ABS}):

$$\text{PI}_{\text{ABS}} = \left[\frac{\gamma_0}{1 - \gamma_0} \right] \left[\frac{\phi_{\text{P}_0}}{1 - \phi_{\text{P}_0}} \right] \left[\frac{\Psi_{\text{E}_0}}{1 - \Psi_{\text{E}_0}} \right]$$

where γ is the fraction of reaction centre chlorophyll (Chl_{RC}) per total chlorophyll (Chl_{RC} + Chl_{Antenna}). Therefore, $\gamma/(1 - \gamma) = \text{Chl}_{\text{RC}}/\text{Chl}_{\text{Antenna}} = \text{RC}/\text{ABS}$.

The log(PI_{ABS}) is defined as the total driving force (DF_{ABS}) for photosynthesis of the observed system, created by summing the partial driving forces

$$\begin{aligned} \text{DF}_{\text{ABS}} &= \log(\text{PI}_{\text{ABS}}) \\ &= \log(\text{RC}/\text{ABS}) + \log(\phi_{\text{P}_0}/1 - \phi_{\text{P}_0}) \\ &\quad + \log(\Psi_{\text{E}_0}/1 - \Psi_{\text{E}_0}). \end{aligned}$$

The actual potential is calculated using as reference the standard potential of the reaction under consideration $\Delta\text{DF} = \text{DF}_{\text{treated}} - \text{DF}_{\text{control}}$.

Above-ground biomass and tree rings: sampling and measurements

All plants were harvested in December 2005. The overall weight of all the above-ground woody parts (stem and branches) was measured at the time of harvesting. Subsequently, the dry weight was determined after drying at 70°C until constant weight. A total of 16 individuals from each species were harvested. Cross-sections were cut from the main stem of each plant at 10-cm above the soil surface. Tree-ring widths of each sample were measured to the nearest 10 μm for the years 2003, 2004 and 2005 using a stereomicroscope (Wild W8 Leica, Solms, Germany) coupled with a Lintab measurement table (Rinntech, Heidelberg, Germany). The data were recorded with the Time Series Analysis Programme (TSAP) software (Rinntech,

Heidelberg, Germany). Tree-ring width can be used as an indicator of whole tree biomass (Dobbertin 2005).

The same samples used for the ring-width measurements were then dissected and separated according to year. The wood samples were finely ground and used for stable carbon isotope analysis. An aliquot of the samples was weighed into tin capsules and then combusted in an Elemental Analyser (EA 1108, Finnigan, Germany). The evolving CO₂ passed into the isotope ratio mass spectrometer (Delta S, Finnigan, Germany), where the ¹³C/¹²C ratio of the sample was determined according to the international PDB reference based on the equation:

$$\delta^{13}\text{C}_{\text{Sample}} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} - 1 \right] \times 1,000$$

The standard error for the repeated analysis of an internal standard was lower than 0.1‰.

Supporting data: exposure and fluxes of ozone

Data on ozone levels and exposure for this same experiment have already been presented in previous papers (Gerosa et al. 2008, 2009; Marzuoli et al. 2009). Exposures are expressed as AOT40, i.e. accumulated ozone concentrations above the threshold of 40 ppb, from April to September. The AOT40 values (measured at the height of 90 cm from the soil) outside the chambers during the experimental periods were 26,995 ppb h in 2004 (21,779 ppb h in the NF and 1,572 ppb h in the CF plots), and 25,166 ppb h in 2005 (16,890 ppb h in the NF and 1,707 ppb h in the CF plots). Maximum hourly ozone concentrations outside the chambers occurred in June 2004 (153 ppb) and in May 2005 (146 ppb). The stomatal fluxes of ozone (F_{st}) were calculated for the years 2004 and 2005. The stomatal conductance (g_{w}) for poplar, which is necessary for flux calculation, was modelled using the classic Jarvis multiplicative approach (Jarvis 1976), and the ozone stomatal flux was calculated at the top canopy leaf, according to other authors (Emberson et al. 2000; Karlsson et al. 2000). Details of the method can be found in Gerosa et al. (2009) and Marzuoli et al. (2009). The results are summarised in Table 2. POP showed the greatest g_{w} and F_{st} in the W plots; QR displayed an intermediate behaviour, whereas the lowest values were detected on FS. Drought conditions caused a 20–25% flux reduction in all species.

Statistics

The experimental design was a complete two-way randomized factorial design, with watering and ozone as the

Table 2 Accumulated ozone dose (for total leaf area) and mean stomatal conductance (g_w) according to the different tree species and experimental conditions

Species	W_{treat}	g_w (mmol m ⁻² s ⁻¹)		AOD (NF) (mmol m ⁻²)		AOD (CF) (mmol m ⁻²)	
		2004	2005	2004	2005	2004	2005
FS	W	23.38	26.55	25.10	26.94	12.11	12.52
	D	18.99	21.11	20.98	24.42	11.26	13.52
QR	W	37.99	42.80	38.13	40.57	18.44	19.04
	D	30.77	33.93	31.74	36.98	17.08	20.63
POP	W	45.62	50.37	39.45	42.65	19.05	19.90
	D	36.34	39.40	32.88	38.10	17.64	21.12

FS *Fagus sylvatica*, QR *Quercus robur*, POP *Populus*, W_{treat} watering treatment, W watered, D dry, AOD accumulated ozone dose

From Gerosa et al. 2009; Marzuoli et al. 2009

fixed main treatment effects. Because chambers were the unit of replication, this gave two replicates per treatment combination. Because ring widths were highly variable prior to the start of the experiment, the 2003 ring widths were used as a covariate in an analysis of covariance. For all experiments, significance was assumed if $p < 0.05$, although $p < 0.10$ was used to indicate probable trends, even though not strictly significant in a traditional sense. We use this approach because the sample sizes involved are small.

For the data analysis, the GLMM module of the package Statistica v 7.1 was employed. A multivariate statistical technique, the principal component analysis (PCA), was then used to investigate and reveal structures of variability and correlations between variables within the measurements. The selection of the principal factors was based on those with eigenvalues greater than 1.

Results

POP displayed the greatest above-ground harvested biomass (even though only the biomass produced in the last year—2005—was considered, see Table 3). QR comes next, followed by FS. Mean annual ring increments for 2004–2005, i.e. the mean value for all treatments, were 3,310 μm in QR and 1,880 μm in FS. POP could not be assessed for this parameter since only the 2005 wood was available; however, the mean 2005 diameter was the greatest amongst the considered plant species (2.5 cm). In 2003, before the treatments began, ring widths were largely variable for FS and QR (Table 3). This may prevent the analysis of variance from being used. For this reason, an analysis of covariance was applied, using the 2003 ring widths as covariate.

Water shortage reduced the above-ground biomass and 2005 ring width in QR, and increased $\delta^{13}\text{C}$ (Table 3) in QR and FS, both in 2004 and in 2005. Photosynthetic

efficiency (expressed with DF) was enhanced in QR under water shortage conditions (in CF OTC, see Fig. 1a). Effects of O_3 were detected on FS with a transitory increment of ring width (in 2004), and there was a suggestion that biomass slightly increased, even though technically, the differences were not significant ($p = 0.059$) (Table 4).

Unlike the other species, all the POP plants had homogeneous initial conditions, from both a genetic point of view (a clone reproduced through cuttings) and in terms of their growth (all plants had been coppiced before the beginning of the season). Thus, in their case, the analysis of variance has been applied. The above-ground biomass and diameter were significantly reduced both by water shortage and ozone exposure. In both kind of stress (water shortage and ozone), the significance of the differences between “treated” and “control” were: $p < 0.01$ for above-ground biomass and $p < 0.05$ for diameter. In D conditions, the biomass and diameter (Table 3) were reduced respectively by 28 and 10% (CF-OTCs), and by 39 and 27% (NF-OTCs), as compared to W conditions. Photosynthetic efficiency was also severely affected by ozone ($p < 0.001$), and the reduction in DF_{ABS} was more marked in D rather in W conditions (Fig. 1a, b).

The results of PCA (principal component analysis) performed taking into account only the common parameters (i.e., those relative to 2005, Table 5), indicated in factor 1 in all species, the most obvious correlations, i.e., that the growth parameters (above-ground biomass and ring width) are correlated. Growth parameters were also positively correlated with the parameters that express the gas exchange rates (O_3 absorbed dose in FS, stomatal conductance in QR and POP). Stomatal conductance correlated inversely with $\delta^{13}\text{C}$ both in FS and QR. Photosynthetic efficiency (DF) also correlated with $\delta^{13}\text{C}$ in all tree species.

The effect of ozone was recognisable only in POP, with negative correlations between ozone dose, growth parameters, DF and $\delta^{13}\text{C}$.

Table 3 Descriptive statistics (mean \pm standard error) of the analysed growth parameters

	Watered		Dry	
	CF	NF	CF	NF
<i>Fagus sylvatica</i>				
A.g. biomass (g)	88.7 \pm 22.2a	130.9 \pm 18.7a	51 \pm 6.2a	135.1 \pm 29.0a
RW 2003 (μm)	1,020 \pm 184b	1,633 \pm 327a	762 \pm 100b	1,115 \pm 97a
RW 2004 (μm)	1,405 \pm 160a	2,103 \pm 101a	967 \pm 183b	1,773 \pm 256a
RW 2005 (μm)	2,293 \pm 291a	216.5 \pm 96a	2,040 \pm 171a	2,575 \pm 578a
$\delta^{13}\text{C}$ 2004 (‰)	-28.3 \pm 0.34a	-28.4 \pm 0.31a	-26.3 \pm 0.38b	-26.9 \pm 0.21b
$\delta^{13}\text{C}$ 2005 (‰)	-28 \pm 0.33a	-28.1 \pm 0.12a	-26.8 \pm 0.42b	-26.5 \pm 0.19b
<i>Quercus robur</i>				
A.g. biomass (g)	330.3 \pm 27.3a	518.5 \pm 108.6a	255.6 \pm 31.2a	216.7 \pm 34.0a
RW 2003 (μm)	822 \pm 154b	1,935 \pm 292a	987 \pm 243a	1,335 \pm 100a
RW 2004 (μm)	2,028 \pm 237a	2,340 \pm 473a	1,628 \pm 189a	1,583 \pm 366a
RW 2005 (μm)	394.5 \pm 23.0a	441.5 \pm 31.5a	296.5 \pm 38.1a	332.5 \pm 38.5a
$\delta^{13}\text{C}$ 2004 (‰)	-27.9 \pm 0.21a	-27.2 \pm 0.27a	-26.4 \pm 0.51a	-26.3 \pm 0.37
$\delta^{13}\text{C}$ 2005 (‰)	-27.2 \pm 0.23a	-27.2 \pm 0.40a	-26.1 \pm 0.30a	-26.1 \pm 0.27a
<i>Populus</i>				
A.g. biomass (g)	813.1 \pm 66.3a	685.4 \pm 71.9b	616.8 \pm 27.9c	282.3 \pm 23.3c
Diameter 2005 (cm)	2.87 \pm 0.11a	2.5 \pm 0.16a	2.6 \pm 0.36a	1.83 \pm 0.50b
$\delta^{13}\text{C}$ 2005 (‰)	-28.2 \pm 0.10a	-28.2 \pm 0.13a	-28.4 \pm 0.20a	-27.9 \pm 0.30a

A.g. biomass above-ground biomass, RW ring width, a.u. arbitrary units

Four plants per treatment; treatment replicates $n = 2$

The same letter under the values indicates no significant differences at $p < 0.05$

RW200x represent the ring width of the year 200x, with $x = 3, 4, 5$

Discussion

Water shortage produced an evident growth reduction in POP, both for biomass and diameter and that reduction was less marked in QR, whereas no effect was detected in FS growth. Ozone caused growth reduction only in POP, and no effects on QR. In FS, we observed a slight and transient increment of radial growth (RW2004) in ozone-treated plants. The observed effect of drought was to a large extent predictable. Several authors have reported that ozone induces growth reduction in fast growing species as *Populus* (cfr. Bortier et al. 2000), but the opposite effect (even though transient) of ozone on FS seems quite surprising. A small number of papers, however, have reported that a transitory increase in growth can be observed during the first treatment period (Rebbeck and Scherzer 2002), or as a consequence of exposure to moderate ozone concentrations (Oksanen et al. 2001).

A combination of ozone and drought can exacerbate the overall effect (Alonso et al. 2001; Yonekura et al. 2001, 2004; Grulke et al. 2004; Ribas et al. 2005; Tausz et al. 2007). Several explanations have been suggested for this behaviour, including an excessive production of reactive oxygen species (ROS) due to the concomitant action of the

two stressors (Tausz et al. 2007) and altered stomatal function due to ozone (stomata remain open or their closure is slowed in drought stress conditions, see Maier-Maercker 1989; McLaughlin and Downing 2002; Grulke et al. 2002).

An opposite and antagonistic effect between ozone and drought stress was observed by Watanabe et al. (2005) on *F. crenata* in Japan: mild drought stress not only induces stomatal closure, but also increases the leaf glutathione concentration thus enhancing metabolic defences. In our study, ozone plus drought stress produced detrimental effects on growth parameters in POP: both biomass and diameter, as well DF_{ABS} , were more affected in NF-D rather than in the other conditions.

In FS and QR, $\delta^{13}\text{C}$ had higher values under D than under W conditions. This increase in $\delta^{13}\text{C}$ is related to WUE, which increases under conditions of drought stress, since stomatal conductance decreases more markedly than net photosynthesis (Adams and Kolb 2004; Egilla et al. 2005; Yin et al. 2005). Enhanced WUE make possible a better exploitation of the carbon also in conditions of partial stomatal closure, and thus feed detoxification processes (Dizengremel et al. 2008), although this is done to the detriment of growth. Ozone fumigation has been found in previous studies to increase $\delta^{13}\text{C}$ values, although not in

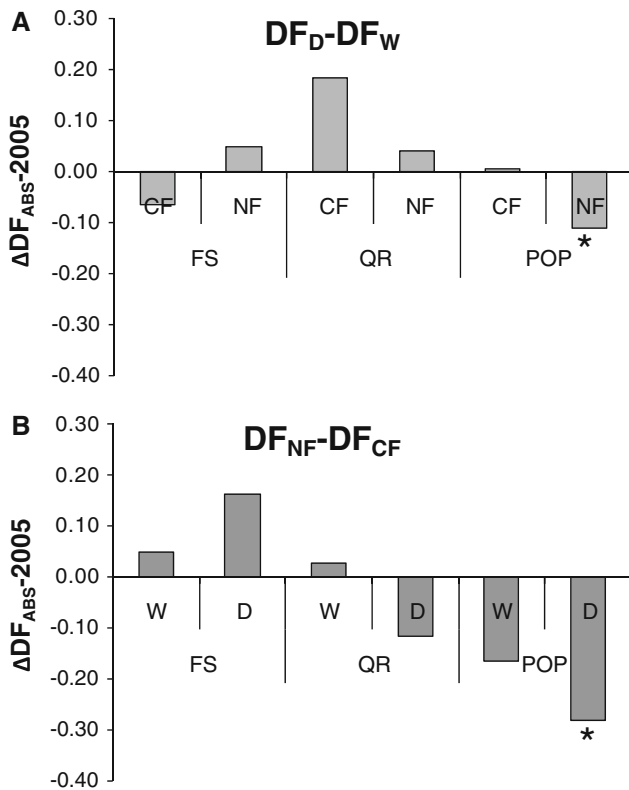


Fig. 1 Values of the average yearly ΔDF_{ABS} 2005 in relation to water stress ($DF_D - DF_W$ **a**) and in relation to ozone ($DF_{NF} - DF_{CF}$ **b**). Four plants per treatment; treatment replicates $n = 2$. Asterisks indicate significant differences between DF_D and DF_W (**a**), and between DF_{NF} and DF_{CF} (**b**)

Table 4 Analysis of covariance on *Fagus sylvatica* and *Quercus robur*, with RW2003 as covariate, to test the differences amongst water status (WS watered and dry) and ozone exposure (O_3 , not filtered and charcoal filtered)

	<i>Fagus sylvatica</i>			<i>Quercus robur</i>		
	WS	O_3	$WS \times O_3$	WS	O_3	$WS \times O_3$
Degrees of freedom	1	1	1	1	1	1
DF_{ABS} 2005	0.749	0.217	0.271	0.013	0.133	0.549
A.g. biomass	0.574	0.059	0.333	0.018	0.750	0.950
RW 2005	0.802	0.630	0.184	0.056	0.929	0.644
RW 2004	0.150	0.014	0.487	0.159	0.446	0.480
$\delta^{13}C$ —2004	0.004	0.320	0.370	0.081	0.756	0.652
$\delta^{13}C$ —2005	0.011	0.650	0.520	0.039	0.852	0.925

p values of the homogeneity of slope test are reported, four plants per treatment; treatment replicates $n = 2$

Significances with $p < 0.05$ are evidenced in bold

DF_{ABS} driving force on absorption basis, A.g. biomass above-ground biomass, RW ring width

combination with reduced stomatal conductance (Martin et al. 1988; Saurer et al. 1995). In relation to ozone, however, no significant variations in $\delta^{13}C$ were observed in

our study in the considered species. In POP, however, the absorbed ozone dose and $\delta^{13}C$ were directly related (Table 5) and such behaviour supports, at least partially, the findings of Novak et al. (2007) that found significant variation only in POP.

Growth parameters (above-ground biomass and ring width) correlated positively in each species to flux parameters (stomatal conductance and absorbed ozone dose, see Table 5), since the conditions that favour growth (moist soils, high gas exchange rates) are also effective in enhancing the uptake of ozone. According to Yamaguchi et al. (2007) in conditions favourable to photosynthesis and growth, such as high nitrogen availability, the relative growth reduction is higher than under less favourable conditions. This behaviour is evident in *Fagus crenata*, but not in *Quercus serrata* (Watanabe et al. 2007). The growth reduction in relation to ozone was evidenced in POP, both for stem diameter and above-ground woody biomass. Stomatal conductance was also inversely related to $\delta^{13}C$ in all species, thus indicating increased WUE. Metabolic adjustments in stress conditions require increased photosynthesis and photosynthetic efficiency (Eamus et al. 1990; Retuerto et al. 2004; Rinnan and Holopainen 2004; Huttunen et al. 2007). In our study, the inverse relation of DF_{ABS} to g_w and $\delta^{13}C$ in POP and QR (and to a lesser extent in FS) give evidence of this physiological behaviour.

Conclusions

The ecological differences of the considered tree species correspond to a gradient in gas exchange rates (stomatal conductance and ozone uptake) and growth between the species (greater in POP and lower in FS). A clear detrimental effect of ozone on the growth and fluorescence parameters was observed only on POP, the most water-demanding species with lower leaf density, especially in dry conditions. This confirmed the sensitivity to ozone of fast-growing species (Reich 1987, 1998; Bortier et al. 2000). This result corresponds to the behaviour of the ‘‘Oxford’’ poplar clone utilised in this study, in relation to foliar symptoms (Gerosa et al. 2009; Marzuoli et al. 2009), and further confirms the findings reported by Novak et al. (2007). The results on FS and QR are less clear. A significant effect on the carbon isotopic discrimination was observed in relation to water shortage, but not to ozone. Growth parameters were negatively affected by drought in QR, but slightly and transiently enhanced by ozone in FS. Photosynthetic efficiency (expressed as DF) was slightly enhanced by drought in CF plots and depressed by ozone in drought conditions. Overall, POP displayed the greater detrimental responses to drought plus ozone; QR behaviour was intermediate and FS seemed to have a transient

Table 5 Results of the multivariate statistic

	<i>Quercus robur</i>		<i>Fagus sylvatica</i>		<i>Populus</i>	
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
DF _{ABS}	0.35	0.862	-0.679	0.151	0.893	0.254
A.g. biomass	0.934	0.179	<i>0.586</i>	<i>0.679</i>	<i>0.637</i>	0.749
Ring width	0.917	-0.028	<i>0.578</i>	<i>0.625</i>	0.741	<i>0.598</i>
O ₃ Dose	0.449	-0.048	-0.051	0.842	-0.827	0.058
g _w	0.914	0.092	0.915	0.141	-0.066	0.938
δ ¹³ C	-0.555	0.706	-0.883	-0.09	<i>0.612</i>	-0.279
Variance displayed	3.181	1.286	2.756	1.61	2.815	1.944
Proportion of the total variance	0.53	0.214	0.459	0.268	0.469	0.324

PCA principal component analysis, Equamax rotation, DF_{ABS} driving force on absorption basis, A.g. biomass above-ground biomass

All parameters refer to 2005

In bold are evidenced the loadings over 0.7; in italic those over 0.5

beneficial effect from ozone. The responsiveness of growth parameters in the different species considered corresponded to the ozone flux rates, and was greater in dry conditions. The onset and spread of foliar symptoms, as reported by Gerosa et al. (2008, 2009), was related to the leaf structure and density (data reported in Bussotti et al. 2007)—with FS being more sensitive than QR—and was greater in watered plots.

The Po valley plain and the associated prealpine regions where this experiment was carried out are amongst the most ozone polluted areas in Europe (Gerosa et al. 2007). The responses observed on tree seedling cannot give direct information about the behaviour of adult trees under air pollution stress (Bussotti 2008), but can be used to predict the fate of the processes of forest regeneration or (in the case of poplar plantations), of the industrial cultures. It is expected that the increasing ozone pressure, in combination with other environmental stresses due to the climate change, may farther differentiate species-specific behaviours and compensative responses on trees (Bytnerowicz et al. 2007).

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