Linking detrimental effect of O_3 on photosynthesis to its uptake into the leaf and resulting damage of mesophyll membranes

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Numerous studies over the past 40 years have improved understanding of biochemical and molecular effects of O_3 on photosynthetic gas exchange. But often it is not possible to determine from the measurements made whether the decline in electron transport (ETR) is due to stomatal closure limiting CO_2 supply or damage of photosynthetic apparatus. Our attempt was to clear up questions on the probable mechanisms of inhibition of photosynthesis and in what parts of the photosynthetic machinery are the damaged targets situated in O_3 exposed leaves.

Leaves of French bean plants were subjected to 3h ozone exposures in differend light and CO_2 conditions. Leaf gas exchange (CO_2, H_2O, O_3) and chlorophyll fluorescence where measured simultaneously. Cell damage was assessed by Evans Blue. Different photosynthetic parameters were related to ozone uptake into the leaf, absorbed ozone dose, and to the percent of dye-permeable cells.

Our results showed:

- No stomatal and photosynthetic response occurred before the O_3 uptake rate exceeded a certain threshold value; at higher rates blue cells appeared. Once started, the number of blue cells continued to increase even after the cessation of O_3.
- Not the ambient O_3 concentration, nor the total absorbed O_3 dose, but an O_3 flux rate of about 36 nmol m^{-2} s^{-1} entering the leaf through the stomata was the critical threshold to induce cell damage and inhibition of photosynthetic parameters.
- Mesophyll conductance (g_m) for CO_2 was a more conservative parameter than stomatal conductance, showing less decrease upon O_3 exposure.
- In order to exclude the possible influence of light, ozonation was performed in the dark (in low CO_2 to keep stomata open). In the dark, the O_3-induced damage progressively developed through the mesophyll from the lower to the upper leaf side. Despite of high O_3 concentrations used, F_m and relative rate constants for photochemistry in open PSII (k_{p0}) were not influenced.
- When the experiment was repeated in the light, the O_3-induced damage was most intense in the palisade parenchyma. Such light dependence suggested that free radicals produced by excess light in chloroplasts might contribute to damage of chloroplast membranes.
- In order to avoid stomatal control in the light, leaves were exposed to O_3 at the photosynthetic CO_2 compensation point. After 3h-exposure O_2 evolution, ETR and F_m' were similar to controls even in leaves with clearly injured plasmalemma. These parameters decreased only in leaves where not only the cell mebrane, but also chloroplast membranes was damaged. This implies that a large part of observed changes in photosynthetic parameters may be due to stomatal closure. O_3 directly influences photosynthesis only after the chloroplast membrane becomes damaged by free radicals.