Mechanisms of Impairment of the Photosynthetic Apparatus in Intact Leaves by Ozone

Thomas Gerhard Reichenauer[a,*] and Harald Romuald Bolhàr-Nordenkampf[b]

[a] Austrian Research Center Seibersdorf, Department of Environmental Research,
A-2444 Seibersdorf, AUSTRIA. Fax: 0043-2254-780-3653.
E-mail: thomas.reichenauer@arcs.ac.at
[b] University of Vienna, Institute of Plant Physiology, Althanstraße 14, A-1090 Vienna, AUSTRIA

* Author for correspondence and reprint requests

Z. Naturforsch. **54c,** 824–829 (1999); received November 28, 1998/April 10, 1999

Light Saturated Net Photosynthesis (A_{sat}), Current Photochemical Capacity (F_v/F_m), Ozone, Oxygen Radicals

Tropospheric ozone has been recognised as a limiting factor for plant growth since late fifties of our century. The decrease in the rate of light saturated net photosynthesis (A_{sat}) was shown to be the major effect of ozone in leaves with negative consequences for plant growth and the development of plant communities. The reasons for the ozone-induced decrease in A_{sat} are still under investigation. Possible mechanisms are an increasing stomatal limitation, an increase in mesophyll limitation including a reduction of the CO_2 fixation in the Calvin cycle and an impairment of the photochemical reactions in the grana membranes of chloroplasts. We conclude from the reviewed literature and from our own experiments that a decrease in carboxylation efficiency (CE) seems to be an early event caused by ozone leading to a decrease in A_{sat}. The loss in current photochemical capacity (F_v/F_m) appears with a lag phase of many days and therefore the loss is thought to be a secondary effect due to a decreased demand of 'assimilatory power'.

Introduction

Tropospheric ozone was first described as a phytotoxic gas by Haagen-Smit (1952). Today it is regarded as one of the most phytotoxic air pollutants with an estimated increase in annual concentration of about 1% (Anfossi and Sandroni, 1994). Ozone has a destructive effect on terrestrial vegetation (for review see Runeckles and Krupa, 1994). One of the first effects of ozone on green leaves is a decrease in the rate of net assimilation of CO_2 under light saturation (A_{sat}) as first described by Todd (1958). Under prolonged ozone exposure, decreases in pigment content of leaves (Reich, 1983; Ballach *et al.,* 1992) and later on necrosis of leaf parts and whole leaves lead to a reduction in photosynthetically active green leaf area and finally to a loss in biomass production (Runeckles and Krupa, 1994). A decrease in A_{sat} caused by ozone could have different reasons (Fig. 1). A decrease in leaf conductance (g_l) due to a closure of stomata, would reduce the supply of CO_2 to mesophyll cells. This would increase the water use efficiency (WUE) calculated according to equation (1):

$$WUE = \frac{A}{E} \qquad (1)$$

where A is the assimilation rate of CO_2 (μmol $m^{-2}s^{-1}$) and E is the transpiration rate of H_2O (mmol $m^{-2}s^{-1}$). Furthermore in case of a limitation of net photosynthesis by a closure of stomata the concentration of intercellular CO_2 (C_i) would decrease leading to less discrimination of carbon isotopes (= a less negative $\delta^{13}C$) by the ribulose bis-phosphat carboxylase/oxygenase (RuBisCO) according to equation (2) proposed by Farquhar *et al.* (1982 and 1989):

$$\delta^{13}C_{plant} = \delta^{13}C_{air} - a - (b-a)(C_i/C_a) \qquad (2)$$

where a is the discrimination due to diffusion via stomata (about 4.4‰), b is the discrimination due to carboxylation (about 27‰), and C_i and C_a are the concentrations of CO_2 inside and outside the leaf.

If the assimilation of CO_2 in the mesophyll cells is not decreased by a limitation of stomatal conductance we would expect an increase in C_i and consequently a decrease in WUE and a more negative $\delta^{13}C$ according to Eqn. (2).

0939–5075/99/0900–0824 $ 06.00

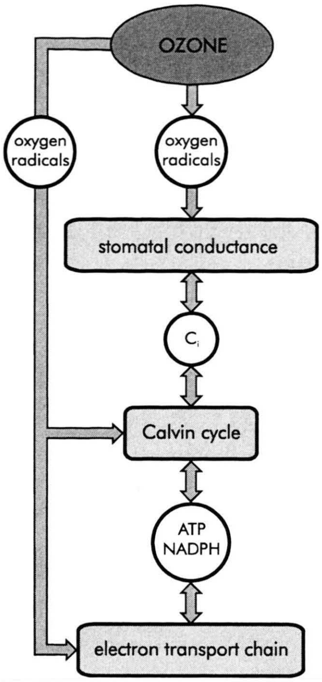

Fig. 1. Possible primary targets (rectangles) for ozone and ozone-generated oxygen radicals in the photosynthetic apparatus causing a decrease in A_{sat} and the relations (circles) of the different parts of the photosynthetic machinery. C_i: CO_2-concentration in the intercellular air space, NADPH: nicotinamide adenine dinucleotide phosphate; further explanations in text.

A decrease in the "mesophyll conductance" again could be caused by an increase in scavenger substances in the cell wall water and the cytoplasm and/or by a reduction in Calvin cycle activity (= carboxylation, the 'dark reaction' of photosynthesis), and/or an impairment of the 'light reaction' (= photochemical reactions in the thylakoid membranes). The activity and amount of RuBisCO can be measured by biochemical methods. A possibility to measure the current carboxylation efficiency (CE) in living attached leaves is the use of A/C_i-curves (Long and Hällgren, 1993). The initial slope of an A/C_i curve is correlated with CE (von Caemmerer and Farquhar, 1981). Information about the 'light reaction' can be gained by measurement of chlorophyll fluorescence after dark adaptation of leaves. The ratio F_v/F_m calculated according to equation (3) is a measure for the current photochemical capacity (= quantum yield of PSII centres) (Bolhàr-Nordenkampf *et al.,* 1989; Bolhàr-Nordenkampf and Öquist, 1993).

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \tag{3}$$

In equation (3) F_0 is the ground fluorescence, F_m is the maximal fluorescence and F_v is the variable fluorescence.

This paper does not intend to give a comprehensive picture of the complete literature about ozone-induced effects on photosynthesis, but tries to give a short review of the possible mechanisms of the well known ozone-induced decrease in the rate of net photosynthesis in whole leaves. The probability of an impairment of (i) stomatal function, (ii) the 'dark reaction' and (iii) the 'light reaction' of photosynthesis caused by ozone, or oxygen radicals generated by ozone will be discussed.

The route of ozone into the leaf and the cell

Ozone reacts with unsaturated hydrocarbons, leading to the production of oxygen radicals *in vitro* (Grimes *et al.,* 1983; Heath, 1987). The cuticle of the leaf consists mostly of saturated hydrocarbons and therefore protects the cells of the epidermis from an ozone attack (Kerstein and Lendzian, 1989). Consequently the stomata are the main route of ozone into the leaf. The ozone concentration in the intercellular air space was shown to be close to zero (Laisk *et al.,* 1989), indicating a fast reaction with chemical compounds of the intercellular air space (Salter and Hewitt, 1992) and the wet internal free surface area of cell walls (Castillo and Creppin, 1988; Chameides, 1989) leading to the generation of oxygen radicals *in vivo* (Mehlhorn *et al.,* 1990; Runeckles and Vaartnou, 1997; Reichenauer *et al.,* 1998). Consequently the amount of oxygen scavengers present in the cell wall water (e.g. ascorbate) and/or produced by the mesophyll cells (gluthatione cycle) is an important factor regarding the ozone sensitivity/resistance of a plant (Lee and Bennett, 1982; Chameides, 1989). Since it is not possible to measure the concentration of ozone in the different cell compartments of a living leaf, it is unclear, how far the ozone molecule itself penetrates into the cell. Due to the short half life of ozone in water it is most likely that the observed decrease in A_{sat} under ozone-exposure is mediated via oxygen radicals which are formed by the reaction of ozone with organic compounds of the cell wall. The reader should be aware of this, when he reads about effects of ozone in the following paragraphs.

Stomatal limitation of photosynthesis?

Contrasting effects of ozone on stomatal conductance among different species have been described (for review see Darrall, 1989). Even cultivars of the same crop species can show distinct changes in the repose of stomatal conductance due to ozone exposure (Gutzy and Heath, 1993). An increasing (Reich and Lassoie, 1984; Barnes *et al.*, 1990; Wallin and Skärby, 1992) or decreasing (Greitner and Winner, 1988; Gutzy and Heath, 1993) g_s was found in ozone-exposed leaves. Thus varying effects of ozone on stomata performance (and stomatal guard cells) are evident from many investigations (for review see Mansfield, 1998). The key question in regard to a possible mechanisms for a decrease in the rate of photosynthesis is, if an observed decrease in stomatal conductance limits heavily the supply of CO_2 to the mesophyll cells and the Calvin cycle (compare with Heath, 1994).

Effects on carbon isotope discrimination ($\delta^{13}C$)

A shift of $\delta^{13}C$ to less negative values has been described as an indicator for exposure of plants to ozone and other air pollutants in woody plants (Martin *et al.*, 1988; Martin and Sutherland, 1990) and clover (Becker *et al.*, 1989).

Greitner and Winner (1988) found only a small shift in $\delta^{13}C$ of +0.3 to +0.7‰ combined with an increase in water use efficiency (WUE) and a decrease in C_i in leaves of radish (*Raphanus sativus* L. cv. Cherrybelle) and soybean (*Glycine max* L. Merr. cv. Williams) exposed to 120 nmol mol^{-1} ozone for 25 days. They concluded that A_{sat} was limited by a decrease in stomatal conductance caused by ozone. Conflicting results were obtained by Saurer *et al.* (1991) who exposed flag leaves of wheat (*Triticum aestivum* L. cv. Albis) to different ozone concentrations in open-top field chambers during the growing season. With increasing ozone concentrations they observed a shift in $\delta^{13}C$ to less negative values, in favour of a stomatal limitation of photosynthesis according to Eqn. (2). At the same time WUE (Eqn. (1)) was decreased, favouring a dominating effect of O_3 on carboxylation in the mesophyll cells. Similarly Matyssek *et al.* (1995) described a less negative $\delta^{13}C$, in parallel to an increase in C_i in birch (*Betula pendula*) exposed to 50, 75 and 100 nmol mol^{-1} ozone throughout one growing season in open-top chambers. The authors of these two studies concluded that the model describing the discrimination of carbon isotopes might not be complete. Other processes like PEP carboxylation, or dark respiration and light respiration might influence the overall fractionation of carbon isotopes (Evans *et al.*, 1986; Farquhar *et al.*, 1989; Farquhar and Richards, 1984). An increase in the content of PEPC was in fact found in pine needles exposed to ozone pointing to enhanced respiration (Lüthy-Krause *et al.*, 1990). In conclusion measuring $\delta^{13}C$ in ozone-exposed leaves seems to produce conflicting results regarding the involvement of stomata in the ozone-induced decrease in A_{sat}.

Gas exchange measurements

In leaves of poplar exposed to 540 nmol mol^{-1} ozone for two hours Furukawa *et al.* (1983) found a decrease in net photosynthesis, whereas transpiration rates remained unchanged. These results were thought to indicate a primary effect of ozone on the mesophyll conductance for CO_2. In contrast, two other poplar species showed a reduction in both the transpiration rate and the rate of net photosynthesis. They concluded that in these species the decrease in net photosynthesis is linked to a decrease in stomatal conductivity. This conclusion is questionable, since g_l is controlled by the rate of net photosynthesis via C_i over a wide range of environmental conditions (Wong, 1985). Thus the transpiration rate could also have been decreased because of a stomatal closure due to an increase in C_i and not the other way round, as was argued by Atkinson (1988). From the exposure of sunflowers (*Helianthus annuus* L.) to 400 nmol mol^{-1} for two hours Furukawa *et al.* (1984) concluded that the observed decrease in stomatal conductivity was only a secondary effect following a decrease in the rate of photosynthesis. After three hours of exposure with 180 nmol mol^{-1} ozone Gupta *et al.* (1991) found a decrease in A_{sat}, but an unchanged g_l in leaves of poplar (*Populus deltoides* × *Populus* cv *caudina*). Our own results demonstrated the long-term effect of ozone on A_{sat} and g_l. In three wheat cultivars exposed to 80 nmol mol^{-1} ozone in a greenhouse, A_{sat} was significantly decreased in soft wheat and durum wheat, whereas stomatal limitation (l) calculated from A/C_i curves according to Jones (1985) remained unchanged (Reichenauer *et al.*, 1998). Thus it seems unlikely that stomatal limitation plays a major role in the ozone-induced decrease of A_{sat}. (Reichenauer *et al.*, 1997; Mansfield, 1998).

"Mesophyll limitation" of photosynthesis?

Schreiber *et al.* (1978) were the first to measure chlorophyll fluorescence in ozone exposed leaves. After 6 hours of exposure with 300 and 500 nmol mol^{-1} ozone they found significant changes in chlorophyll fluorescence parameters. They concluded that ozone caused an impairment of water oxidation in the electron transport chain. Since carboxylation efficiency was not measured the observed effect on the 'light reaction' could also have been a secondary effect due to a depression of CO_2-assimilation in the Calvin cycle.

Only a few studies were performed where the effects of ozone on 'dark reaction' and 'light reaction' of photosynthesis were investigated simultaneously:

Wheat exposed to charcoal filtered air, unfiltered air and ozone enriched air (100 nmol mol^{-1} for 8 h d^{-1}) in open-top chambers showed a decrease in A_{sat} with increasing ozone concentration. This was not the result of changes in g_l, or synthesis of Ribulose Bis-Phosphate (RuBP), but due to a reduced amount of RuBisCO present in the ozone-exposed leaves (Lehnherr *et al.*, 1987). Farage *et al.* (1991) measured CE (by A/C_i-curves), oxygen evolution, F_v/F_m and the amount of D1 protein of PSII. After exposure of wheat (*Triticum auestivum* L. cv. Avalon) to 200 and 400 nmol mol^{-1} ozone for 16 h they found a decrease in A_{sat} that was only to a minor part due to stomatal limitation. The current photochemical capacity (F_v/F_m) was only significantly decreased under the highest ozone concentration after 16 h and no change in the amount of the D1 protein could be detected, indicating that changes in the carboxylation efficiency are early in the response to ozone and therefore are thought to be the primary cause for the decrease in rate of net photosynthesis. Our own investigations demonstrated the long-term effect of ozone on the photosynthetic machinery in leaves of *Populus nigra* (clone T107) exposed to ambient and elevated ozone concentrations in open-top chambers (Reichenauer *et al.*, 1997). It could be shown that the significant decrease in A_{sat} was not due to a stomatal limitation whereas CE calculated from A/C_i curves was reduced significantly already in young leaves exposed to ambient ozone concentrations. In contrast F_v/F_m stayed unchanged compared to control leaves in charcoal filtered air throughout the experiment. Under artificially elevated ozone concentrations (ambient + 50 nmol mol^{-1} ozone for 8 h d^{-1}) F_v/F_m decreased only in older leaves, when CE has decreased already by about 60% (Fig. 2).

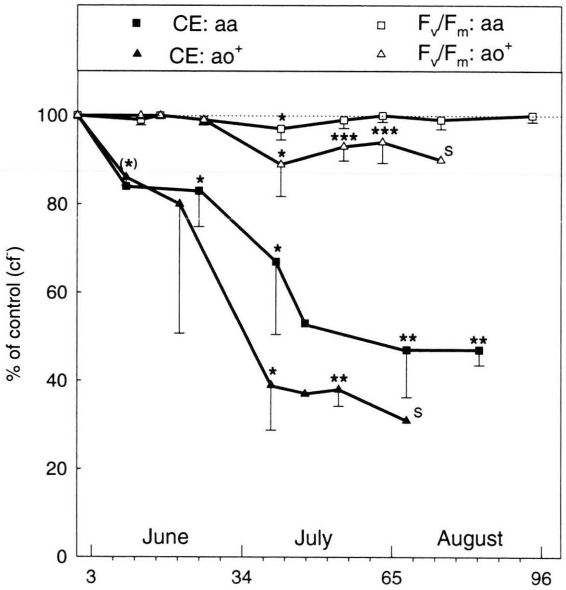

Fig. 2. Current photochemical capacity of PSII (F_v/F_m) and carboxylation efficiency (CE) in leaves of *Populus nigra* exposed to ozone. aa: ambient air, ao^+: ambient air + 50 nmol mol^{-1} ozone, cf $^-$: charcoal filtered air, single leaf. Significant differences between mean values are indicated: (*) $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (ANOVA); $n = 3$ (CE); $n = 7-12$ (F_v/F_m); error bars = standard deviation. (From Reichenauer *et al.*, 1997).

An early effect of ozone on the 'dark reaction' of photosynthesis is supported by measurements of the amount and activity of RuBisCO in ozone-exposed leaves (for review see Pell *et al.*, 1994). Ozone was shown to reduce the activity and the concentration of RuBisCO. The amount of rbcS mRNA (for the small subunit of RuBisCO), which is encoded in the nucleus is reduced by ozone, whereas the rbcL mRNA (for the large subunit of RuBisCO) shows much less sensitivity. Nie *et al.* (1993) exposed wheat (cv. Avalon) to 150 nmol mol^{-1} ozone (7 h d^{-1}) and measured A_{sat}, F_v/F_m and RuBisCO concentration among other parameters. They found a decrease in A_{sat} and concentration of RuBisCO, without a change in F_v/F_m and concluded that ozone acts by inducing a loss in RuBisCO.

Conclusion

In most plant species exposed to moderate concentrations of ozone, stomatal limitation seems not to be the cause for an observed decrease in A_{sat}. An impairment of the carboxylation efficiency caused by a decrease in the conductivity for CO_2 from intercellular spaces via the plasmalemma and the cytoplasm to the chloroplast and/or a reduced activity and concentration of the RuBisCO appears to be a primary effect of ozone on photosynthesis. Effects on the electron transport in the grana membranes are regarded as secondary ones, caused by a consistent high ΔpH across the thylakoid membrane due to a decreased demand of ATP and $NADPH^+$ in the Calvin cycle.

Anfossi D. and Sandroni S. (1994), Surface ozone at mid latitudes in the past century. Il nuovo cimento **17C,** 199–208.

Atkinson C. J., Robe S. V. and Winner W. E. (1988), The relationship between changes in photosynthesis and growth for radish plants fumigated with SO_2 and O_3. New Phytol. **110,** 173–184.

Ballach H.-J., Mooi J. and Wittig R. (1992), Premature aging in *Populus nigra* L. after exposure to air pollutants. Angew. Bot. **66,** 14–20.

Barnes J. D., Eamus D., Davison A. W., Ro-Poulsen H. and Mortensen L. (1990), Persistent effects of ozone on needle water loss and wettability in Norway spruce. Environ. Poll. **63,** 345–363.

Becker K., Sauerer M., Egger A. and Fuhrer J. (1989), Sensitivity of white clover to ambient ozone in Switzerland. New Phytol. **112,** 235–243.

Bolhàr-Nordenkampf H. R. and Lechner E. G. (1989), Synopse streßbedingter Modifikationen der Anatomie und Physiologie von Nadeln als Frühdiagnose einer Disposition zur Schadensentwicklung bei Fichte. Phyton (Austria) **29,** 255–301.

Bolhàr-Nordenkampf H. R. and Öquist G. (1993), Chlorophyll Fluorescence as a Tool in Photosynthesis Research, (D. O. Hall, J. M. O. Scurlock, H. R. Bolhàr-Nordenkampf, R. C. Leegood, and S. P. Long, eds.), chap. 12, Chapman & Hall, London, 193–206.

Castillo F. J. and Greppin H. (1988), Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone esposure. Environ. Exp. Bot. **28,** 231–238.

Chameides W. L. (1989), The chemistry of ozone deposition to plant leaves: Role of ascorbic acid. Environ.Sci.Technol. **23,** 595–600.

Darrall N. M. (1989), The effect of air pollutions on physiological processes in plants. Plant Cell Environ. **12,** 1–30.

Evans J. R., Sharkey T. D., Berry J. A. and Farquhar G. D. (1986), Carbon isotope discrimination measured concrrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust. J. Plant Physiol. **13,** 292.

Farage P. K., Long S. P., Lechner E. G. and Baker N. R. (1991), Sequence of changes within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiol. **95,** 529–535.

Farquhar G. D. and Sharkey T. D. (1982), Stomatal conductance and photosynthesis. Ann. Rev.Plant Physiol. **33,** 317–345.

Farquhar G. D., Ehleringer J. R. and Hubick K. T. (1989), Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. **33,** 317–345.

Furukawa A., Katase M., Ushijima T. and Totsuka T. (1983), Inhibition of photosynthesis of poplar species and sunflower by O_3. Res. Rep. Natl. Inst. Environ. Stud. **65,** 77–86.

Furukawa A., Masaaki Y., Ushijima T. and Totsuka T. (1984), The effects of NO_2 and/or O_3 on photosynthesis of sunflower leaves. Res. Rep. Natl. Inst. Environ. Stud., Japan. **65,** 89–97.

Greitner C. S. and Winner W. E. (1988), Increases in $\delta^{13}C$ values of radish and soybean plants caused by ozone. New Phytol. **108,** 489–494.

Grimes H. D., Perkins K. K. and Boss W. F. (1983), Ozone degrades into hydroxyl radical under physiological conditions. A spin trapping study. Plant Physiol. **72,** 1016–1020.

Gupta A. S., Alscher R. G. and McCune D. (1991), Response of photosynthesis and cellular antioxidants to ozone in *Populus* leaves. Plant Physiol. **96,** 650–655.

Guzy M. R. and Heath R. L. (1993), Responses to ozone of varieties of common bean (*Phaseolus vulgaris* L.). New Phytol. **124,** 614–625.

Haagen-Smit A. (1952), Chemistry and physiology of Los Angeles smog. Ind. Engin. Chem. **44,** 1342–1346.

Heath R. L. (1987), The biochemistry of ozone attack on the plasma membrane of plant cells. Rec. Adv. Photochem. **21,** 29–54.

Heath R. L. (1994), Possible mechanisms for the inhibition of photosynthesis by ozone. Photosynth. Res. **39,** 439–451.

Jones H. G. (1985), Partitioning stomatal and non-stomatal limitations to photosynthesis. Plant, Cell Environ. **8,** 95–104.

Kersteins G. and Lendzian K. J. (1989), Interactions between ozone and plant cuticles. II. water permeability. New Phytol. **112,** 21–27.

Laisk A., Kull O. and Moldau H. (1989), Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiol. **90,** 1163–1167.

Lee E. H. and Bennett J. H. (1982), Superoxide dismutase. A possible protective enzyme against ozone injury in snap beans (*Phaseolus vulgaris* L.). Plant Physiol. **69,** 1444–1449.

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

Long S. P. and Hällgren J.-E. (1993), Measurement of CO_2 Assimilation by Plants in the Field and Laboratory, in: Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual (D. O. Hall, J. M. O. Scurlock, H. R. Bolhàr-Nordenkampf, R. C. Leegood, and S. P. Long, eds.), chap. 9, Chapman & Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras, 129–167.

Lüthy-Krause B., Pfenninger I. and Landolt W. (1990), Effects of ozone on organic acids in needles of Norway spruce and Scots pine. Trees **4,** 198–204.

Mansfield T. A. (1998), Stomata and plant water relations: does air pollution create problems? Environ. Poll. **101,** 1–11.

Martin B., Bytnerowicz A. and Thorstenson Y. R. (1988), Effects of air pollutants on the composition of stable carbon isotopes, $\delta^{13}C$, of leaves and wood, and on leaf injury. Plant Physiol. **88,** 218–223.

Martin B. and Sutherland E. K. (1990), Air pollution in the past recorded in width and stable carbon isotope composition of annual growth rings of Douglas-fir. Plant Cell Environ. **13,** 839–844.

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

Mehlhorn H., Tabner B. J. and Wellburn A. R. (1990), Electron-spin resonance evidence for the formation of free radicals in plants exposed to ozone. Physiol. Plan. **79,** 377–383.

Nie G.-Y., Tomasevic M. and Baker N. R. (1993), Effects of ozone one the photosynthetic apparatus and leaf proteins during leaf development in wheat. Plant Cell Environ. **16,** 643–651.

Pell E. J., Eckardt N. A. and Glick R. E. (1994), Biomolecular and molecular basis for impairment of photosynthetic potential. Photosynth. Res. **39,** 453–462.

Reich P. B. (1983), Effects of low concentrations of O_3 on net photosynthesis, dark respiration, and chloropyhll contents in aging hybrid poplar leaves. Plant Physiol. **73,** 291–296.

Reich P. B. and Lassoie J. P. (1984), Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant Cell Environ. **7,** 661–668.

Reichenauer T. G., Bolhàr-Nordenkampf H. R., Ehrlich U., Soja G., Postl W. F. and Halbwachs F. (1997), The influence of ambient and elevated ozone concentrations on photosynthesis in *Populus nigra*. Plant Cell Environ. **20,** 1061–1069.

Reichenauer T. G., Goodman B. A., Kostecki P. and Soja G. (1998), Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury, photosynthetic activity and free radical content. Physiol. Plant. **104,** 681–686.

Runeckles V. C. and Krupa S. V. (1994), The impact of UV-B radiation and ozone on terrestrial vegetation. Environ. Poll. **83,** 191–213.

Runeckles V. C. and Vaartnou M. (1997), EPR evidence for suproxide anion formation in leaves during exposure to low levels of ozone. Plant Cell Environ. **20,** 306–314.

Salter L. and Hewitt C. N. (1992), Ozone-hydrocarbon interactions in plants. Phytochemistry **31,** 4045–4050.

Saurer M., Fuhrer J. and Siegenthaler U. (1991), Influence of ozone on the stable carbon isotope composition, $\delta^{13}C$, of leaves and grain of spring wheat (*Triticum aestivum* L.). Plant Physiol. **97,** 313–316.

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

Todd G. W. (1958), Effect of ozone and ozonated 1-hexene on respiration and photosynthesis of leaves. Plant Physiol. **33,** 416–420.

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

Wallin G. and Skärby L. (1992), The influence of ozone on the stomatal and non-stomatal limitations of photosynthesis in Norway spruce, *Picea abies* (L.) Karst, exposed to soil moisture deficit. Trees **6,** 128–136.