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Ozone effects on stomatal responses to environmental parameters (blue light, red light, CO₂ and VPD) in three poplar genotypes and the study of gene expression on guard cells

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Ozone effects on stomatal responses to environmental parameters (blue light, red light, CO₂ and VPD) in three poplar genotypes and the study of gene expression on guard cells.

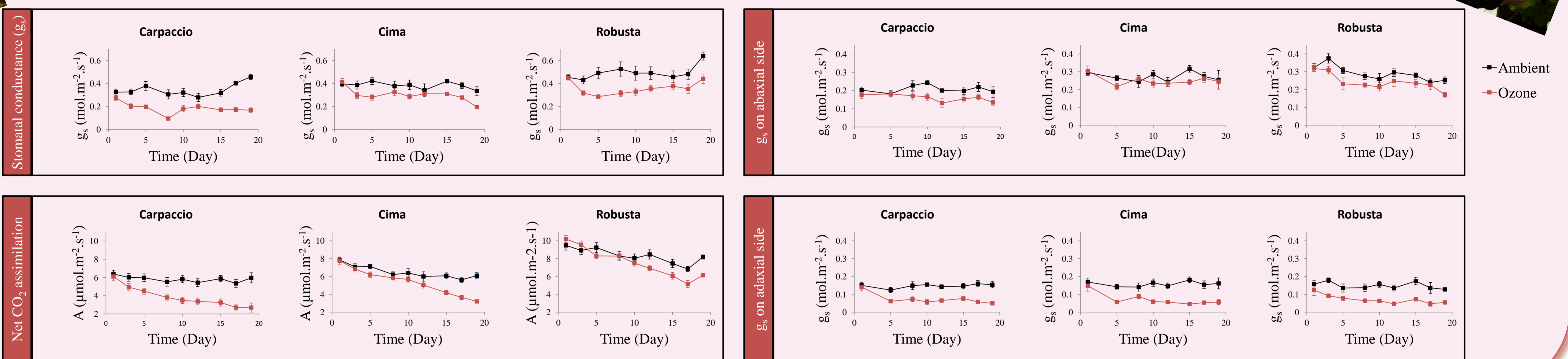
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Introduction : Tropospheric ozone acts as a phytotoxin which produces an oxidative stress in plants. Its concentration increases and represents a significant threat. Two ways of defense are used by plants against ozone (O₃). Ozone enters in the leaf by stomata and is degraded in Reactive Oxygen species (ROS). Stomatal conductance can regulate the ozone flux like a mechanical defense, preventing from an excessive ozone flux. In a second time, there is a biochemical defense corresponding in a complex induced detoxification system. The stomatal conductance is important to regulate the gas exchanges, in function to environmental conditions, but ozone could lead to a stomatal sluggishness (Paoletti and Grulke, 2010).

Objectives: This study try to understand what is exactly the effect of ozone on stomatal functioning and how ozone can alter the stomatal movement leading to sluggish responses.

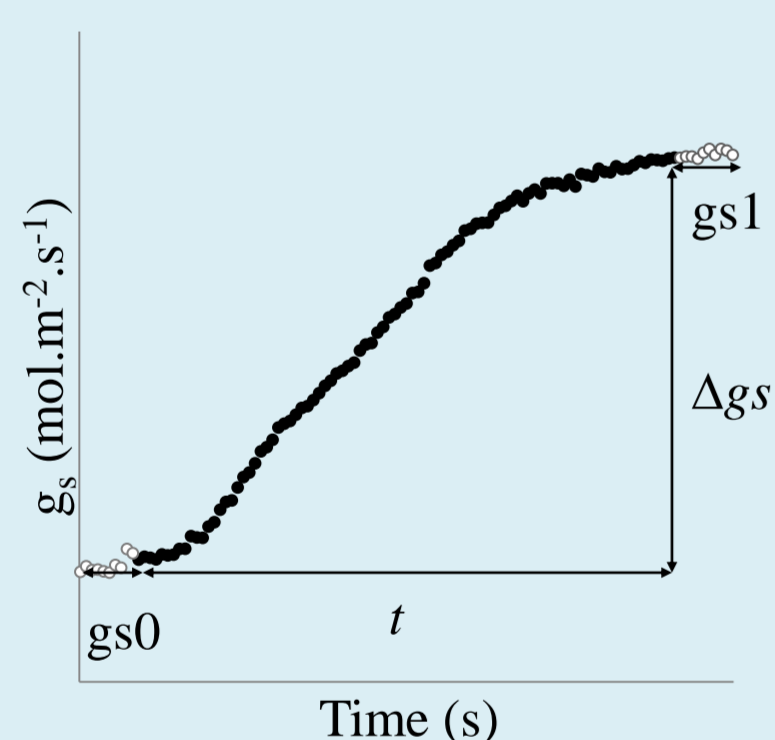
Materials : 3 euramerican poplar genotypes (Carpaccio, Cima and Robusta) were submitted to control (air charcoal filtered air) or ozone fumigation (120nmol.mol⁻¹ 13h/day) during three weeks, in phytotronic chambers.

Effect of ozone on gas exchange



How O₃ impacts the regulation of stomatal conductance to main environmental parameters ?

• We studied, with three intercalibrated Li-6400, the effects of ozone on the stomatal conductance (g_s) response to increase of a **unique** parameter (blue light, red light, CO₂ or VPD), focusing on the amplitude (Δg_s) and the speed of reactions (v = Δg_s/t).



Environmental parameters\	Blue light	Red light	VPD	CO ₂
CO ₂ (μmol CO ₂ . mol ⁻¹)	Initial 400 ± 2.0	400 ± 3.0	100 ± 1.0	400 ± 3.0
Final	400 ± 2.0	400 ± 3.0	100 ± 1.0	1200 ± 3.0
Temperature (°C)	Initial 25 ± 0.3	25 ± 0.3	25 ± 0.3	25 ± 0.4
Final	25 ± 0.3	25 ± 0.3	25 ± 0.3	25 ± 0.4
Light red/blue (μmol.m ⁻² .s ⁻¹)	Initial 0/0	200/30 ± 5.0	0/0	800/30 ± 5.0
Final	0/30 ± 1	800/30 ± 5.0	0/0	800/30 ± 5.0
VPD (kPa)	Initial 0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Final	0.8 ± 0.2	0.8 ± 0.2	3.0 ± 0.3	0.8 ± 0.2

Results : Blue light : decrease of Δg_s and v VPD : decrease of v
Red light : decrease of v CO₂ : decrease of v

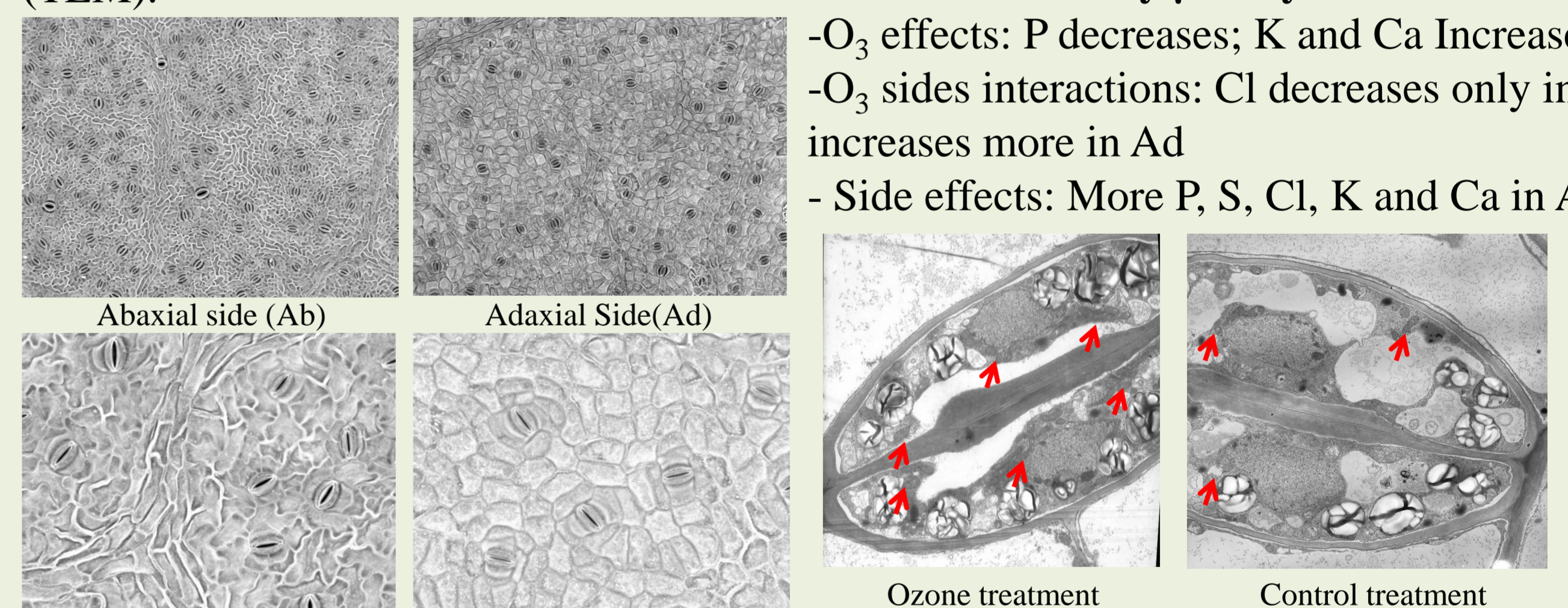
We also observed a decrease of photosynthesis and an increase of respiration

How to explain the difference between sides?

• We studied with Scanning Electron Microscopy (SEM) the stomatal density, the size of stomata and by X-ray analyses, the element contents of guard cells. We completed this with observation of ultrastructure of guard cells on each side by transmission electron microscopy (TEM).

X-Ray analyses results:

- O₃ effects: P decreases; K and Ca Increase
- O₃ sides interactions: Cl decreases only in Ab, Ca increases more in Ad
- Side effects: More P, S, Cl, K and Ca in Ab

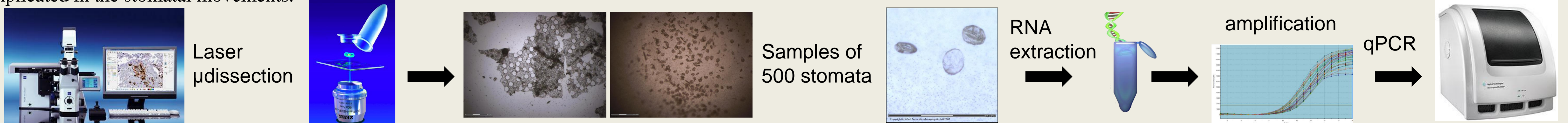


Ad:40% of stomata and Ab:60%
no difference of size

Ozone treatment Control treatment
no difference between sides,
increase of number of mitochondria (↗)with ozone

Impact of ozone on gene expression of channels implicated in stomatal movements

• We microdissected stomata of the two faces of leaves separately by laser microdissection, and we extracted RNA. After an amplification, we performed qPCR analyses on 46 genes of channels implicated in the stomatal movements.



Conclusion

- Stomatal conductance of the adaxial side of the leaf decreases more and faster than the other side with ozone treatment.
- Ozone leads to sluggish stomatal movements in response to variation of blue light, red light, CO₂ and VPD characterized by slower reactions and/or lower amplitude of response. This sluggishness must be taken into account in stomatal conductance models used for the Phytotoxic Ozone Dose calculation (Jarvis, 1976 and Emberson *et al.* 2000).
- The number of mitochondria increases with the ozone treatment inside the guard cells.

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