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Ozone effects on primary metabolism in three poplar genotypes differing in sensitivity

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Introduction: Tropospheric ozone acts as a phytotoxin which produces an oxidative stress in plants. Its concentration increases and represents a significant threat as it leads to foliar injuries, decreased photosynthesis, reduced biomass and thus, important economic losses in agriculture and forestry. Differences of sensitivity to ozone have been observed between species but also between genotypes. We studied the effect of ozone on primary metabolism of three Euramerican poplar genotypes (*Populus deltoides x nigra*): Carpaccio, Cima and Robusta.

Material and Methods:

The plant material was grown from cuttings in growth chambers for 5 weeks at 23/21°C (14/10h day/night).

They were transferred in 8 phytotronic chambers (4 for each treatments) for 1 week of acclimation with the same environmental conditions before to start the treatments: Control (charcoal-filtered air) and elevated ozone (120ppb for 13h each day).

The 10th and 11th leaves from the top marked at the beginning of the experiment were collected in 4 replicates always at 15h after 2, 4, 11, 15 and 17 days of treatment. Chemical analysis were performed by GC/MS from freeze-dried samples extracted in MeOH.



Fig 1: Visible ozone-injuries on poplar leaves after 17 days compared to control (Dumont *et al.*, 2014)

Genotypes	Chlorophyll content	Stomatal conductance	Net CO ₂ assimilation	Total biomass
Carpaccio	-55%	-57%	-55%	-20%
Cima	-49%	-54%	-70%	-41%
Robusta	-49%	-34%	-51%	-18%

Table 1: Effect of ozone after 17 days in 3 poplar genotypes on chlorophyll content, stomatal conductance and net CO₂ assimilation in saturating light conditions and total biomass, expressed as % relative to control

Ozone symptoms:

- Ozone led to visible injuries (Fig 1) and increased leaf fall (Dghim *et al.*, 2013), especially in Robusta
- Ozone induced a decrease of chlorophyll content, stomatal conductance, photosynthesis and biomass, especially in Cima (Tab. 1)
- Based on those parameters and other parameters not shown, **Carpaccio has been characterized as the most resistant genotype**

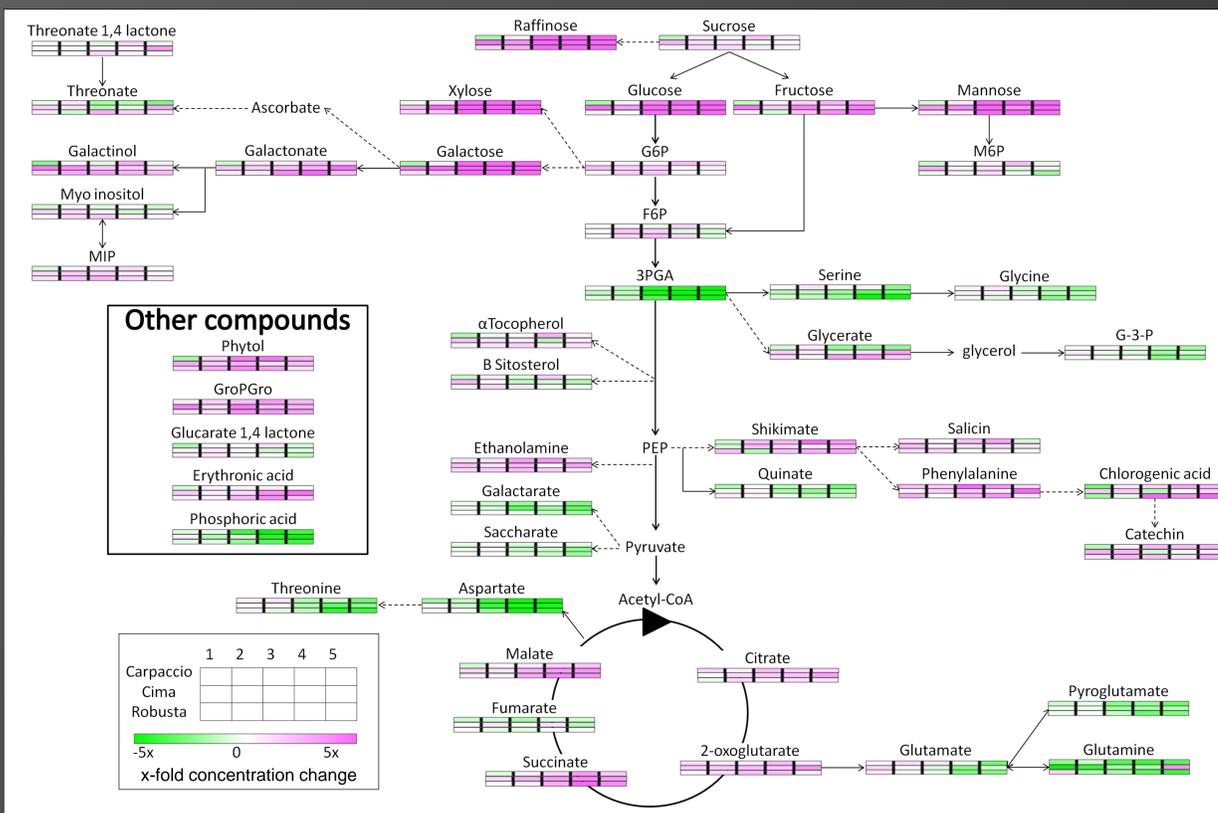


Fig 2: Heat maps placed on metabolic pathways when possible, representing the effects of ozone on primary metabolites in Carpaccio, Cima and Robusta at five sampling dates (1, 2, 3, 4 and 5 corresponding to 2, 4, 11, 15 and 17 days, respectively)

Results :

- Ozone increased strongly the sugars concentrations in leaves especially Glucose, Mannose, Raffinose, Xylose and Galactose (Fig 2)
- Ozone induced a global increase of the shikimate pathway involved in phenolic compounds metabolism and of the metabolites involved in the TCA cycle (Fig 2)
- Most of the amino acids were decreased by ozone except those supporting the detoxification process (Fig 2)
- The ozone effects appeared earlier in Cima than in the resistant genotype, Carpaccio (Fig 3)
- The increase in sugars was the strongest in the resistant genotype, Carpaccio (Fig 4)
- Carpaccio was characterized by higher constitutive sugar concentrations than the other genotypes (Fig 4)

References:

Dumont *et al.*, (2014) Ozone affects ascorbate and glutathione biosynthesis as well as amino acid contents in three Euramerican poplar genotypes. *Tree Physiology* 34, 253-266.
Dghim *et al.*, (2013) Capacity for NADPH regeneration in the leaves of two poplar genotypes differing in ozone sensitivity. *Physiologia Plantarum* 148, 36-50.

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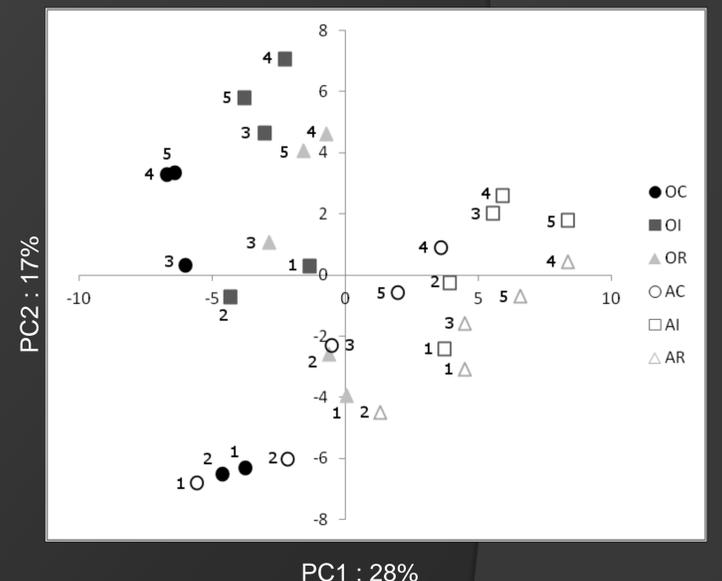


Fig 3: Principal Component Analysis separating the two treatments (O: Ozone and A: Control) and the three genotypes (C: Carpaccio, I: Cima and R: Robusta). Each dot is the centered value of the 4 replicates. 1, 2, 3, 4 and 5 corresponding to 2, 4, 11, 15 and 17 days, respectively

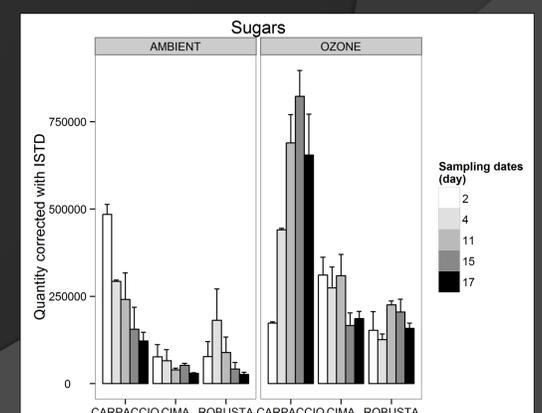


Fig 4: Sugar content (sum of fructose, galactose, glucose, mannose, raffinose and xylose) in three poplar genotypes exposed to control or ozone treatment at day 2, 4, 11, 15, 17 (mean ± SE). Artificial values corrected with sample dry weight and peak area of internal Standard (Glycerol-d8).