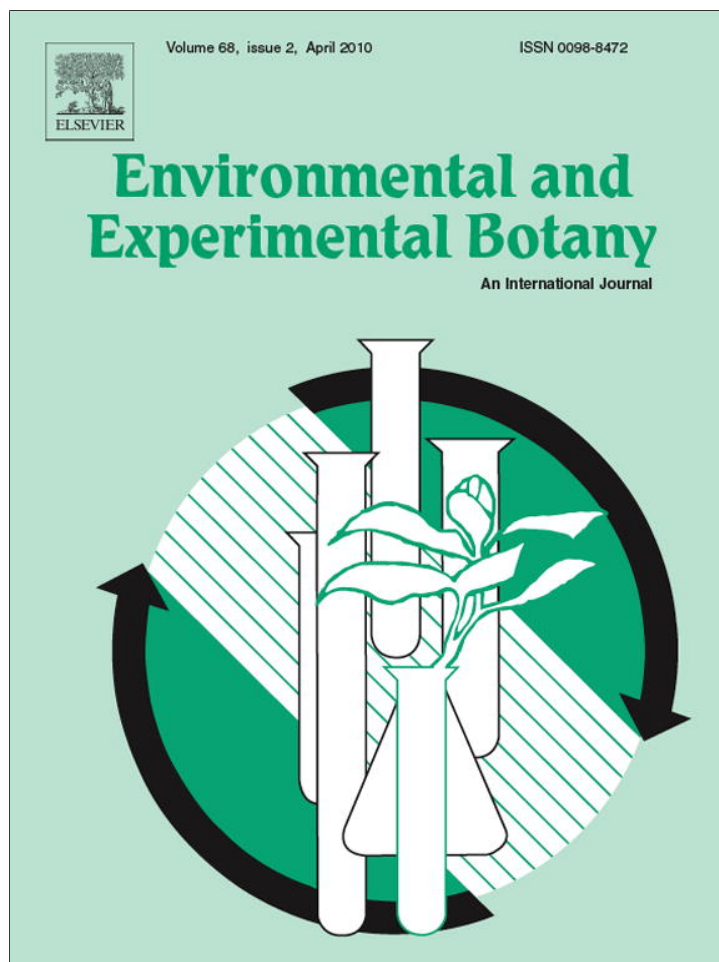


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbotFoliar responses to ozone of *Fagus sylvatica* L. seedlings grown in shaded and in full sunlight conditionsChiara Cascio^a, Marcus Schaub^b, Kristopher Novak^c, Rosanna Desotgiu^a, Filippo Bussotti^{a,*}, Reto J. Strasser^d^a University of Florence, Department of Plant Biology, Piazzale delle Cascine 28, 50144 Firenze, Italy^b Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland^c Environmental Media Assessment Group, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Mail Drop B243-01, 109 TW Alexander Dr., Research Triangle Park, NC 27711, USA^d University of Geneva, Bioenergetics Laboratory, CH-1254 Jussy-Geneva, Switzerland

ARTICLE INFO

Article history:

Received 17 July 2009

Received in revised form 21 October 2009

Accepted 27 October 2009

Keywords:

Competition
 High light stress
 Net photosynthesis
 OKJIP fluorescence
 Open-top chambers
 Ozone

ABSTRACT

Ozone (O₃) is well known to affect photosynthesis on tree seedlings under experimental conditions. In natural conditions, young trees grow under a competitive environment that may alter light availability. Such conditions were simulated in an open-top chamber (OTC) experiment carried out in Southern Switzerland (Lattecaldo). The experimental set-up consisted of *Fagus sylvatica* (beech) seedling plots (planted within the same chamber) either in a monospecific population (Mono, leaves grown in full sunlight conditions—FL) or under competition with taller plants of *Viburnum lantana* (Mixed, shaded leaves—SH). These cultures were treated with ambient air (NF, not filtered, 92% of ozone in ambient air) and with charcoal filtered air (CF, 50% of ozone in ambient air). Measures of net photosynthesis (P_N) and chlorophyll a fluorescence (with the analysis of the OKJIP transient) were performed over a growing season, from June to September. Net photosynthesis (P_N) and the maximum quantum yield of electrons reaching the acceptor side ($\varphi_{Ro} = RE_0/ABS$) were higher in Mono conditions (full light leaves). Vice versa, quantum yield of primary photochemistry ($\varphi_{Po} = TR_0/ABS = F_v/F_M$) and Performance Index on absorption basis (PI_{ABS}) were higher in Mixed plots (shaded leaves). Changes due to ozone occurred only at the end of the growing season (September). Ozone affected mainly P_N, that was lower in the NF-OTCs, both Mono (−19%) and Mixed (−28%) plots, compared to the CF ones. Fluorescence parameters were affected much more by light conditions (full light vs. shaded leaves) than ozone exposure, but ozone enhanced the action of high light by lowering the values of TR₀/ABS and PI_{ABS}. The most specific effect of ozone concerned the decrease in RE₀/ABS, that indicates the inactivation of the end acceptors of electrons, so producing an excess of oxidative pressure. Results are discussed in relation to the impact of ozone on the processes of the regeneration of forests.

© 2009 Elsevier B.V. All rights reserved.

Abbreviations: OTC, open top chamber; CF, charcoal filtered chamber; NF, non-filtered chamber; FL, full light; SH, shaded; Aot40, accumulated exposure of ozone over the threshold of 40 ppb; PAR, photosynthetic active radiation; PAR_i, PAR inside the cuvette; PAR_o, PAR outside the cuvette; ROS, reactive oxygen species; O₃, ozone; HL, high light; FT, fluorescence transient; F₀, fluorescence at time zero; F_M, maximal fluorescence; F_v, variable fluorescence; PSI, photosystem I; PSII, photosystem II; Q_A, plastoquinone; $\varphi_{Po} = TR_0/ABS$, quantum yield of primary photochemistry; $\Psi_{Eo} = ET_0/TR_0$, efficiency with which a trapped exciton can move an electron into the electron transport chain further than; Q_A[−], $\varphi_{Eo} = ET_0/ABS = \varphi_{Po}$; Ψ_{Eo} , quantum yield of electron transport; φ_{D0} , DI₀/ABS quantum yield for energy dissipation; $\varphi_{Ro} = RE_0/ABS$, maximum quantum yield of an electron reaching the acceptor side beyond the PSI; RC/CS₀, concentration of active PSII reaction centres per excited cross section; PI_{ABS}, performance index on absorption basis; P_N, net photosynthesis; g_w, stomatal conductance to water vapour; Ci, intercellular carbon; WUE, water use efficiency; LMA, leaf mass per area; LD, leaf density; LT, leaf thickness.

* Corresponding author. Tel.: +39 055 3288369; fax: +39 055 360137.

E-mail address: filippo.bussotti@unifi.it (F. Bussotti).

1. Introduction

Most research studies on ozone (O₃) effects on trees have investigated young plants (seedlings, cuttings) grown under optimal conditions (well watered, not shaded). The use of seedlings does not allow researchers to reproduce the physiological behaviour of adult trees and, even less so, forest ecosystems (Kolb and Matyssek, 2001; Kolb et al., 1997). In fact, seedlings are considered sensitive to O₃, and when adult trees were chosen as targets in free-air O₃ fumigation experiments very few effects were detected on growth and overall physiology (Matyssek et al., 2007). Mature forest ecosystems proved to be resilient to oxidative stress and O₃ (Bussotti, 2008). Under specific conditions, such as regeneration areas at the edge of the woodlands, where young trees prevail, sensitivity to O₃ may be enhanced. In the regeneration phases, young trees are affected by the competition exerted by forbs and other components of the ground vegetation layer. For shade tolerant tree seedlings

this is an advantage, since they are protected against the excess of sun radiation. Under competition, leaves growing in the shade may be exposed to sun flecks, i.e., sudden exposure to sunlight for a limited period of time. It is well known that, under these conditions, leaves transiently increase their photosynthesis activity considerably, as compared to leaves growing in a steady and stable light condition (Küppers and Schneider, 1993; Naumburg and Ellsworth, 2000; Oguchi et al., 2003). This favours a marked increase in hydrogen peroxide which can temporarily exceed the leaf's detoxification capacity, enhancing the O₃ effects (Wei et al., 2004a,b).

Light is the most powerful factor determining morphological and physiological variations in leaves. The different features of sun and shade leaves have been described from many years (Boardman, 1977; Lichtenthaler et al., 1981, 2007; Anderson and Osmond, 1987; Anderson et al., 1995; Kim et al., 2005; Terashima et al., 2006; Tateno and Taneda, 2007). Sun leaves display a higher leaf mass per area (LMA) and thickness than shade leaves (Groom and Lamont, 1997; Tattini et al., 2000; Evans and Poorter, 2001; Temesgen and Weiskittel, 2006; Gratani et al., 2006). Chloroplast acclimation in sun leaves consists in shorter thylakoids, and a greater amount of photosystem I (PSI) and Rubisco, respect to shade leaves (Maxwell et al., 1999). Moreover, sunlight stimulates a high level of antioxidants in the leaves (García-Plazaola et al., 2004) and the production of screening and antioxidant pigments (Neill and Gould, 2003), so enhancing the general resistance of plants to stress factors (cross-resistance, see Mittler, 2006; Bussotti, 2008). Sun leaves are able also to manage actively the flux of electrons by reducing the antenna size in the PSII and enhancing the controlled dissipation of energy (Ballottari et al., 2007). The content and activity of Rubisco are higher in sun acclimated leaves (Chow and Anderson, 1987a,b), thus allowing a higher velocity of reduction of carbon.

The interaction between high light (HL) and O₃ stress is controversial. It is commonly assumed that shade leaves increase their response to O₃ (Volin et al., 1993; Tjoelker et al., 1993, 1995; Topa et al., 2001, 2004; Wei et al., 2004a,b). This behaviour has been explained with the unbalanced net photosynthesis – P_N – respect to the stomatal conductance to water vapour – g_w (Fredericksen et al., 1996). Foliar visible ozone symptoms, however, are generally lower in the inner parts of the crown and in shaded leaves (Novak et al., 2008; Gielen et al., 2007). Davison et al. (2003) consider sunlight an essential factor in triggering the pathway of anthocyanin synthesis, enhancing the related symptomatology. According to several authors (Mikkelsen and Ro-Poulsen, 1994; Mikkelsen et al., 1995; Guidi et al., 2000, 2002; Guidi and Degl'Innocenti, 2008) high levels of O₃ enhance the photoinhibition processes induced by high light, so amplifying the overall response.

Biochemical and structural changes in sun leaves, due to acclimation processes to high light are believed to make sun leaves more resistant to ozone stress (cross-resistance, see Bussotti, 2008; Matyssek and Innes, 1999), but an excess of oxidative pressure, originated from the additive action of O₃ + HL could overpass these defences. Shade leaves, not acclimated to high light, are subjected to strong photoinhibition when suddenly exposed to high light, and that inhibition can be enhanced by O₃. For that reason shaded seedlings, subjected to sun flecks (for example, at the forest edges or in forest gaps), likely are vulnerable to ozone.

Seedlings growing in the understory (below the tree canopies), however, are not subjected to sun flecks and, in these conditions, vegetation and regeneration are believed to be protected from stress because the low light irradiation limits both direct oxidative pressure and stomatal opening, so avoiding ozone uptake. This is the hypothesis that has been checked in this paper.

2. Material and methods

2.1. Site characteristics and experimental design

A two-year study was conducted at an open-top chamber (OTC) facility located in the pre-alpine region of southern Switzerland within the Lattecaldo Cantonal Forest Nursery in the Valle di Mugello, Canton Ticino (09°03'E, 45°51'N, 600 m a.s.l.) (VanderHeyden et al., 2001; Novak et al., 2003, 2005). General meteorological features for the experimental year are reported in Table 1A. The experimental design consisted of two O₃ treatments in open-top chambers with four non-filtered (NF) OTCs with approximately 92% of the ambient O₃ concentrations, and four charcoal filtered (CF) OTCs with approximately 47% of the ambient O₃ concentrations. Within each OTC, fiberglass separations were installed 50 cm into the soil to divide the plot into equal thirds. In early spring of 2003, each third was planted with seedlings arranged in a grid design using 20 cm spacing between plants. One section of the chamber was planted with a monospecific population (Mono) of 4-year-old *Fagus sylvatica* L. (beech) seedlings, with leaves exposed in full sunlight (FL), a second section with a monospecific population of 2-year-old *V. lantana* L. seedlings and the third section with a 1:1 mixture (Mixed) of the two species (14 seedlings of beech and 14 seedlings of *Viburnum*), where beech leaves were shaded (SH) from the tallest *Viburnum* plants (see Novak et al., 2008). The O₃ treatments started at the end of April 2003. To minimize edge effects, all measurements and assessments focused on the inner eight individuals of each section, only. All plots were watered routinely throughout the experiment to minimize drought stress to the plants.

In a previous paper by Novak et al. (2008), findings from the same experiment were reported in relation to ozone foliar symptoms, growth and biomass allocation. Ozone foliar symptoms affected mainly beech plants grown in NF plots in Mono conditions, and became visible only late in the season. No differences in height growth were found across the different experimental conditions.

During the 2004 growing season, measurements were carried out, once a month, at four different dates: 8 June; 5 July; 3 August; 11 September. Four *F. sylvatica* plants for each chamber (2 Mono and 2 Mixed) were considered at each time.

2.2. Ozone measurements

Ozone concentrations were continuously monitored throughout the 2003 and 2004 growing seasons, from 21 April to 18 October, using a Monitor Labs model ML 8810 O₃ monitor, which was calibrated monthly. Based on a 20-min sampling interval, two-minute air samples were repeatedly drawn at 1 m height from each OTC and one ambient plot. Table 2 reports the mean O₃ concentrations and peaks over the growing season. The overall exposure levels (AOT40) at were 21.8 ppm h in NF and 4.1 ppm h in CF OTC.

2.3. Fluorescence transient (FT) analysis

Chl *a* fluorescence transients of intact leaves were measured with an ADC FIM 1500 fluorimeter (direct fluorescence). Fluorescence was measured, at the dates indicated above, on 5 leaves of each plant. Before each measurement, the leaves were dark-adapted for 20 min with leaf clips. The rising fluorescence transients were induced by a red light (peak at 650 nm) of 600 Wm⁻² provided by an array of six light-emitting diodes; they were recorded for 1 s, starting from 50 μs after the onset of illumination, with 12-bit resolution. On a logarithmic time scale, the fluorescence transient from F_0 (F at 50 μs, when all the reaction centres of the photosystem II – PSII – are open, i.e., when the primary acceptor quinone Q_A is fully oxidised) to F_P (where $F_P = F_M$ under saturating excitation light, when the excitation intensity is high enough to ensure the closure

Table 1
(A) General meteorological conditions. *T* (air), air temperature; *T* (otc), temperature within the OTCs; RH=relative humidity; PAR, maximal PAR; Rain, overall monthly precipitation. (B) Conditions at the cuvette level, at the assessment dates.

A	Month	<i>T</i> (air)	<i>T</i> (air)	<i>T</i> (air)	<i>T</i> (otc)	<i>T</i> (otc)	<i>T</i> (otc)	RH	PAR	Rain
		°C	°C	°C	°C	°C	°C	%	μmol m ⁻² s ⁻¹	mm
		Mean	Min	Max	Mean	Min	Max	Mean	Max	Sum
	April	12.9	5.4	24.3	13.7	5.2	27.0	60.3	1459	87
	May	12.6	2.5	25.5	13.6	1.9	28.2	66.9	1575	202
	June	19.0	9.2	30.5	20.2	9.5	33.8	58.9	1573	24
	July	20.4	11.3	32.4	21.2	10.4	35.4	59.7	1579	75
	August	20.0	12.2	30.2	20.8	11.6	32.7	64.3	1556	165
	September	16.6	6.1	28.6	17.2	6.0	30.4	65.5	1324	61

B	Month	Plant assemblage	<i>T</i> (cuv)	<i>T</i> (cuv)	<i>T</i> (leaf)	<i>T</i> (leaf)	VPD	VPD	PARo	PARo
			°C	°C	°C	°C	kPa	kPa	μmol m ⁻² s ⁻¹	μmol m ⁻² s ⁻¹
			Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
	June	Mono	27.6	0.18	28.9	0.60	1.95	0.09	1039	302
Mixed		27.7	0.19	29.4	0.59	2.05	0.06	497	335	
	July	Mono	31.0	0.22	30.6	0.68	2.26	0.09	1052	252
Mixed		31.0	0.23	31.1	0.52	2.39	0.10	568	392	
	August	Mono	31.1	0.30	31.0	0.13	2.31	0.13	853	253
Mixed		31.1	0.22	31.0	0.09	2.36	0.17	579	417	
	September	Mono	26.1	0.93	24.8	0.16	1.96	0.21	697	368
Mixed		26.0	0.66	24.8	0.13	1.95	0.15	355	276	

T (cuv), temperature inside the cuvette; *T* (leaf), foliar temperature; VPD=vapor pressure deficit; PARo, PAR outside the cuvette (PARI, PAR inside the cuvette, is constant = 1500 μmol m⁻² s⁻¹).

of all reaction centres of PSII, i.e., the full reduction of all reaction centres) had a polyphasic behaviour (Strasser and Govindjee, 1992; Strasser and Strasser, 1995). The fluorescence values at 50 μs (*F*₀, step 0), 100 μs (*F*₁₀₀), 300 μs (*F*₃₀₀), 2 ms (step J), 30 ms (step I) and maximal (*F*_M, step P), were taken into consideration.

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). The JIP-test refers to a translation of the original data to biophysical parameters, all referring to time zero (onset of fluorescence induction), that quantify PSII behaviour. Among these parameters, in this paper we have considered the flux ratios or yields, i.e., the maximum quantum yield of primary photochemistry ($\varphi_{P_0} = TR_0/ABS$), the efficiency ($ET_0/TR_0 = \Psi_{E_0} = ET_0/TR_0$) with which a trapped exciton can move an electron into the electron transport chain further than *Q*_A⁻, the quantum yield of electron transport ($\varphi_{E_0} = ET_0/ABS = \varphi_{P_0} \cdot \Psi_{E_0}$), the quantum yield for energy dissipation ($\varphi_{D_0} = DI_0/ABS$), and the concentration of active PSII reaction centres per excited cross section (RC/CS₀). Recently, new concepts describing the activity of PSI were discussed for an enrichment of the JIP-test (Smit et al., 2008; Tsimilli-Michael and Strasser, 2008). The term RE₀, for reduction of end acceptors of PSI, has been

introduced based on the probability that an electron is transported from reduced PQ to the electron acceptor side of PSI. The maximum quantum yield of an electron reaching the acceptor side is expressed by $\varphi_{R_0} = RE_0/ABS$.

Three functional steps regulate the initial stage of photosynthetic activity of an RC complex, namely absorption of light energy (ABS), trapping of excitation energy (TR₀) and conversion of excitation energy to electron transport (ET₀). Strasser et al. (2000) introduced a multi-parametric expression of these three independent steps contributing to photosynthesis, the so-called performance index (PI_{ABS}):

$$PI_{ABS} = \left[\frac{\gamma_0}{1 - \gamma_0} \right] \left[\frac{\varphi P_0}{1 - \varphi P_0} \right] \left[\frac{\psi_0}{1 - \psi_0} \right]$$

where γ is the fraction of reaction centre chlorophyll (Chl_{RC}) per total chlorophyll (Chl_{RC} + Antenna). Therefore, $\gamma/(1 - \gamma) = Chl_{RC}/Chl_{Antenna} = RC/ABS$. The PI combines three parameters favourable to photosynthetic activity: (1) the density of reaction centres (expressed on an absorption basis); (2) the quantum yield of primary photochemistry and (3) the ability to feed electrons into the electron chain between photosystem II and photosystem I.

2.4. Gas-exchange measurements

Leaf gas-exchange measurements including net photosynthesis (*P*_N) and stomatal conductance to water vapour (*g*_w) were conducted simultaneously (two leaves per plant) to the fluorescence measurements with a LiCor 6200 portable photosynthesis system using a 0.25 l cuvette (LiCor Inc., Lincoln, NE). Before each measurement period, the system was calibrated to known concentrations of CO₂ (0 and 400 ppm). Measurements were taken at constant light provided by supplied by a “cold” lamp (LED type), adjusted at 1500 μmol m⁻² s⁻¹ (PARI, i.e., PAR inside the cuvette). For each measurement period (09:00–12:00), two consecutive measurements were taken. A total of 12 measurements per species, measurement period, and treatment were conducted.

Table 2
General features of the ozone pollution at the experimental site.

	Ambient Air		NF-OTC		CF-OTC	
	μ	Peak	μ	Peak	μ	Peak
April	40	79	36	71	19	49
May	41	93	38	86	20	61
June	48	112	44	103	24	73
July	56	124	52	122	27	80
August	56	108	43	99	27	67
September	34	95	31	88	15	52
October	18	49	17	47	8	30
Season mean	40	124	37	122	19	80

Ambient air, outside the chambers; NF-OTC, inside the not filtered OTCs; CF-OTC, inside the charcoal filtered OTCs. μ, monthly mean; Peak, maximum hourly mean. All data are reported in nl l⁻¹.

The values for the environmental factors at the measurement time are reported in Table 1B.

2.5. Leaf morphology and chlorophyll content

At the end of the growing season, a random sample of full light (from Mono plots) and shaded (from Mixed plots) beech leaves was gathered. Four leaves were collected from each individual plant included in the measurements over the growing season. Overall, 64 full light and 64 shaded leaves were collected. From a subsample, a small part of the lamina was removed to measure the leaf thickness; and, also from each leaf, depending on its extension, either 2 or 3 discs measuring 2.54 cm² each, were taken, and their dry weight (DW) was obtained after drying in an oven at 70 °C (until constant weight). From the interveinal areas of each leaf, two 2 mm² wide fragments of lamina were taken and, then, cross-sections were obtained by Vibrotome 1000 Plus (St. Lois, MO, USA). Four cross-sections of each leaf were measured with a light microscope Nikon Eclipse E400 (Japan) equipped with a calibrated micrometric grid. Leaf mass per area (LMA) and leaf density (LD) were calculated using the initial area and dry weight measurements (LMA = DW LA⁻¹, mg cm⁻²; LD = DW (LALT)⁻¹, mg cm⁻³). On the same leaves, chlorophyll content was also measured using a CL-01 Chlorophyll Content Meter (Hansatech Instruments, Pentney–Norfolk, UK). Relative chlorophyll content is measured using dual wavelength optical absorbance (620 and 940 nm wavelength) from leaf samples. The values are reported, in arbitrary units, on a proportional scale.

2.6. Statistics

Fluorescence data were processed through Biolyzer software (R. Maldonado-Rodriguez, Bioenergetics Lab., Geneva, CH). Combined elaborations were performed by relating fluorescence data, and data on leaf injury and photosynthesis, to each individual plant. The NF/CF (not filtered/charcoal filtered) and FL/SH (full light/shaded leaves) ratios were taken in most of the findings as the expression of the extent of response to the treatment. All data were organized in Excel (Microsoft) spreadsheets and processed by means of Statistica 6.0 (Statsoft 2001, Tulsa OK).

3. Results

3.1. Overall results

Full light leaves were smaller (in terms of leaf area) than the shaded ones, but the first were thicker and had a higher leaf mass per area (LMA) and leaf density (LD). The total leaf thickness (LT) and the thickness of the mesophyll (spongy + palisade) were higher in full light than in shaded leaves, and had a higher percentage of palisade parenchyma (calculated on the whole mesophyll). The content of chlorophyll was lower in full light leaves. Findings are presented in Table 3.

The results of the variance analysis (two-ways ANOVA), according to the variables light exposure (full light vs. shaded) and ozone treatment (NF vs. CF), are shown in Table 4 for the whole year. The *p* values show that most of the considered parameters are more markedly influenced by light exposure conditions rather than by ozone treatment. The maximum quantum yield of primary photochemistry (TR₀/ABS), the probability that the energy of a trapped exciton is used for electron transport beyond Q_A⁻ (ET₀/TR₀), the quantum yield of electron transport (ET₀/ABS), the density of active reaction centers per cross section (RC/CS₀), and the performance index (PI_{ABS}) were significantly lower in full light leaves, both in CF and NF-OTCs, whereas the maximum quantum yield of an electron reaching the acceptor side beyond the PSI (RE₀/ABS) and the

Table 3

Morphological features of FL (full light, in monospecific plots) and SH (shaded, in mixed plots) beech leaves, collected at the end of the growing season.

	No.	FL (Mono)		SH (Mixed)	
		M	St. dev.	M	St. dev.
Leaf area (1 leaf), cm ²	16	12.4±	2.2	17.0±	3.5
Upper epidermis, μm	16	10.5±	2.2	10.2±	1.6
Palisade parenchyma, μm	16	52.0±	5.9	32.0±	3.1
Spongy parenchyma, μm	16	58.0±	6.6	45.4±	1.5
Lower Epidermis, μm	16	8.9±	0.9	8.3±	0.6
Total leaf thickness, μm	16	129.4±	5.1	96.0±	2.7
Leaf mass per area, mg cm ⁻²	16	7.2±	0.3	4.5±	0.7
Leaf density, mg cm ⁻³	16	557.8±	25.3	470.4±	78.3
Percent of palisade, %	16	47.3±	4.8	41.3±	2.7
Chlorophyll content (a.u.)	16	5.74±	1.27	7.94±	1.56

quantum yield for energy dissipation (DI₀/ABS) had the opposite behaviour. In most cases, the FL/SH ratios had higher values in NF plots, with the exception of RE₀/ABS. Water Use Efficiency (WUE) was significantly lower only in NF plots (Fig. 1A). No significant differences were found according to ozone treatment (Fig. 1B).

At monthly basis, some parameters became significantly influenced by O₃ only in the last assessment month (September), both in full light and shaded leaves (Fig. 1C). They were RE₀/ABS and P_N (significantly lower in O₃ treated plots, respectively with *p* = 0.036 and with *p* = 0.011). Some others parameters, even not significant, decreased considerably their *p* level (TR₀/ABS was lower in O₃ treated plots, with *p* = 0.092).

Table 5 shows the correlations values (*r* and *p*) between the selected JIP-test and the gas-exchange parameters. P_N correlated positively with the maximum quantum yield of an electron reaching the acceptor side beyond the PSI (RE₀/ABS), but *p* was significant (<0.05) only in the full light leaves. In the shaded leaves, P_N correlated positively with the maximum quantum yield for primary photochemistry (TR₀/ABS) and negatively with the quantum yield for dissipation (DI₀/ABS). Stomatal conductance (g_w) correlated negatively with the density of reaction centers (RC/CS₀) and with the performance index (PI_{ABS}). The intercellular carbon (C_i) correlated negatively with most fluorescence parameters, while WUE had the opposite trend.

Table 4

Results of the Factorial ANOVA for each considered parameter. In bold are indicated the significant factors.

	A. Ozone treatment		B. Light exposure		Interaction A × B	
	F	P	F	P	F	P
φ _{P0} = TR ₀ /ABS	2.334	0.129	31.077	0.000	1.441	0.232
ψ _{E0} = ET ₀ /TR ₀	0.582	0.447	8.429	0.004	1.237	0.270
φ _{E0} = ET ₀ /ABS	0.076	0.784	14.819	0.000	0.299	0.585
φ _{D0} = DI ₀ /ABS	2.334	0.129	31.077	0.000	1.441	0.232
φ _{R0} = RE ₀ /ABS	0.024	0.876	5.556	0.020	0.638	0.426
RC/CS ₀	0.051	0.822	9.608	0.002	0.765	0.383
PI _{ABS}	0.034	0.854	9.262	0.003	0.591	0.444
P _N	1.070	0.303	9.401	0.003	0.193	0.662
g _w	0.245	0.622	3.103	0.081	1.392	0.240
C _i	1.704	0.194	3.434	0.066	0.424	0.516
WUE	1.289	0.258	4.315	0.040	0.447	0.505

φ_{P0} = TR₀/ABS = maximum quantum yield of primary photochemistry; ψ_{E0} = ET₀/TR₀ = efficiency with which a trapped exciton can move an electron into the electron transport chain further than Q_A⁻; φ_{E0} = ET₀/ABS = φ_{P0}. ψ_{E0} = quantum yield of electron transport; φ_{D0} = DI₀/ABS = quantum yield for energy dissipation; φ_{R0} = RE₀/ABS = maximum quantum yield of an electron reaching the acceptor side; RC/CS₀ = concentration of active PSII reaction centres per excited cross section; PI_{ABS} = Performance Index on absorption basis; P_N = net photosynthesis; g_w = stomatal conductance to water vapour; C_i = concentration of intercellular carbon; WUE = water use efficiency (P_N/g_w).

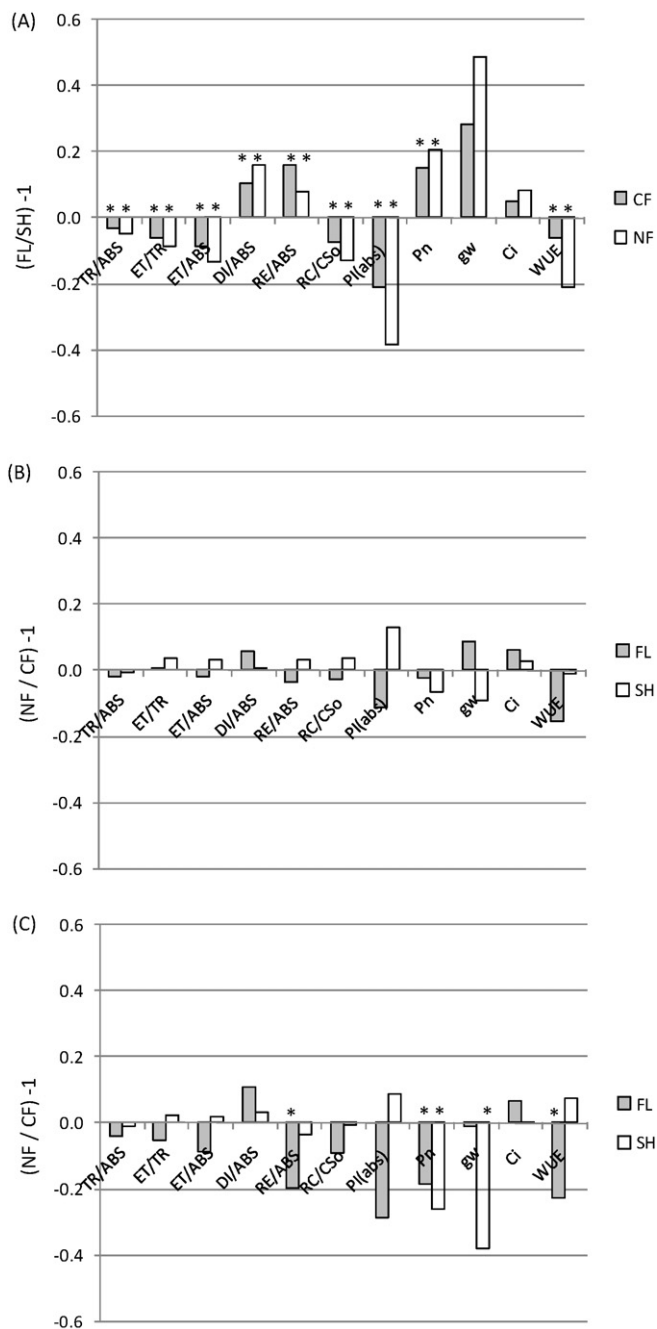


Fig. 1. Relative values of selected photosynthesis parameters. (A and B) all assessment data, and the relative parameters are reported according to the condition light exposure (FL/SH–1, A) and according to ozone exposure (NF/CF–1, B). C reports the relative values for ozone exposure (NF/CF–1) only for the last assessment (September). The straight line at the “0” level represents the control, and histograms represent the relative variations. Findings significantly different from the control are indicated with an asterisk (see also the results of the ANOVA, Table 4). TR₀/ABS = maximum quantum yield of primary photochemistry; ET₀/TR = efficiency with which a trapped exciton can move an electron into the electron transport chain further than Q_A⁻; ET₀/ABS = quantum yield of electron transport; DI₀/ABS = quantum yield for energy dissipation; RE₀/ABS = maximum quantum yield of an electron reaching the acceptor side; RC/CS₀ = concentration of active PSII reaction centres per excited cross section; PI_{ABS} = Performance Index on absorption basis; P_N = net photosynthesis; g_w = stomatal conductance to water vapour; C_i = concentration of intercellular carbon; WUE = water use efficiency.

3.2. Temporal trends

According to ozone treatment (NF/CF, Fig. 2A), the maximum quantum yield of an electron reaching the acceptor side beyond the PSI (RE₀/ABS) decreased over time, and that behaviour was significant in September in full light but not in shaded leaves. According to light exposure (FL/SH, Fig. 2B), RE₀/ABS was significantly higher in CF but not in NF plots.

P_N values (Fig. 3A) were always higher in full light as compared to shaded leaves, but dropped in September in NF plots in both conditions of light exposure. The reduction of P_N attributable to ozone (in NF respect to CF plots) was 28% in shaded leaves and 19% in full light leaves. A similar behaviour was observed for stomatal conductance (Fig. 3B). Intercellular carbon increased over time, without any evident differences between experimental conditions (Fig. 3C), whereas WUE followed the opposite trend (Fig. 3D). It may be interesting to note that the highest values of C_i and the lowest of WUE were observed in the full light leaves–NF plots. When data are presented on leaf mass per area basis (i.e., assuming LMA = 1, see Tatenò and Taneda, 2007), P_N was constantly higher shaded leaves (Fig. 4A), and the g_w increased over the time in shaded respect to full light leaves.

3.3. ΔV curves

The rising curves of the fluorescence transients OJIP were normalized between F₀ and F_M to make them comparable. The so-called ΔV (= V_{treated} – V_{reference}) curves were calculated by subtracting, at each point in time, the relative variable fluorescence intensity V values recorded in the CF plants from the corresponding NF point (i.e., NF–CF, see Fig. 5A and B), and the relative variable fluorescence intensity V values recorded in the shaded from the corresponding full light leaves (FL–SH, see Fig. 5C and D). The ΔV curves indicate the points where the fluorescence increases, in particular the J and I steps at about 2 and 30–50 ms (ΔV_J and ΔV_I peaks). The main effect of O₃ were the enhanced ΔV_J, ΔV_I peaks late in the season (September), in full light leaves (Fig. 5A). In shaded leaves the responses were less marked, but a ΔV_I peak was observed also in these conditions (Fig. 5B). In relation to the light exposure, ΔV curves showed two different kinds of patterns, both in NF (Fig. 5C) and CF plots (Fig. 5D). There is an “early” pattern (June and July), characterized by a peak at the J and a “depression” at the I steps; and a “late” pattern (August and September) with the opposite behaviour.

4. Discussion

Light induced morphological and physiological changes in the beech growing in the monospecific plots, respect to the mixed ones. Full light leaves had a lower maximum quantum yield of primary photochemistry (TR₀/ABS) than the shaded leaves. This finding contrasts with Lichtentaler and Babani (2004) and Sarijeva et al. (2007), who described an opposite trend. In the ecological conditions South of the Alps, where the sun radiations are very high, photoinhibitory damage can be avoided through the increase in controlled non-photochemical de-excitation processes (enhanced quantum yield of dissipation – DI₀/ABS – and reduced maximum quantum yield of primary photochemistry – TR₀/ABS – and density of reaction centers per cross section – RC/CS₀). This behaviour, however, is consistent with the behaviour in high light conditions described by Adams and Demmig-Adams (2004) and Gilmore (2004).

Another important feature of the acclimation to high light is represented by the changes in the shape of the I–P region in the fluorescence transient in full light leaves. This behaviour is expressed by the higher value of the maximum quantum yield of an electron

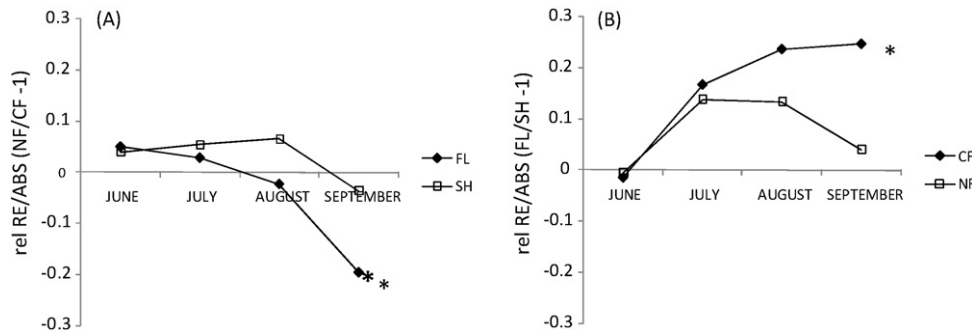


Fig. 2. Variation, over the growth season, of the relative values of RE_0/ABS (maximum quantum yield of an electron reaching the acceptor side) according to ozone (NF/CF -1, Fig. 2A) in Mono and Mixed conditions, and light exposure (FL/SH -1, Fig. 2B) in CF and NF plots. The straight line at the “0” level represents the control, and histograms represent the relative variations. Findings significantly different from the control are indicated with an asterisk.

reaching the acceptor side beyond the $PSI-RE_0/ABS$ (Fig. 2B) in full light respect to shaded leaves, and by the marked negative peak ΔV_1 in the latest part of the ΔV curve (Fig. 5C and D) The I-P region of the fluorescence transient (and the JIP-test parameter RE_0/ABS) reflects the velocity of the reduction of ferredoxine beyond the PSI (Schansker et al., 2003) or, in other words, it expresses a relative abundance of PSI respects to the $PSII$. Full light leaves have a high $PSI/PSII$ ratio (Maxwell et al., 1999), that allows a quick reduction of ferredoxine and, consequently, high P_N rates (P_N is correlated to RE_0/ABS in full light leaves). Only in the late season, in full light leaves, the ΔV_1 peak became positive, both in CF and NF plots (in NF plots that happens earlier than in the CF ones). In shaded leaves (Mixed plots), P_N correlated positively with TR_0/ABS and negatively with DI_0/ABS . This is part of a strategy to capture and preserve the sun energy at low radiation conditions. The photosynthesis of shaded leaves, in mixed plots, is saturated at lower PAR values, and

the PAR_0 measured at the time of gas-exchange assessment are enough to assure the photosynthetic saturation (Bilger et al., 1995; Beaudet et al., 2000; Wittmann et al., 2001).

Effects of O_3 were recognizable mainly at the end of the season (Topa et al., 2001). Changes in the JIP-test parameters were more pronounced in full light respect to the shaded leaves. In the ΔV curves (NF-CF in full light and, in a lesser extent, also in the shaded leaves), a ΔV_1 peak became evident in the September assessment, corresponding to a decrease in the value of the maximum quantum yield of an electron reaching the acceptor side beyond the $PSI-RE_0/ABS$. This effect indicates a slowing of the reduction of ferredoxine (Schansker et al., 2003) that, together with the inactivation of Rubisco (that can occur as a consequence both of O_3 and high light stress, see Jactap et al., 1998; Dann and Pell, 1989; Fontaine et al., 2003; Brendley and Pell, 1998; Inclan et al., 2005; Guidi et al., 2009), can explicate the decline of P_N in September. This

Table 5
Correlation coefficients of singular relative JIP-test parameters with gas-exchange parameters (abbreviations: see legend Table 4).

	TR_0/ABS	ET_0/TR_0	ET_0/ABS	DI_0/ABS	RE_0/ABS	RC/CS_0	PI_{ABS}
All plots							
P_N	$r=0.1176$ $p=0.186$	$r=0.0456$ $p=0.609$	$r=0.0583$ $p=0.513$	$r=-0.1176$ $p=0.186$	$r=0.1603$ $p=0.071$	$r=-0.0153$ $p=0.864$	$r=-0.0620$ $p=0.487$
g_w	$r=0.0642$ $p=0.471$	$r=-0.1684$ $p=0.057$	$r=-0.1343$ $p=0.131$	$r=-0.0642$ $p=0.471$	$r=-0.0739$ $p=0.407$	$r=-0.2732$ $p=0.002$	$r=-0.2552$ $p=0.004$
C_i	$r=-0.1037$ $p=0.244$	$r=-0.3683$ $p=0.000$	$r=-0.3438$ $p=0.000$	$r=0.1037$ $p=0.244$	$r=-0.2474$ $p=0.005$	$r=-0.5079$ $p=0.000$	$r=-0.4580$ $p=0.000$
WUE	$r=0.0851$ $p=0.339$	$r=0.3492$ $p=0.000$	$r=0.3237$ $p=0.000$	$r=-0.0851$ $p=0.339$	$r=0.2269$ $p=0.010$	$r=0.4910$ $p=0.000$	$r=0.4480$ $p=0.000$
Mono (FL)							
P_N	$r=0.1801$ $p=0.155$	$r=0.1929$ $p=0.127$	$r=0.2048$ $p=0.105$	$r=-0.1801$ $p=0.155$	$r=0.3237$ $p=0.009$	$r=0.1422$ $p=0.262$	$r=0.0200$ $p=0.875$
g_w	$r=0.1938$ $p=0.125$	$r=-0.0571$ $p=0.654$	$r=-0.0059$ $p=0.963$	$r=-0.1938$ $p=0.125$	$r=-0.0181$ $p=0.887$	$r=-0.2639$ $p=0.035$	$r=-0.1867$ $p=0.140$
C_i	$r=0.0089$ $p=0.944$	$r=-0.3175$ $p=0.011$	$r=-0.2800$ $p=0.025$	$r=-0.0089$ $p=0.944$	$r=-0.2594$ $p=0.038$	$r=-0.5131$ $p=0.000$	$r=-0.3988$ $p=0.001$
WUE	$r=-0.0305$ $p=0.811$	$r=0.2868$ $p=0.022$	$r=0.2485$ $p=0.048$	$r=0.0305$ $p=0.811$	$r=0.2185$ $p=0.083$	$r=0.4789$ $p=0.000$	$r=0.3858$ $p=0.002$
Mixed (SH)							
P_N	$r=0.4026$ $p=0.001$	$r=0.0413$ $p=0.746$	$r=0.1118$ $p=0.379$	$r=-0.4026$ $p=0.001$	$r=-0.1278$ $p=0.314$	$r=-0.0204$ $p=0.873$	$r=-0.0002$ $p=0.999$
g_w	$r=0.1173$ $p=0.356$	$r=-0.2013$ $p=0.111$	$r=-0.1535$ $p=0.226$	$r=-0.1173$ $p=0.356$	$r=-0.1903$ $p=0.132$	$r=-0.2309$ $p=0.066$	$r=-0.2455$ $p=0.050$
C_i	$r=-0.0900$ $p=0.479$	$r=-0.3690$ $p=0.003$	$r=-0.3399$ $p=0.006$	$r=0.0900$ $p=0.479$	$r=-0.3257$ $p=0.009$	$r=-0.4689$ $p=0.000$	$r=-0.4630$ $p=0.000$
WUE	$r=0.0468$ $p=0.713$	$r=0.3508$ $p=0.004$	$r=0.3158$ $p=0.011$	$r=-0.0468$ $p=0.713$	$r=0.3328$ $p=0.007$	$r=0.4579$ $p=0.000$	$r=0.4470$ $p=0.000$

In bold are evidenced the correlations significant with $p < 0.05$.

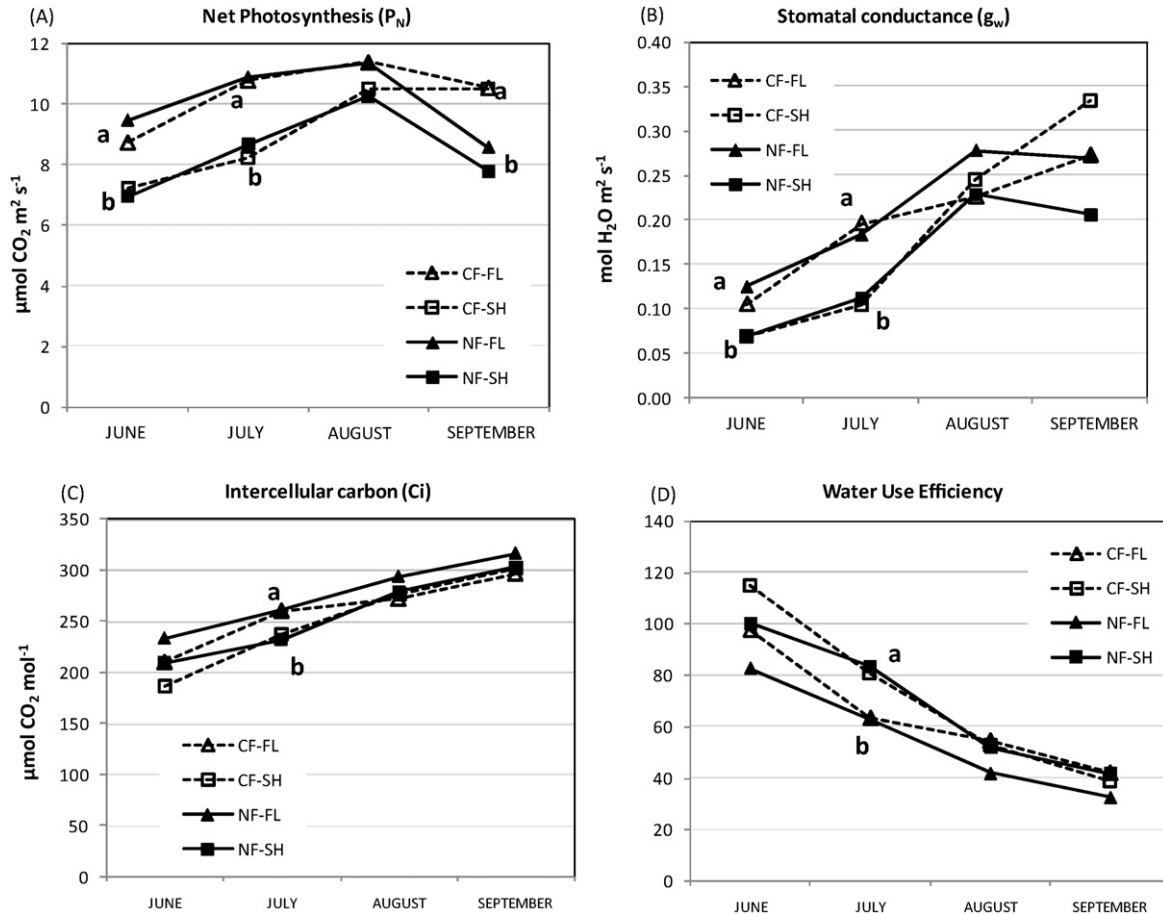


Fig. 3. Variation, over the growth season, of the gas-exchange parameters: net photosynthesis (P_N , A), stomatal conductance (g_w , B), intercellular carbon (C_i , C) and Water Use Efficiency (WUE, Fig. 3D). Different letters indicate significant differences.

decline in the ozone treated plots is a well know finding (see also Gravano et al., 2004; Novak et al., 2005; Calatayud et al., 2007) and was greater in shaded leaves respect to the full light ones. When data are expressed on leaf mass per area (LMA) basis, the shade leaves increase their stomatal conductance (g_w) at the end of the season, so they are more susceptible to ozone uptake.

In September, both high light and O_3 produced positive ΔV_i peaks, with a lowering of RE_0/ABS relative values. That may create an unbalance between electrons sent by the ET (electron trans-

port) chain and those reaching the acceptors beyond the PSI, and namely the ferredoxine. Under high light conditions we suppose that the “free” electrons (those coming from the PSI, but that did not reach the end acceptors), can activate the oxygen (from fundamental to excited status: Powles, 1984; Krieger-Liszky, 2005) with production of ROS, so inducing photo-oxidation processes of the cellular content. According to Hald et al. (2008), if the flow of electrons through the electron transport chain exceeds the capacity of metabolism to consume the reductant produced, then poten-

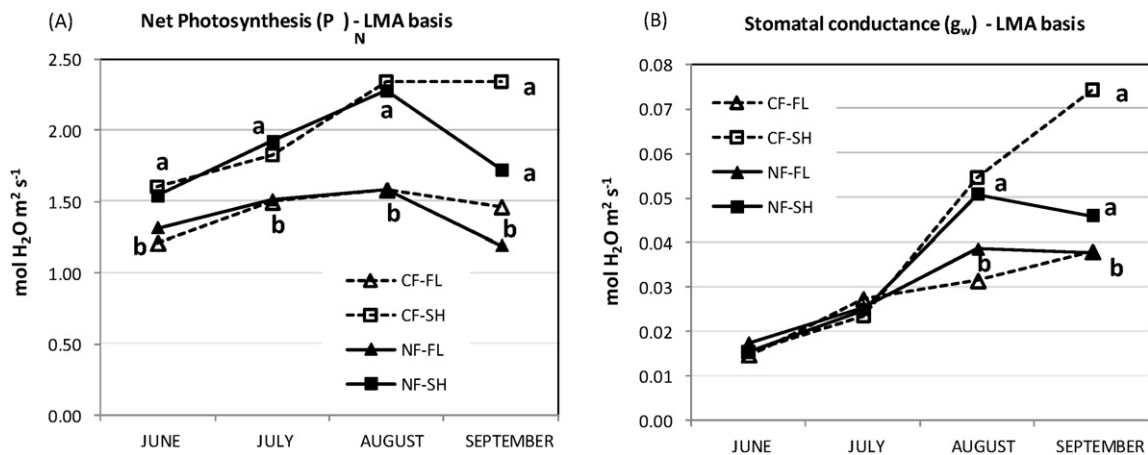


Fig. 4. Variation, over the growth season, of the gas-exchange parameters, expressed on leaf mass per area (LMA) basis, i.e., assuming LMA = 1. (A) net photosynthesis (P_N); (B) stomatal conductance (g_w). Different letters indicate significant differences.

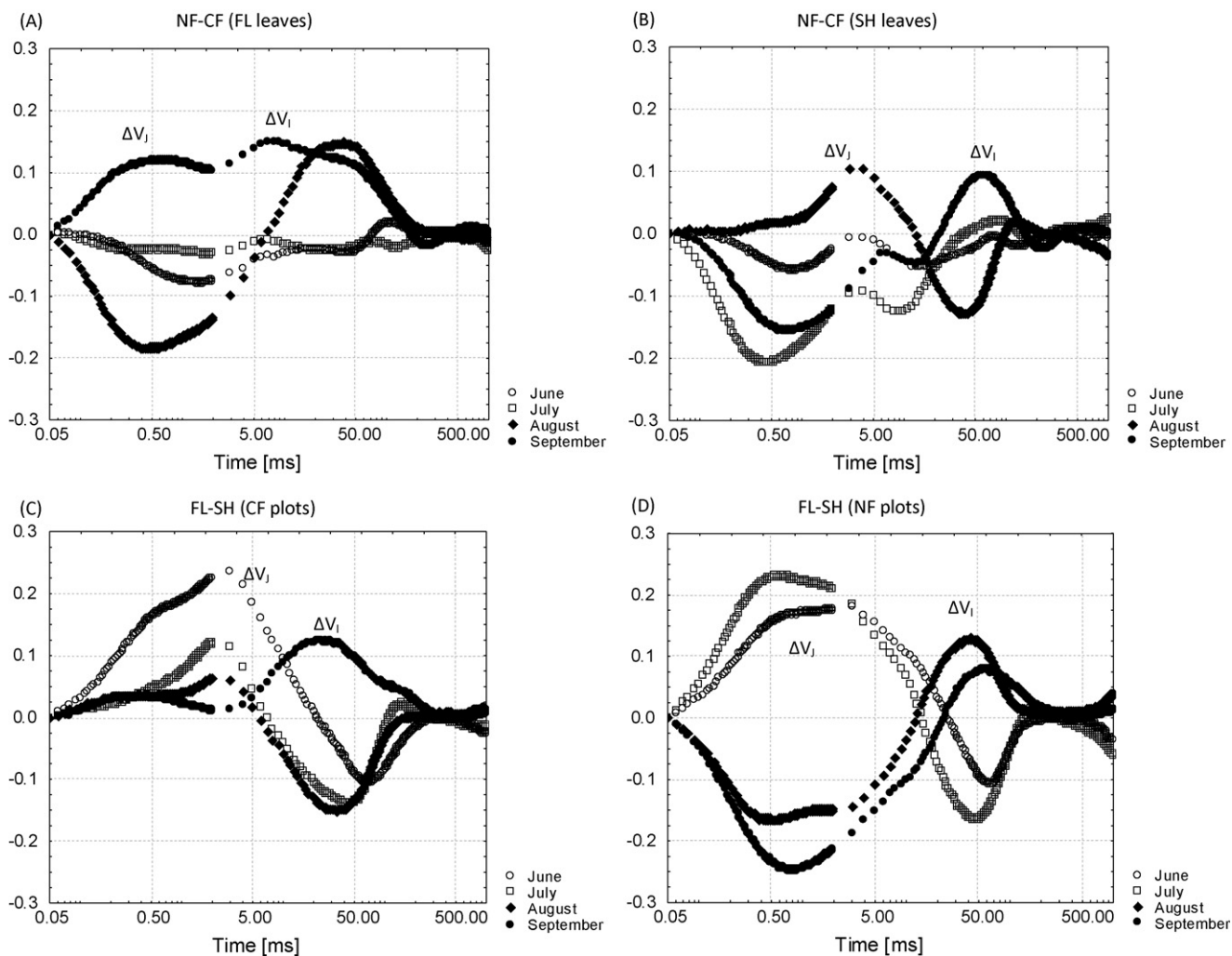


Fig. 5. ΔV curves, obtained by subtraction from the original fluorescence transients. (A and B) ΔV curves according to ozone exposure (NF–CF), in full light (FL, A) and shaded (SH, B) leaves. (C and D) ΔV curves according to light conditions (FL–SH), in charcoal filtered (CF, C) and not filtered (NF, D) plots.

tially harmful side reactions are liable to occur. In these conditions O_3 increased the oxidative pressure producing additive effects, so explicating the foliar symptoms (bronzing) observed in the NF–FL leaves (Novak et al., 2008).

5. Conclusions

The relationships between O_3 and high light conditions are complex. In the present study shaded leaves demonstrated to be more susceptible to ozone uptake (increasing g_w), and reduced the P_N more than full light leaves (see also Topa et al., 2001, 2004). These latter, however, showed the most relevant changes as far the chlorophyll fluorescence parameters are concerned. The changes induced by O_3 in chlorophyll fluorescence appeared very similar to those induced by high light (with special reference to the decreased maximum quantum yield of primary photochemistry and increased quantum yield of dissipation). The maximum quantum yield of an electron reaching the acceptor side beyond the PS I – RE_0/ABS – showed a more specific behaviour, because this parameter is enhanced by light and depressed by O_3 . The alleged ozone sensitivity of sun leaves is based on the onset of the foliar visible symptoms, that were not recorded in shaded conditions (Novak et al., 2008). Our results suggest that the leaf bronzing, usually attributed to ozone (Vollenweider et al., 2003; Bussotti et al., 2005), derives from an excess of oxidative pressure pro-

duced by the concomitant action of high light and O_3 when, at the end of the season, the capacity to feed the final electron receptors declines.

The action of sunlight on the processes of beechwood regeneration (i.e., seedlings growing in forest gaps) had been discussed in several papers (Tognetti et al., 1994, 1998; García-Plazaola and Becerril, 2000; Valladares et al., 2002; Reynolds and Frochot, 2003). All the authors underline the role of photoinhibition, especially when shade-acclimated seedlings are suddenly exposed to full sunlight. Our results suggest that the understory environment protects beech seedling from high irradiance, but not from ozone. Responses to ozone stress, however, are produced on individual basis according to the genotype (Farage, 1996; Cascio et al., 2008), and a possible scenario foresees that the most ozone-resistant genotypes will be selected for the future forest regeneration.

Acknowledgements

Funding for this research project was provided by the Swiss Agency for the Environment, Forests and Landscape (SAEFL). The research was conducted in collaboration with the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland and the Pennsylvania State University, USA. The project would not have been possible without the exceptional support provided by Mr. Giuseppe Tettamanti and his nursery staff at the Lattecaldo research site.

We also thank the Canton Ticino for the continuous support of the Lattecaldo research facility.

References

- Adams III, W.W., Demmig-Adams, B., 2004. Chlorophyll fluorescence as a tool to monitor plant response to the environment. In: Papageorgiou, G., Govindjee, G. (Eds.), *Advances in Photosynthesis and Respiration Series. Chlorophyll fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, NL, pp. 583–604.
- Anderson, J.M., Osmond, C.B., 1987. Shade-sun responses: compromises between acclimation and photoinhibition. In: Kyle, D.J., Osmond, C.B., Arntzen, C.J. (Eds.), *Photoinhibition*. Elsevier, Amsterdam, NL, pp. 1–38.
- Anderson, J.M., Chow, W.S., Park, Y.I., 1995. The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research* 46, 129–139.
- Ballottari, M., Dall'Osto, L., Morosinotto, T., Bassi, R., 2007. Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. *Journal of Biological Chemistry* 282, 8947–8958.
- Beaudet, M., Messier, C., Hilbert, D.W., Lo, E., Wang, Z.M., Lechowicz, M.J., 2000. Leaf- and plant-level carbon gain in yellow birch, sugar maple, and beech seedlings from contrasting forest light environments. *Canadian Journal of Forest Research* 30, 390–404.
- 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.
- Brendley, B.W., Pell, E.J., 1998. Ozone-induced changes in biosynthesis of Rubisco and associated compensation to stress in foliage of hybrid poplar. *Tree Physiology* 18, 81–90.
- Boardman, N.K., 1977. Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* 28, 355–377.
- Bussotti, F., 2008. Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview. *Global Change Biology* 14, 2727–2739.
- Bussotti, F., Agati, G., Desotgiu, R., Matteini, P., Tani, C., 2005. Ozone foliar symptoms in woody plants assessed with ultrastructural and fluorescence analysis. *New Phytologist* 166, 941–955.
- Cascio, C., Bussotti, F., Strasser, R.J., Marzuoli, R., 2008. Variability of photosynthetic performances in tree seedlings under ozone and water stress. In: Allen, J.F., Gantt, E., Golbeck, J.H., Osmond, B. (Eds.), *Photosynthesis. Energy from the sun*. 14th International Congress of Photosynthesis Glasgow 2007. Springer, Dordrecht, NL, pp. 1417–1420.
- Calatayud, V., Cerveró, J., Sanz, M.J., 2007. Foliar, physiological and growth responses of four maple species exposed to ozone. *Water Air and Soil Pollution* 185, 239–254.
- Chow, W.S., Anderson, J.M., 1987a. Photosynthetic responses of *Pisum sativum* to an increase in irradiance during growth I. Photosynthetic activities. *Australian Journal of Plant Physiology* 14, 1–8.
- Chow, W.S., Anderson, J.M., 1987b. Photosynthetic responses of *Pisum sativum* to an increase in irradiance during growth II. Thylakoid membrane component. *Australian Journal of Plant Physiology* 14, 9–19.
- Dann, M.S., Pell, E.J., 1989. Decline of activity and quantity of ribulosediphosphate carboxylase/oxygenase and net photosynthesis in ozone-treated potato foliage. *Plant Physiology* 91, 427–432.
- Davison, A.W., Neufeld, H.S., Chapellek, A.A.H., Wolff, K., Finkelstein, P.L., 2003. Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, *Rudbeckia laciniata* L. *Environmental Pollution* 125, 61–70.
- Evans, J.R., Poorter, H., 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment* 24, 755–767.
- Farage, P.K., 1996. The effect of ozone fumigation over one season on photosynthetic processes of *Quercus robur* seedlings. *New Phytologist* 134, 279–285.
- Fontaine, V., Cabane, M., Dizengremel, P., 2003. Regulation of phosphoenolpyruvate of carboxylase in *Pinus halepensis* needles submitted to ozone and water stress. *Physiologia Plantarum* 117, 445–452.
- Fredericksen, T.S., Kolb, T.E., Skelly, J.M., Steiner, K.C., Joyce, B.J., Savage, J.E., 1996. Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. *Tree Physiology* 16, 485–490.
- García-Plazaola, J.I., Becerril, J.M., 2000. Photoprotection mechanisms in European beech (*Fagus sylvatica* L.) seedlings from diverse climatic origins. *Trees* 14, 339–343.
- García-Plazaola, J.I., Becerril, J.M., Hernández, A., Niinemets, Ü., Kollist, H., 2004. Acclimation of antioxidant pools to the light environment in a natural forest canopy. *New Phytologist* 163, 87–97.
- Gielen, B., Löw, M., Deckmyn, G., Metzger, U., Heerdt, C., Matyssek, R., Valcke, R., Ceulemans, R., 2007. Chronic ozone exposure affects leaf senescence of adult beech trees: a chlorophyll fluorescence approach. *Journal Experimental Botany* 58, 785–795.
- Gilmore, A.M., 2004. Excess light stress: probing excitation dissipation mechanisms through global analysis of time- and wavelength-resolved chlorophyll a fluorescence. In: Papageorgiou, G., Govindjee, C. (Eds.), *Advances in Photosynthesis and Respiration Series. Chlorophyll Fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, NL, pp. 555–581.
- Gratani, L., Covone, F., Larcher, W., 2006. Leaf plasticity in response to light of three evergreen species of the Mediterranean maquis. *Trees* 20, 549–558.
- Gravano, E., Bussotti, F., Strasser, J.R., Schaub, M., Novak, K., Skelly, J., Tani, C., 2004. Ozone symptoms in leaves of woody plants in open top chambers: ultrastructural and physiological characteristics. *Physiologia Plantarum* 121, 620–633.
- Groom, P.K., Lamont, B.B., 1997. Xerophytic implications of increased sclerophylly: interactions with water and light in *Hakea psilorrhyncha* seedlings. *New Phytologist* 136, 231–237.
- Guidi, L., Tonini, M., Soldatini, G.F., 2000. Effects of high light and ozone fumigation on photosynthesis in *Phaseolus vulgaris*. *Plant Physiology Biochemistry* 38, 717–725.
- Guidi, L., Degl'Innocenti, E., Soldatini, G.F., 2002. Assimilation of CO₂, enzyme activation and photosynthetic electron transport in bean leaves, as affected by high light and ozone. *New Phytologist* 156, 377–388.
- Guidi, L., Degl'Innocenti, E., 2008. Ozone effects on high light-induced photoinhibition in *Phaseolus vulgaris*. *Plant Science* 174, 590–596.
- Guidi, L., Degl'Innocenti, E., Martinelli, F., Piras, M., 2009. Ozone effects on carbon metabolism in sensitive and in sensitive *Phaseolus* cultivars. *Environmental and Experimental Botany* 66, 117–125.
- Hald, S., Nandha, B., Gallois, P., Johnson, G.N., 2008. Feedback regulation of photosynthetic electron transport by NADP(H) redox poise. *Biochimica et Biophysica Acta* 1777, 433–440.
- Inclan, R., Gimeno, B.S., Dizengremel, P., Sanchez, M., 2005. Compensation processes of Aleppo pine (*Pinus halepensis* Mill.). *Environmental Pollution* 137, 517–524.
- Jactap, V., Bhargava, S., Steb, P., Feierabend, J., 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. *Journal of Experimental Botany* 49, 1715–1721.
- Kim, G.T., Yano, S., Kozuka, T., Tsukaya, H., 2005. Photomorphogenesis of leaves: shade-avoidance and differentiation of sun and shade leaves. *Photochemistry Photobiology Science* 4, 770–774.
- Kolb, T.E., Fredericksen, T.S., Steiner, K.C., Skelly, J.M., 1997. Issues in scaling tree size and age responses to ozone: a review. *Environmental Pollution* 98, 195–208.
- Kolb, T.E., Matyssek, R., 2001. Limitations and perspectives about ozone impacts in trees. *Environmental Pollution* 15, 373–393.
- Krieger-Liszskay, A., 2005. Singlet oxygen production in photosynthesis. *Journal of Experimental Botany* 56, 337–346.
- Küppers, M., Schneider, H., 1993. Leaf gas exchange of beech (*Fagus sylvatica* L.) seedlings in lightflecks: effects of fleck length and leaf temperature in leaves grown in deep and partial shade. *Trees* 7, 160–168.
- Lichtenthaler, H.K., Babani, F., 2004. Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In: Papageorgiou, G., Govindjee, C. (Eds.), *Advances in Photosynthesis and Respiration Series. Chlorophyll Fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, NL, pp. 713–736.
- Lichtenthaler, H.K., Buschmann, C., Döll, M., Fietz, H.-J., Bach, T., Kozel, U., Meier, D., Rahmsdorf, U., 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research* 2, 115–141.
- Lichtenthaler, H.K., Ač, A., Marek, M.V., Kalina, J., Urban, O., 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry* 45, 577–588.
- Matyssek, R., Innes, J.L., 1999. Ozone—a risk factor for trees and forest in Europe? *Water Air and Soil Pollution* 116, 199–226.
- Matyssek, R., Bahnweg, G., Ceulemans, R., Fabian, P., Grill, D., Hanke, D.E., Kraigher, H., Osswald, W., Rennenberg, H., Sandermann, H., Tausz, M., Wieser, G., 2007. Synopsis of the CASIROZ case study: Carbon sink strength of *Fagus sylvatica* L. in a changing environment—experimental risk assessment of mitigation by chronic ozone impact. *Plant Biology* 9, 163–180.
- Maxwell, K., Marrison, J.L., Leech, R.M., Griffiths, H., Horton, P., 1999. Chloroplast acclimation in leaves of *Guzmania monostachia* in response to high light. *Plant Physiology* 121, 89–95.
- Mikkelsen, T.N., Ro-Poulsen, H., 1994. Exposure of Norway spruce to ozone increases the sensitivity of current year needles to photoinhibition and desiccation. *New Phytologist* 128, 153–163.
- Mikkelsen, T.N., Dodel, B., Lütz, C., 1995. Changes in pigment concentration and composition in Norway spruce induced by long-term exposure to low-levels of ozone. *Environmental Pollution* 87, 197–205.
- Mittler, R., 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* 11, 15–19.
- Naumburg, E., Ellsworth, D.S., 2000. Photosynthetic sunfleck utilization potential of understory saplings growing under elevated CO₂ in FACE. *Oecologia* 122, 163–174.
- Neill, S.O., Gould, K.S., 2003. Anthocyanins in leaves: light attenuators or antioxidants? *Functional Plant Biology* 30, 865–873.
- Novak, K., Skelly, J.M., Schaub, M., Kraeuchi, N., Hug, C., Landolt, W., Bleuler, P., 2003. Ozone air pollution and foliar injury development. *Environmental Pollution* 125, 41–52.
- Novak, K., Schaub, M., Fuhrer, J., Skelly, J.M., Hug, C., Landolt, W., Bleuler, P., Kräuchi, N., 2005. Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plants species. *Environmental Pollution* 136, 33–45.
- Novak, K., Schaub, M., Fuhrer, J., Skelly, J.M., Frey, B., Kräuchi, N., 2008. Ozone effects on visible foliar injury and growth of *Fagus sylvatica* and *Viburnum lantana* seedlings grown in monoculture or in mixture. *Environmental and Experimental Botany* 62, 212–220.
- Oguchi, R., Hikosaka, K., Hirose, T., 2003. Does the photosynthetic light acclimation need change in leaf anatomy? *Plant, Cell and Environment* 26, 505–512.

- Powles, S.B., 1984. Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology* 35, 15–44.
- Reynolds, P.E., Frochot, H., 2003. Photosynthetic acclimation of beech seedlings to full sunlight following a major windstorm event in France. *Annals of Forest Science* 60, 701–709.
- Sarijeva, G., Knapp, M., Lichtentaler, H.K., 2007. Differences in photosynthetic activity, chlorophyll and carotenoid levels, and in chlorophyll fluorescence parameters in green sun and shade leaves of *Ginkgo* and *Fagus*. *Journal of Plant Physiology* 164, 950–955.
- Schansker, G., Srivastava, A., Govindjee, Strasser, R.J., 2003. Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. *Functional Plant Biology* 30, 785–796.
- Smit, M.F., Krüger, G.H.J., van Heerden, P.D.R., Pienaar, J.J., Weissflog, L., Strasser, R.J., 2008. Effect of trifluoroacetate, a persistent degradation product of fluorinated hydrocarbons on C3 and C4 crop plants. In: Allen, J.F., Gantt, E., Golbeck, J.H., Osmond, B. (Eds.), *Photosynthesis. Energy from the sun*. 14th International Congress of Photosynthesis Glasgow 2007. Springer, Dordrecht, NL, pp. 1501–1504.
- Strasser, R.J., Govindjee, 1992. The F_0 and the O-J-I-P fluorescence rise in higher plants and algae. In: Argyroudi-Akoyunoglou, J.H. (Ed.), *Regulation of Chloroplast Biogenesis*. Plenum Press, New York, USA, pp. 423–426.
- Strasser, R.J., Strasser, B.J., 1995. Measuring fast fluorescence transients to address environmental questions: the JIP test. In: Mathis, P. (Ed.), *Photosynthesis: From Light to Biosphere*, V. Kluwer Academic Publishers, Dordrecht, NL, pp. 977–980.
- Strasser, A., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. Taylor & Francis, London, UK, pp. 445–483.
- Strasser, A., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the fluorescence transient. In: Papageorgiou, G., Govindjee, C. (Eds.), *Advances in Photosynthesis and Respiration Series. Chlorophyll fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, NL, pp. 321–362.
- Tateno, M., Taneda, H., 2007. Photosynthetically versatile thin shade leaves: a paradox of irradiance-response curves. *Photosynthetica* 45, 299–302.
- Tattini, M., Gravano, E., Pinelli, P., Mulinacci, N., Romani, A., 2000. Flavonoid secreted by glandular trichomes play a key role in the acclimation mechanism of *Phillyrea latifolia* L. to high solar radiation. *New Phytologist* 112, 215–220.
- Temesgen, H., Weiskittel, A.R., 2006. Leaf mass per area relationships across light gradients in hybrid spruce crowns. *Trees* 20, 522–530.
- Terashima, I., Hanba, Y.T., Tazoe, Y., Vyas, P., Yano, S., 2006. Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *Journal of Experimental Botany* 57, 343–354.
- Tognetti, R., Michelozzi, M., Borghetti, M., 1994. Response to light of shade-grown beech seedlings subjected to different watering regimes. *Tree Physiology* 14, 751–758.
- Tognetti, R., Minotta, G., Pinzauti, S., Michelozzi, M., Borghetti, M., 1998. Acclimation to changing light conditions of long-term shade-grown beech (*Fagus sylvatica* L.) seedlings of different geographic origins. *Trees* 12, 326–333.
- Tsimilli-Michael, M., Strasser, R.J., 2008. In vivo assessment of stress impact on plant's vitality: applications in detecting and evaluating the beneficial role of mycorrhization on host plants. In: Varma, A. (Ed.), *Mycorrhiza: Genetics and Molecular Biology, Eco-function, Biotechnology, Eco-physiology, and Structure and Systematic*. Springer, Berlin, DE, pp. 679–703.
- Tjoelker, M.G., Volin, J.C., Oleksyn, J., Reich, P.B., 1993. Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh. and hybrid *Populus* L. I. In situ net photosynthesis, dark respiration and growth. *New Phytologist* 124, 627–636.
- Tjoelker, M.G., Volin, J.C., Loksyn, J., Reich, P.B., 1995. Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. *Plant Cell and Environment* 18, 895–905.
- Topa, M.A., Vanderklein, D.W., Corbin, A., 2001. Effects of elevated ozone and low light on diurnal and seasonal carbon gain in sugar maple. *Plant Cell and Environment* 24, 663–677.
- Topa, M.A., McDermitt, D.J., Yun, S.C., King, P.S., 2004. Do elevated ozone and variable light alter carbon transport to roots in sugar maple? *New Phytologist* 162, 173–186.
- Valladares, F., Chico, J.M., Aranda, I., Balaguer, L., Dizengremel, P., Manrique, E., Dreyer, E., 2002. The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees* 16, 395–403.
- VanderHeyden, D.J., Skelly, J.M., Innes, J.L., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. *Environmental Pollution* 111, 321–331.
- Volin, J.C., Tjoelker, M.G., Oleksyn, J., Reich, P.B., 1993. Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh. and hybrid *Populus* L. I. Diagnostic gas exchange and leaf chemistry. *New Phytologist* 124, 637–646.
- Vollenweider, P., Ottiger, M., Günthard-Goerg, M.S., 2003. Validation of leaf ozone symptoms in natural vegetation using microscopical methods. *Environmental Pollution* 124, 101–118.
- Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J.E., Stevenson, R.E., Davis, D.D., 2004a. Responses of hybrid poplar clones and red maple seedlings to ambient O_3 under different light within a mixed hardwood forest. *Environmental Pollution* 130, 199–214.
- Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J.E., Stevenson, R.E., Davis, D.D., 2004b. Influence of light fleck and low light on foliar injury and physiological response on two hybrid poplar clones to ozone. *Environmental Pollution* 130, 215–227.
- Wittmann, C., Aschan, G., Pfanz, H., 2001. Leaf and twig photosynthesis of young beech (*Fagus sylvatica*) and aspen (*Populus tremula*) trees grown under different light regime. *Basic and Applied Ecology* 2, 145–154.