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The impact of ozone fumigation and fertilization on chlorophyll fluorescence of birch leaves (*Betula pendula*)

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Abstract The impact of ozone fumigation on chlorophyll *a* fluorescence parameters and chlorophyll content of birch trees grown at high and low fertilization were studied for 6-, 8-, and 12-week old leaves. Fluorescence parameters were measured with a portable fluorometer with its fibre optics tightly inserted in a gas exchange cuvette at light intensities from 0 to 220 µmol photons m^{-2} s^{-1}. Ozone caused significant changes of primary photosynthetic reactions: a decrease of the quantum yield of photosystem II and an increase of non-photochemical quenching. In all leaves a biphasic light response of non-photochemical quenching was observed. Ozone fumigation shifted the onset of the second phase from a PFD of about 60 µmol m^{-2} s^{-1} to about 30 µmol m^{-2} s^{-1}. While the fertilizer concentration had no influence on this character, high fertilization supply of plants partially reduced O_3-induced damage. The light responses of Ft, Fm′ and NPQ observed in birch leaves grown in O_3-free air indicate the existence of at least two different processes governing energy conversion of the photosynthetic apparatus at PS II in the range of PFD 0–200 µmol photons m^{-2} s^{-1}. The first phase was attributed to a rather slowly relaxing type of non-photochemical quenching, which, at least at low PFD, is thought to be related to a state 1–2 transition. The further changes of the fluorescence parameters studied at higher PFD might be explained by an increase of energy-dependent quenching, connected with the energization of the thylakoid membrane and zeaxanthin synthesis. A major effect of ozone treatment was a lowering of PS II quantum yield. This reflects a reduction of PS II electron transport and corresponds to the reduction of CO_2-fixation observed in ozonated leaves.

Key words Ozone fumigation · Chlorophyll fluorescence · Fertilization · Light intensity · Senescence

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Introduction

Ozone is known to potentially limit biomass production in trees (Miller 1973; Peterson et al. 1987; Reich 1987; Pye 1988). Whole-plant production is determined by the crown foliage available (Körner 1991) and therefore may be reduced by O_3-induced leaf loss. However, preceding leaf destruction, the carbon uptake capacity of the single leaves, which is the ultimate prerequisite for growth, is also decreased by ozone (Sasek and Richardson 1989; Schweizer and Arndt 1990). The declining CO_2 assimilation can be traced back to the biochemical level: quantity and activity of rubisco were found to be lowered under O_3 stress (Dann and Pell 1989; Landry and Pell 1993), while measurements of chlorophyll fluorescence also proved that the light reactions of photosynthesis were affected (Schreiber et al. 1978; Schmidt et al. 1990; Ruth and Weisel 1993; Mikkelsen and Ro-Poulsen 1994; Reiling and Davison 1994). The chloroplasts are probably impaired by an overall disturbed cell metabolism rather than by direct O_3 impact, because most ozone (in the concentrations prevailing in the ambient air) is absorbed in the apoplast and plasmalemma (Urbach et al. 1989; Luwe et al. 1993). Nevertheless, deformations have been observed in chloroplasts adjacent to cell walls (Fink 1991).

The present study aims at elucidating the impact of ozone on chlorophyll fluorescence under different nutrient supply, in order to broaden the mechanistic basis for

clarifying the role of nutrition in the O_3 sensitivity of trees (Matyssek et al. 1995). The birch clone (*Betula pendula* Roth) chosen had responded to ozone by declining CO_2 assimilation and whole plant production as well as markedly altered leaf differentiation and whole plant carbon allocation (Matyssek et al. 1991, 1992; Günthardt-Goerg et al. 1993; Maurer et al. 1997; Maurer and Matyssek 1997). Fluorescence was investigated by the 'pulse amplitude modulated approach' (Schreiber et al.1986; Bolhar-Nordenkampf et al.1989). How do the chlorophyll *a* fluorescence parameters of birch leaves respond to O_3 stress and nutrient supply under different light conditions in the PFD range 0–200 µmol photons $m^{-2}s^{-1}$?

Materials and methods

From 13 April through 4 October, 1993, cuttings of one birch clone (*Betula pendula*, Roth) were grown in 10 l pots filled with sand and a basal layer of inert synthetic clay beads (1 plant/pot). When transferred to the Birmensdorf field fumigation chambers on 3 May, the plants were separated into two O_3 treatments (80 plants/treatment, 8 plants/chamber): charcoal-filtered air with either<3 (control) or 90/40 nl O_3 l^{-1} (day/night, i.e. 0700–2100 hours/2100–0700 hours, until 4 October). In the latter regime, O_3 was generated from pure oxygen (Fischer, model 502) and in both treatments continuously monitored with a 'Monitor Labs 8810' instrument. Each regime was split into high and low soil nutrient supply (Table 1) by watering plants with either a 0.05% or 0.005% solution of a fertilizer (Hauert, Nährsalz Typ A/Anzucht) which contained macro and micro nutrients.

The birch clone and fumigation chambers were the same as described in Matyssek et al. (1991, 1992; see also Landolt et al. 1989). On clear sunny days, a shading roof limited the photosynthetic photon flux density (PFD) to a maximum of about 600 µmol m^{-2} s^{-1} to prevent over-heating in the kind of open-top chambers used. Otherwise (overcast and cloudy conditions, dawn, dusk) the roof was not employed. For the study 6-, 8- and 12-week old leaves were used. Their appearance was estimated according to the following classification (according to Günthardt-Goerg et al. 1993): 0 – no injury; 1 – light-green dots spread over leaf; 2 – light-green or bronze-green discoloration and 3 – bronze-green discoloration with small necrotic areas. The external applied dose of ozone was likely to correlate with this classification of macroscopic leaf injury, and the daily rate of ozone uptake was found to be similar in low and high fertilized trees (Maurer et al. 1997). Thus, leaves grown under different nutrient supplies most probably absorbed similar amounts of ozone.

Chlorophyll fluorescence of the leaves was determined with a PAM-2000 (Walz, Germany). The measurements of fluorescence reported here were part of a larger study on leaf gas exchange (Maurer et al.1997) and were conducted on attached complete leaves sealed into a thermo-electrically climate-controlled cuvette system (Walz, Germany; Matyssek et al. 1991). The fibre optics of the PAM-2000 providing measuring and actinic light as well as saturating light pulses, were tightly introduced into the gas exchange cuvette and oriented to the abaxial leaf side (from 1 cm distance and 60º angle). This leaf side was chosen to diminish the possible photoinhibitory effect of saturating light intensities during gas exchange measurements which had been monitored before from the same leaf with the light source position above the adaxial leaf side. The total PFD on the abaxial leaf side did not exceed 240 µmol photons m^{-2} s^{-1} during a measuring day.

The measured leaf area was about 1 cm in diameter and close to (but excluding) the central leaf vein. During the measurements, leaf temperature was 19 ºC, Δw=10 mmol mol^{-1} (i.e. the leaf/air difference in the mole fraction of water vapor), and the CO_2 concentration of the cuvette air was controlled to stay within 340±5 µl l^{-1}. PFD of measuring light provided by the PAM-2000 unit was<1 µmol photons m^{-2} s^{-1}. Actinic light from the light-emitting diode inside the PAM-2000 was increased by 10 steps between 0 and 220 µmol photons m^{-2} s^{-1} (maximum supply from PAM-2000) and measured with a miniaturised quantum sensor of the unit (sensor diameter 1.5 mm, resolution 1µmol photons m^{-2} s^{-1}, calibrated with a LI-190, LICOR). The fluorescence characteristics under steady-state light conditions were determined 5 min after changing the light level. Ft (the steady state level of fluorescence) and Fm′ (the amplitude of fluorescence induced by a single pulse of saturating light intensity under ambient light conditions) were measured after F0 and Fm parameters had been obtained during dark adaptation of the leaf (30 min) at the beginning of the experiment. The saturation pulses (duration 0.8 s) released 1000 µmol photons m^{-2} s^{-1}. Measurements were made after plants had been brought the evening before to the measuring site close to the exposure chambers (plants were shielded from direct sunlight and rain during experiments). The PS II quantum yield (ΔF/Fm′) values were calculated from the equation ΔF/Fm′=(Fm′–Ft):Fm′ (Genty et al. 1989). Non-photochemical quenching coefficients (NPQ) were calculated from NPQ=Fm/Fm′–1.

Each set of experiments was repeated 6–7 times per treatment with different plants. Standard biostatistical calculations were used. In the figures data from a single set of experiments representing the characteristic light response curves are shown.

The chlorophyll concentration of the leaf area exposed to the fibre optics was determined according to Lichtenthaler and Wellburn (1983) after each experimental day.

Table 1 The effect of O_3 exposure and nutrient supply on 8-week-old leaves. Treatment differences were calculated with ANOVA (*: P<0.05; **: P<0.005; ***: P<0.001; *ns*=not significant; N=7). No O_3×N interactions were found.

O_3 concentration day/night (nl l^{-1}):	Treatments				Statistics	
	<5/<5	<5/<5	90/40	90/40	O_3	N
nutrient supply (0.05%=HF; 0.005%=LF):	HF	LF	HF	LF		
Class of visual O_3 injury in leaves: (see text)	0	0	2–3	2–3		
Chlorophyll concentration, total: (mg g^{-1} DW)	9.2±1.1	6.0±0.9	7.4±0.8	4.2±1.4	***	***
Chl *a*/Chl *b*	2.6±0.1	2.8±0.1	2.5±0.2	2.5±0.1	***	ns
CO_2 assimilation rate, A_{amb} (see text) (mg g^{-1} h–1):	34.2±6.0	32.3±7.2	14.0±5.1	7.4±2.4	***	*
Fo (mV)	191±43	254±50	237±53	289±33	*	**
Fm (mV)	1058±265	1283±274	1056±214	1090±231	ns	ns
Fv/Fm	0.818±0.008	0.802±0.010	0.775±0.026	0.726±0.056	***	**

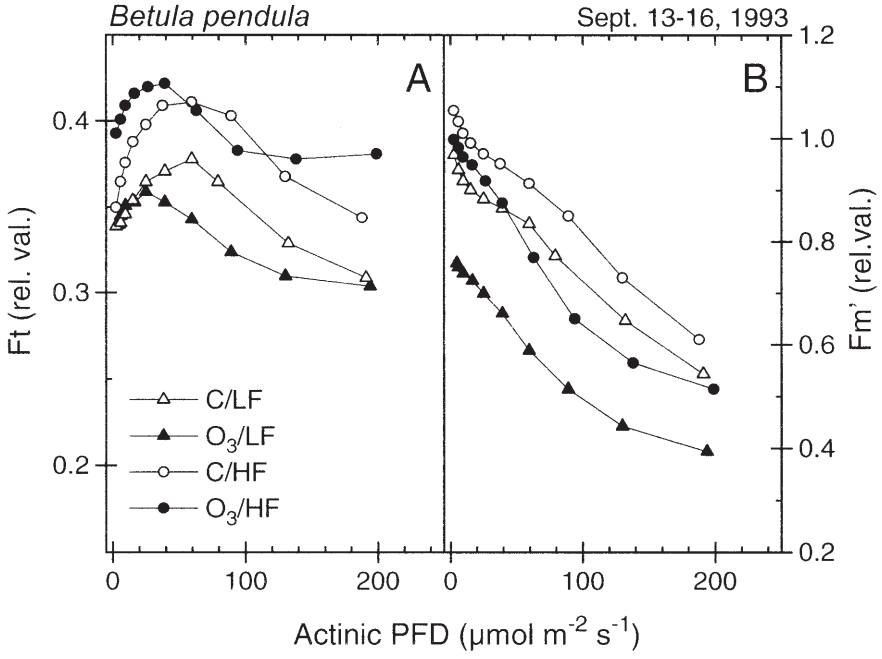

Fig. 1 Dependence of the fluorescence parameters Ft (**A**) and Fm′ (**B**) on actinic PFD in mid-September (leaves 8 weeks old). (*C* – control plants in O_3-free air; *O_3* – ozone-exposed plants; *HF* – high nutrient supply; *LF* – low nutrient supply. Each line represents one leaf)

Results

The leaves used for the experiments were 8 weeks old and are characterized in Table 1. O_3 treated leaves appeared light-green to bronze-green, reflecting a damage class of 2–3 according to Günthardt-Goerg et al. (1993). Total chlorophyll concentration was highest in high-fertilized control plants and declined under O_3 exposure (Table 1). The Chl *a*/Chl *b* ratio was increased in the low-fertilized control plants and only slightly reduced by O_3, regardless of the nutrient supply. However, ozone markedly lowered the CO_2 assimilation rate of the given injury classes, while high fertilization increased the CO_2 uptake rate relative to low nutrient supply significantly only in the O_3-treated plants (Table 1). In Table 1 fluorescence values of dark adapted leaves are also shown. Leaves grown under high nutrient supply in O_3-free air conditions demonstrated the lowest F0 level whereas the low nutrient supply- and O_3-treatments caused an increase of F0 (Table 1). The highest F0 developed under combined effect of two factors – low fertilization and ozonation. Fm values under different treatments did not show any considerable differences, but optimal quantum yield of PS II (Fv/Fm) declined under ozonation.

Fluorescence light response curves of 8-week-old control (O_3-free air) leaves showed a maximum of Ft at about 60 µmol photons m^{-2} s^{-1}, regardless of the nutrient supply (Fig. 1A). This maximum shifted to a PFD of 25–40 µmol photons m^{-2} s^{-1} under O_3 exposure (Fig. 1A). Fm′ declined with increasing irradiance but displayed a shoulder between 15 and 60 µmol photons m^{-2} s^{-1} (Fig. 1B), indicating a transition between two different states of the photosynthetic apparatus. The length and the location of this bend in the light curve depended on the treatment. The fertilizer concentration had no influence on these characteristics, whereas ozone shortened the shoulder of

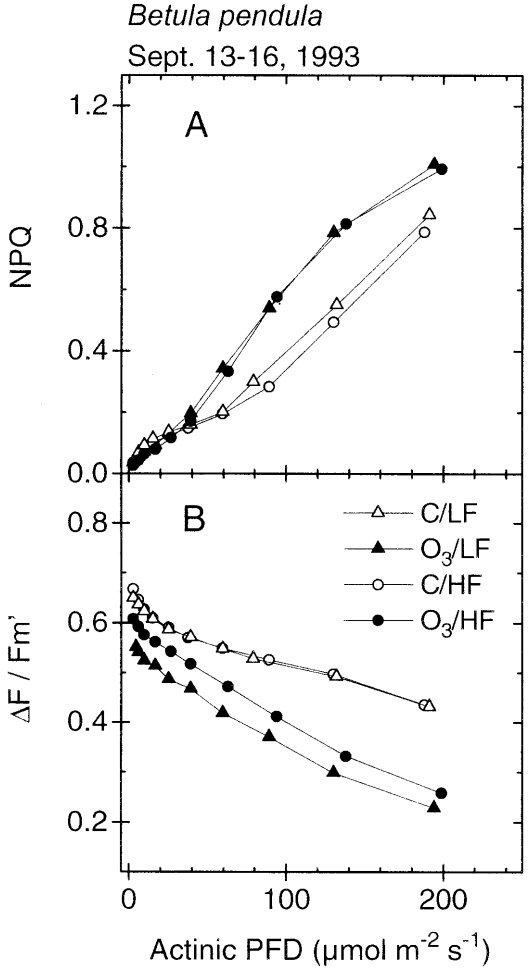

Fig. 2 Dependence of non photochemical quenching, NPQ, (**A**), and effective PS II quantum yield, ΔF/Fm′, (**B**) on actinic PFD. Same leaves and symbols as in Fig. 1

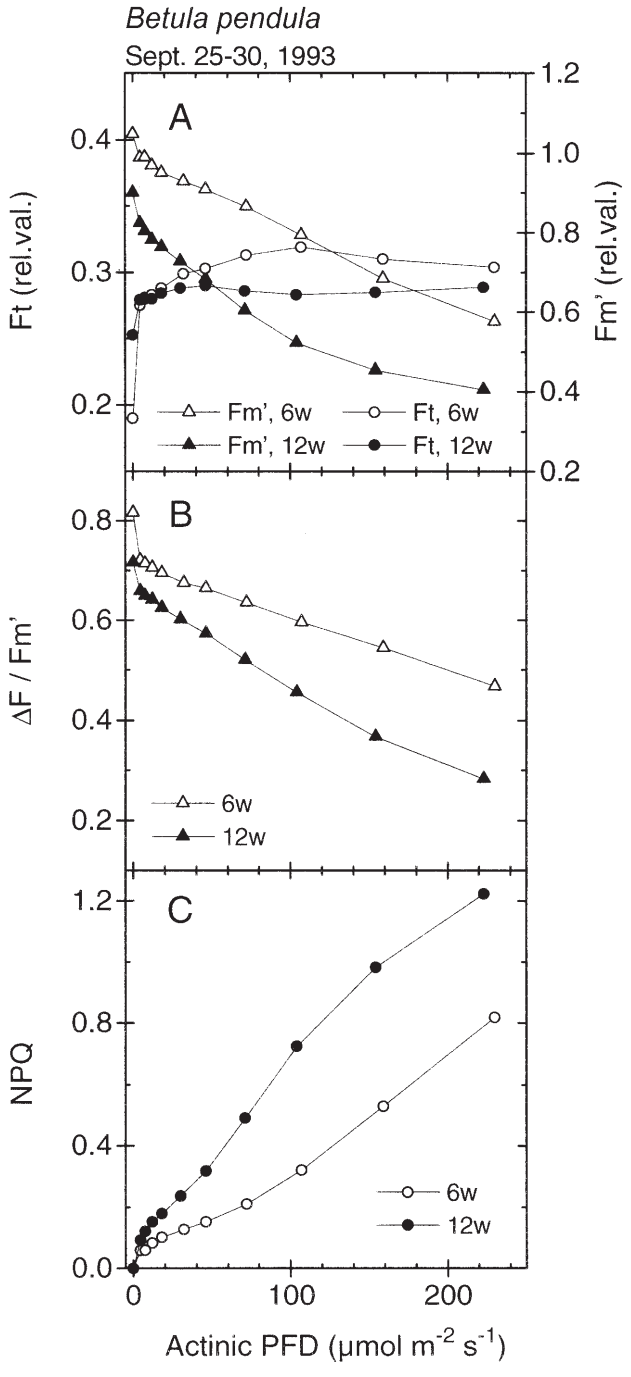

Fig. 3 Light response curves of Ft and Fm′ values (**A**), effective PS II quantum yield, ΔF/Fm′, (**B**), and non photochemical quenching, NPQ, (**C**) in leaves of high fertilized plants exposed for 6 (*6w*) or 12 (*12w*) weeks to ozone fumigation. Measurements were in late September

the Fm′ response curve and shifted it to the range of 5–20 µmol photons m^{-2} s^{-1} (Fig. 1B). Comparison of Fig. 1A and B shows that the first phase of Fm′ decline corresponds to the increase of Ft to the maximum, while the second phase coincides with the decrease of Ft.

In low fertilized ozonated plants at PFD>100 µmol photons m^{-2} s^{-1} Ft fell below the F0 level, which did not allow calculation of photochemical quenching in our experiments. The decrease of Ft below F0 is apparently connected with F0-quenching appearing in leaves under conditions of strong non-photochemical quenching (Bilger and Schreiber 1986). It should be noted that this drop was characteristic only for ozone-treated plants and was not observed in control treatments. The existence of such a quenching under normal physiological conditions is a result of pronounced chloroplast energization in this treatment.

Given the overall consistency in the light response of Ft and Fm′, Fig. 2 shows the fluorescence parameters NPQ and ΔF/Fm′. The derived parameters obviously reflected the changes of primary photosynthetic reactions in birch leaves indicated by the shape of Ft and Fm′ light curves in the range 10–50 µmol photons m^{-2} s^{-1}. The ΔF/Fm′ parameter, which reflects the quantum yield of PS II electron transport (Genty et al.1989), was lowered by ozone (Fig. 2B) which was even more pronounced at low nutrient supply (Fig. 2B). NPQ increased with rising PFD in the control leaves in two phases, corresponding to the biphasic decline of Fm′ (Fig. 1). The light dependent increase of NPQ was affected by ozone almost exclusively in the second phase of the curve while the first phase was similar to the control leaves.

As an example of leaf ageing under O_3 exposure (i.e. increasing O_3 dose because of the longer exposure), Fig. 3 demonstrates the fluorescence parameters of 6- and 12-week-old leaves under high nutrient supply in late September. Principally, similar effects between the two leaf age classes could be observed as when comparing ozone treated and control plants (Figs. 1, 2). In older leaves, which had received higher doses of ozone, the F0 value was also higher (Fig. 3A). This effect was combined with the shift of the Ft-maximum and the shoulder in the Fm′ graph to lower PFD. NPQ increased with leaf age, while the bend of the plot described above shifted towards low irradiance. ΔF/Fm′ declined with leaf age.

Discussion

The results show that prolonged treatment under O_3 concentrations, which correspond to concentrations on the experimental site during sunny summer days, cause significant changes of chlorophyll fluorescence parameters in birch leaves. These changes include a decrease in the quantum yield of photosystem II, and an increase of non-photochemical quenching. The light dependence of NPQ revealed two different phases. O_3 treatment had no apparent effect on the first phase whereas the second one was already induced at lower PFD values. In the illuminated state an adaptation of the photosynthetic apparatus to O_3-induced stress was connected to the rise of radiationless energy dissipation. All these effects became more obvious with an increase of ozone dose in leaves during their exposure to the fumigation.

The light responses of Ft, Fm′ and NPQ observed in birch leaves grown in O_3-free air indicate the existence of at least two different processes governing energy con-

version of the photosynthetic apparatus at PS II in the range of PFD 0–200 µmol photons m^{-2} s^{-1}. An inflection in the light response curve of NPQ has also been observed in other studies (e.g. Demmig-Adams and Winter 1988). The first phase was attributed to a rather slowly relaxing type of NPQ, which, at least at low PFD, is thought to be related to a state 1–2 transition (Chow et al. 1981; Barber 1983; Quick and Stitt 1989; Krause and Weis 1991; Walters and Horton 1991; Allen 1992).

According to the mechanism of the state 1–2 transition, state 1 occurs at rather low irradiance with low $\Delta\mu H^+$ across the thylakoid membrane and most plastoquinone (PQ) being oxidised (for a review, see Allen 1992). An increase in irradiance from 0 to 50 µmol photons m^{-2} s^{-1} caused an increase in Ft, indicating the reduction of Q_A, the primary acceptor of PS II (Fig. 1). Q_A reduction is combined with an increase of $\Delta\mu H^+$, PQ pool reduction and activation of the phosphorylation of PS II light harvesting complexes (LHCII) (Allen 1992). It is assumed that phosphorylated LHCII shifts from PS II to PS I which is an effective quencher of fluorescence at room temperature. Thus, NPQ at low irradiance might reflect a decrease of excitation energy flow to PS II. An alternative view was proposed by Walters and Horton (1993). These authors suggest that NPQ induced at very low PFD is due to antenna located dissipation induced by phosphorylation of the LHCII.

Ozone treatment had no appreciable effect on the first phase of NPQ (Figs. 2, 3). This could have been caused by an insensitivity of LHC II phosphorylation to ozone induced damage. At the lowest PFDs the electron pressure provided by PS II is limiting electron transport. Reduced Fv/Fm values and a lowered apparent quantum yield of CO_2 fixation as determined by gas exchange measurements (Maurer 1995) indicated a decreased PS II activity. Hence, one would rather expect a decreased NPQ in ozone treated leaves. Close inspection of Fig. 2A revealed a tendency for lower NPQ in ozone fumigated leaves. However, the effect was too weak to be significant, possibly because the decrease of PS II quantum yield was not sufficiently large. Farage et al. (1991) showed only marginal effects on the maximal quantum yield of photosynthesis of wheat after short term fumigation with high ozone doses.

The further changes of Ft, Fm′, NPQ and ΔF/Fm′ in the leaves at higher PFD might be explained by an increase of energy-dependent quenching, connected with the energization of the thylakoid membrane and zeaxanthin synthesis (Krause and Weis 1991). The contribution of these processes to energy conversion at PS II strongly rises at intermediate PFDs when photosynthesis becomes light saturated (Quick and Stitt 1989; Demmig-Adams 1990). Therefore, the shift of the start of the second phase of NPQ to lower PFD observed in ozone treated leaves may indicate an earlier transition between limitation of photosynthesis by light or by energy utilization in the Calvin cycle.

A major effect of ozone treatment was a lowering of PS II quantum yield (ΔF/Fm′). This reflects a reduction of PS II electron transport and paralleled the reduction of CO_2-fixation observed (Table 1). A remarkable O_3-caused loss of biomass productivity at the whole-tree level was established in both low and high fertilized trees, as a result of the reduced CO_2 assimilation rate, a reduced whole-tree foliage area, and an increased demand of assimilates for repair or detoxification (for details see Maurer and Matyssek 1997). ΔF/Fm′ was reduced by ozone to a lesser extent under light limited conditions (Fig. 2B) and after dark adaptation (Table 1), which indicates rather that a limitation of electron consumption in the Calvin cycle occurred than direct damage to PS II. However, the given decrease in Fv/Fm, especially in the LF treatment, also points to a certain effect on PS II itself. One could argue that the natural daily illumination of the leaves during fumigation caused photoinhibition in the leaves where photosynthesis was partially inhibited. Although we can not exclude photoinhibition, it seems rather unlikely, since incident PFD was experimentally restricted to values below 600 µmol m^{-2} s^{-1} and Fv/Fm was determined from the side of the leaves averted from the light.

Improved mineral nutrition alleviated the damaging effects of ozone (Fig. 2, Table 1) in chlorophyll fluorescence, whereas in control plants no significant influence of fertilization on Fv/Fm, NPQ or ΔF/Fm′ could be detected. The photosynthetic capacity increased with high fertilization and was considerably reduced by ozonation in both fertilization treatments (Table 1). Maurer et al. (1997) found that ozone caused a stronger relative decrease in the CO_2 assimilation rate than in the stomatal conductance, irrespective of the fertilization regime. In the short-term, stomatal narrowing may have directly inhibited the Calvin cycle activity, but this is not evident for long-term fumigation with ozone. The effects of ozone fumigation seen in chlorophyll fluorescence were strongly dependent on the duration of exposure. Ozonated, 6-week-old leaves showed fluorescence characteristics very similar to those of 8-week-old control leaves. (Figs. 2, 3). On the other hand, in leaves exposed only 2 weeks longer to ozone, clear signs of damage appeared (Fig. 2). This damage apparently did not greatly increase during the following 4 weeks (Fig. 3).

In contrast to CO_2 gas exchange measurements where various ozone-induced effects have been reported from annual (Fuhrer et al. 1989) and perennial plants (Matyssek et al. 1995), few data on chlorophyll fluorescence under elevated, low-level ozone concentrations are available. Ruth (1990) and Ruth and Weisel (1993) observed a decline in the Rfd-ratio after ozonation of spruce. However, a lowering of this parameter may reflect either a reduction of maximal fluorescence yield or an inhibition of fluorescence quenching. Therefore, discrimination of damage to PS II from damage to other photosynthetic reactions is not possible. Fumigation with non-environmentally high concentrations (450 nlO_3 $l^{-1)}$ resulted in a decrease of the photosynthetic electron flux as measured by chlorophyll fluorescence (Urbach et al. 1989). Young *Fagus sylvatica* showed no effects after 2 months

of low ozone fumigation but revealed a moderately reduced Fv/Fm after 5 months (Lippert et al. 1996).

In conclusion, our results showed that ozone fumigation with realistic concentrations resulted in a time-dependent manner in a strong inhibition of electron transport through PS II. This was caused by an effect after PS II, most probably by an inhibition of the Calvin cycle activity. It appears that the primary target of ozone is here since intrinsic quantum yield of PS II was affected to a lesser extent.

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References

Allen JF (1992) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098:275–335

Barber J (1983) Membrane conformational changes due to phosphorylation and the control of energy transfer in photosynthesis. Photobiochem Photobiophys 5:181–190

Bilger W, Schreiber U (1986) Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. Photosynth Res 10:303–308

Bolhar-Nordenkampf HR, Long SP, Baker NR, Öquist G, Schreiber U, Lechner EG (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct Ecol 3:497–514

Chow WS, Telfer A, Chapman DJ, Barber J (1981) State 1- state 2 transition in leaves and its association with ATP-induced chlorophyll fluorescence quenching. Biochim Biophys Acta 638:60–68

Dann MS, Pell EJ (1989) Decline of activity and quantity of ribulose bisphosphate caboxylase/oxygenase and net photosynthesis in ozone-treated potato foliage. Plant Physiol 91:427–432

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta 1020:1–24

Demmig-Adams B, Winter K (1988) Characterisation of three components of non-photochemical fluorescence quenching and their response to photoinhibition. Aust J Plant Physiol 15:163–177

Farage PK, Long SP, Lechner E, Baker NR (1991) The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiol 95:529–535

Fink S (1991) Unusual patterns in the distribution of calcium oxalate in spruce needles and their possible relationships to the impact of pollutants. New Phytol 119:41–51

Fuhrer J, Egger A, Lehnherr B, Grandjean A, Tschannen W (1989) Effects of ozone on the yield of spring wheat (*Triticum aestivum* L. cv. Albis) grown in open-top field chambers. Environ Pollut 60:273–289

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Günthardt-Goerg MS, Matyssek R, Scheidegger C, Keller T (1993) Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentration. Trees 7:104–114

Körner C (1991) Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. Funct Ecol 5:162–173

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349

Landolt W, Pfenninger I, Lüthi-Krause B (1989) The effects of ozone and season on the pool size of cyclitols in Scots pine (*Pinus sylvestris*). Trees 3:85–88

Landry LG, Pell EJ (1993) Modification of Rubisco and altered proteolytic activity in O_3-stressed hybrid poplar (*Populus maximowizii trichocarpa*). Plant Physiol 101:1355–1362

Lichtenthaler HK; Wellburn AR (1983) Determination of total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 603:591–592

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

Luwe M, Takahama U, Heber U (1993) Role of ascorbate in detoxifying ozone in the apoplast of spinach *Spinacia oleracea*. Plant Physiol 101:969–976

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

Matyssek R, Günthardt-Goerg MS, Saurer M, Keller T (1992) Seasonal growth, $\delta^{13}C$ in leaves and stem, and phloem structure of birch (*Betula pendula*) under low ozone concentration. Trees 6:69–76

Matyssek R, Reich PB, Oren R, Winner WE (1995) Response mechanisms of conifers to air pollutants. In: Smith WK, Hinkley TM (eds) Ecophysiology of coniferous forests. Academic Press, San Diego, pp 255–308

Maurer S (1995) Einfluss der Nährstoffversorgung auf die Ozonempfindlichkeit der Birke (*Betula pendula*). Inauguraldissertation Universität Basel

Maurer S, Matyssek R (1997) Nutrition and the ozone sensitivity of birch (*Betula pendula*). II. Carbon balance, water-use efficiency and nutritional status of the whole plant. Trees 12:11–20

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

Mikkelsen TN, Ro-Poulsen H (1994) Exposure of Norway spruce to ozone increases the sensitivity of current year needles to photoinhibition and desiccation. New Phytol 128:153–163

Miller PR (1973) Oxidant-induced community change in a mixed conifer forest. Adv Chem Ser 122:101–117

Peterson DL, Arbaugh MJ, Wakefield VA, Miller PR (1987) Evidence of growth reduction in ozone-stressed Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) in Sequoia and Kings Canyon National Parks. J Air Pollut Contr Assoc 37:906–912

Pye JM (1988) Impact of ozone on the growth and yield of trees: a review. J Environ Qual 17:347–360

Quick WP, Stitt M (1989) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim Biophys Acta 977:287–296

Reich PB (1987) Quantifying plant response to ozone: a unifying theory. Tree Physiol 3:63–91

Reiling K, Davison AW (1992) The response of native, herbaceous species to ozone: growth and fluorescence screening. New Phytol 120:29–37

Reiling K, Davison AW (1994) Effects of exposure to ozone at different stages in the development of *Plantago major* L on chlorophyll fluorescence and gas exchange. New Phytol 128:509–514

Ruth B (1990) The fluorescence induction kinetics as a non-destructive tool for investigating spruce treated with ozone. Rad Environ Biophys 29:57–73

Ruth B, Weisel B (1993) investigations on the photosynthetic system of spruce affected by forest decline and ozone fumigation in closed chambers. Environ Pollut 79:31–35

Sasek TW, Richardson CJ (1989) Effects of chronic doses of ozone on loblolly pine: photosynthetic characteristics in the third growing season. For Sci 35:745–755

Schmidt W, Neubauer C, Kolbowski J, Schreiber U, Urbach W (1990) Comparison of effects of air pollutants (SO_2, O_3, NO_2) on intact leaves by measurements of chlorophyll fluorescence and P700 absorbance changes. Photosynth Res 25:241–248

Schreiber U, Vidaver W, Runeckles VC, Rosen P (1978) Chlorophyll fluorescence assay for ozone injury in intact plants. Plant Physiol 61:80–84

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10:51–62

Schweizer B, Arndt U (1990) CO_2/H_2O gas exchange parameters of one- and two-year-old needles of spruce and fir. Environ Pollut 68:275–292

Urbach W, Schmidt W, Kolbowski J, Rümmele E, Reisberg E, Steigner W, Schreiber U (1989) Wirkungen von Umweltschadstoffen auf Photosynthese und Zellmembranen von Pflanzen. *In:* Reuther M, Kirchner M (eds) Statusseminar der PBWU zum Forschungsschwerpunkt Waldschäden. GSF, Munich, pp 195–206

Walters RG, Horton P (1991) Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. Photosynth Res 27:121–133

Walters RG, Horton P (1993) Theoretical assessment of alternative mechanism for non- photochemical quenching of PS 2 fluorescence in barley leaves. Photosynth Res 36:119–139