Reducing power dependent on metabolic changes could improve the determination of ozone risk threshold for higher plants

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Introduction

Tropospheric ozone (O_3) is now considered as the major air pollutant affecting vegetation, causing reduction in photosynthesis, growth and yield. Initially, the critical level of ozone exposure associated to biomass losses was based on the seasonal sum of the external concentrations of the pollutant above 40 nL.L^{-1} (AOT 40). To improve the determination of ozone risk assessment for plants a concept of effective ozone flux has been proposed. This concept takes into account the ozone flux in the leaf through stomata and the internal defense capacity of the foliar tissues. We propose to also consider some parameters linked to the generation/ state of the reducing power, essential to sustain the functioning of the detoxifying systems. In this report, we present preliminary results (NAD(P)H pool and PEPc activity) got on poplar leaves exposed to chronic ozone fumigations.

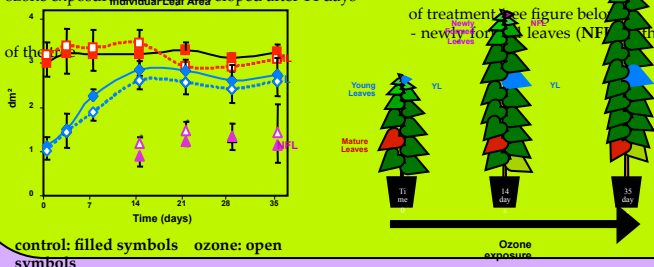
Plant material and experimental

- Micropropagated six-month-old poplar trees (*Populus tremula* L. x *alba* L., INRA 717-1-B4 clone) grown in controlled conditions in 8 fumigation chambers with charcoal-filtered air (control, *n*=48) or with charcoal-filtered air + 100 to 120 ppb of ozone 13 h daily during 35 days (*n*=48).

- Leaf stages: - mature leaves (**ML**), fully developed at day 0

- young leaves (**YL**), expanding during ozone exposure and fully developed after 14 days of treatment (see figure below)

- newly formed leaves (**NFL**) at the apex of the tree

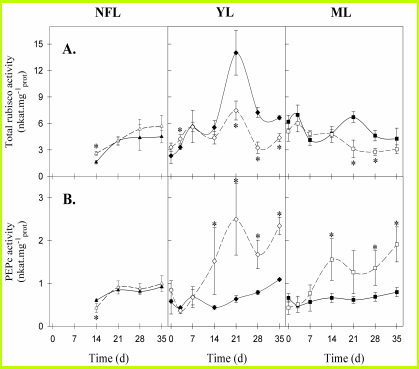


control: filled symbols ozone: open symbols

Results

Carboxylase activities

- In vitro determination of rubisco and PEPc activities was determined by spectrophotometric assays



* Significative difference between ozone-treated and control samples ($p < .05$)

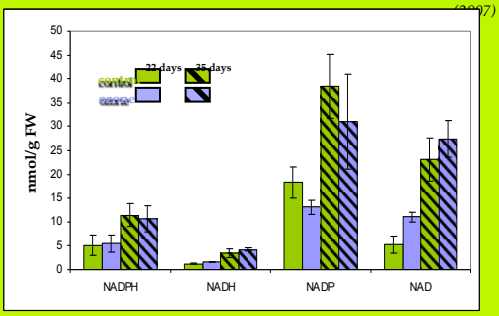
control: filled symbols ozone: open symbols

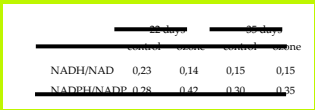
►In ML and YL, total rubisco activity was hugely inhibited (by up to 40%) from 14 days of ozone exposure

►On the opposite, in response to ozone fumigation, PEPc activity was stimulated 2- to 3-fold in ML and YL. This stimulation occurred as soon as the second week of fumigation

Pyridine nucleotide pool in YL leaves

- Pyridine nucleotide amounts were determined according Queval and Noctor, Analyt. Biochem, 363 (2007) 58-69





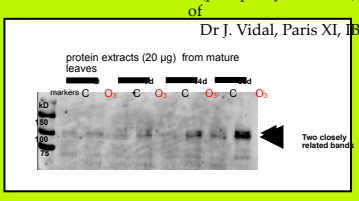
►In YL, the pyridine nucleotide pool increased as the leaf gets older

Poor changes occured as a response to ozone treatment: the NAD amounts increased at least after 22 days of ozone fumigation

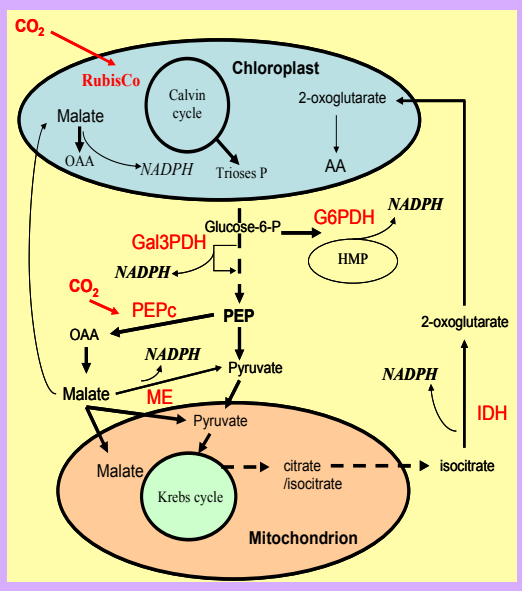
►The changes in the reduced on oxidized ratio were mainly the consequence of a modification in the amounts of the oxidized forms (NAD or NADP).

PEPc amount

- Western blot was performed with anti-N-terminal (phosphorylation site) peptide antibodies (gift of Dr J. Vidal, Paris XI, IBP, Orsay)



►In ML, two PEPc isoforms (≈ 110 kD) were detected. In response to ozone, the amount of the isoforms increased



Conclusion

As a consequence to chronic ozone fumigation, the PEPc activity was increased in poplar leaves. This effect, considered as an usual reaction of the carbon metabolism in response to ozone, should be at least due to a higher amount of the protein.

The reaction catalyzed by PEPc allows the subsequent formation of malate (cf schema). Malate is know to play a central role in plant cell metabolism, particularly to provide NAD(P)H in different cell compartments. Thus, we suggest that the containment of the NAD(P)H pool could be partly explained by the stimulation of the PEPc activity. However preliminary experiments also showed that the activity of other enzymes susceptible to provide NADPH were also stimulated.

Actually additional investigations are performed to valid the occurrence of these enzymes to sustain the detoxication processes.