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

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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 (\(A_{\text{sat}}\)) 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 \(A_{\text{sat}}\) 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 \(A_{\text{sat}}\). The loss in current photochemical capacity (\(F_{\text{J}}/F_{\text{m}}\)) 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’.

\textbf{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 (\(A_{\text{sat}}\)) 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 \(A_{\text{sat}}\) caused by ozone could have different reasons (Fig. 1). A decrease in leaf conductance (\(g_1\)) 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):

\[
WUE = \frac{A}{E}
\]

where \(A\) is the assimilation rate of \(CO_2\) (\(\mu\)mol m\(^{-2}\)s\(^{-1}\)) and \(E\) is the transpiration rate of \(H_2O\) (\(\mu\)mol 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):

\[
\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{air}} - a - (b-a)(C_i/C_a)
\]

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).
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 Hallgren, 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) (Bolhär-Nordenkampf et al., 1989; Bolhär-Nordenkampf and Öquist, 1993).

$$\frac{F_{v}}{F_{m}} = \frac{F_{m} - F_{0}}{F_{m}}$$

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%o 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. (1). 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 $g_s$ 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 $g_i$ in leaves of poplar (Populus deltoides × Populus cv. caudina). Our own results de-
monitored the long-term effect of ozone on $A_{sat}$ and $g_i$. 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 $g_i$, or synthesis of Ribulose Bis-Phosphate (RuBP), but due to a reduced amount of RuBiSCO present in the ozone-exposed leaves (Lehnerr 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 aestivum* 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 thereby 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: (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (ANOVA); $n = 3$ (CE); $n = 7–12$ ($F_v/F_m$); error bars = standard deviation. (From Reichenauer et al., 1997).](image-url)

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 plasma-lemma 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 causes, caused by a consistent high \(\Delta p\)H across the thylakoid membrane due to a decreased demand of ATP and NADPH\(^+\) in the Calvin cycle.