Improving the determination of the effective ozone flux in tree leaves for issuing a sub-model to be integrated in models predicting ozone risks to forest ecosystems

Pierre P. Dizengremel, Yves Y. Jolivet, Didier Le Thiec

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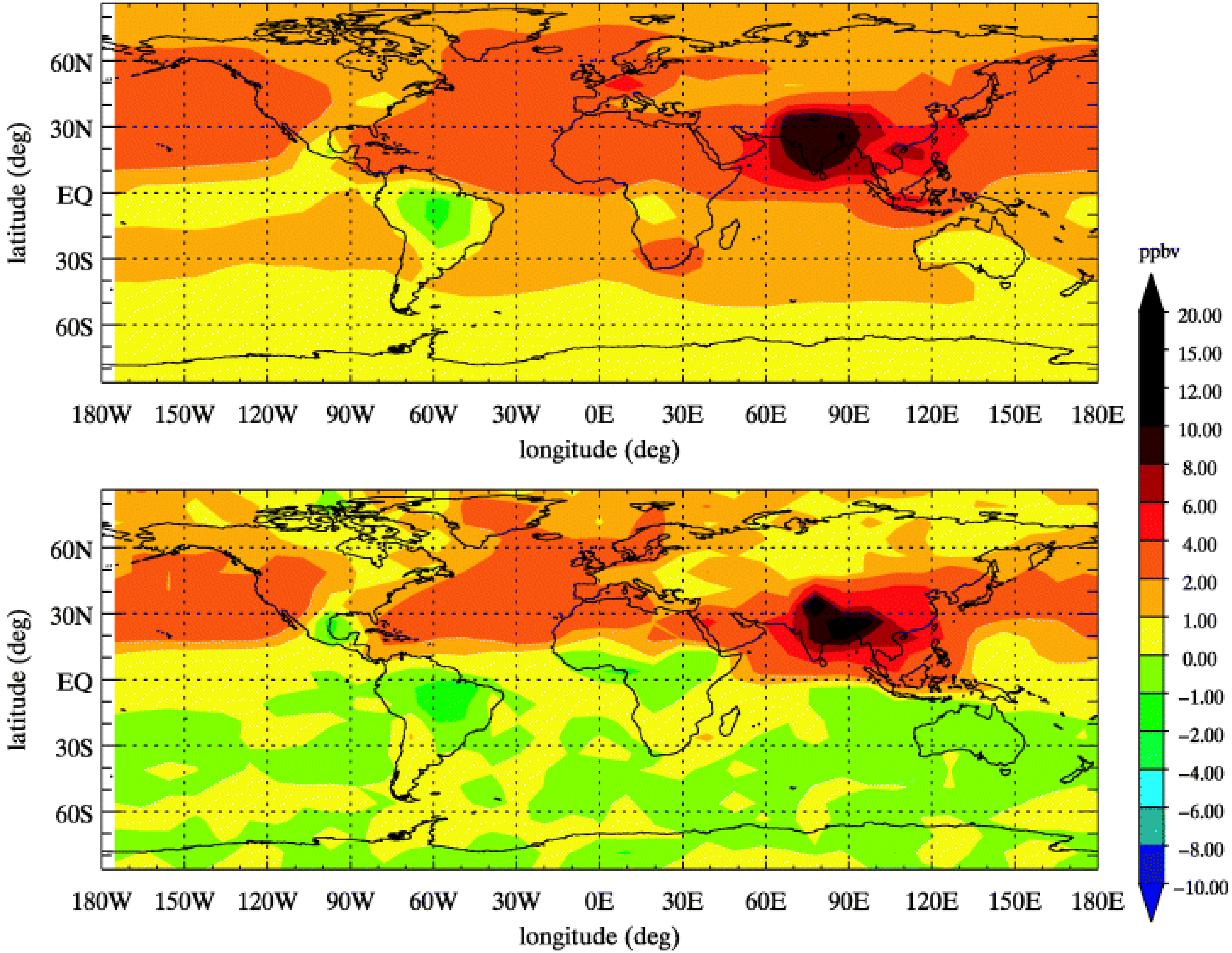
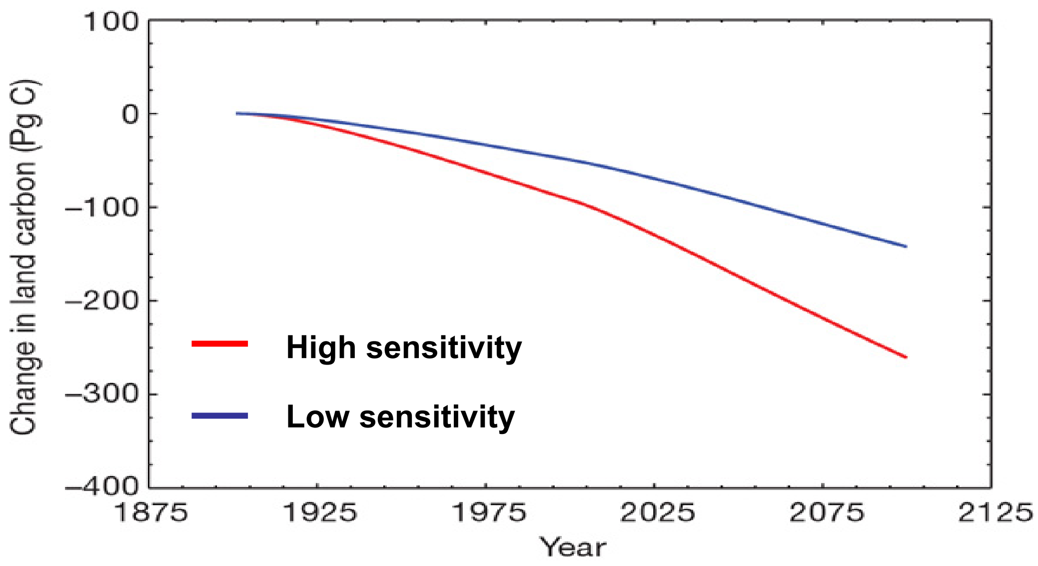
COST FP0903, Rome, October 2010

The concentration of OZONE, greenhouse gas, is expected to increase in the near future



Predicted differences in decadal annual mean surface ozone concentrations from the 1990s to the 2020s, for two global chemistry-transport models, under a 'Current Legislation' scenario. The upper diagram presents predictions for the TM3 model and the lower diagram presents predictions for the STOCHEM model. This figure is reproduced from fig. 11(a) of Dentener *et al*. (2005).

Ozone is linked to temporal changes in land carbon storage

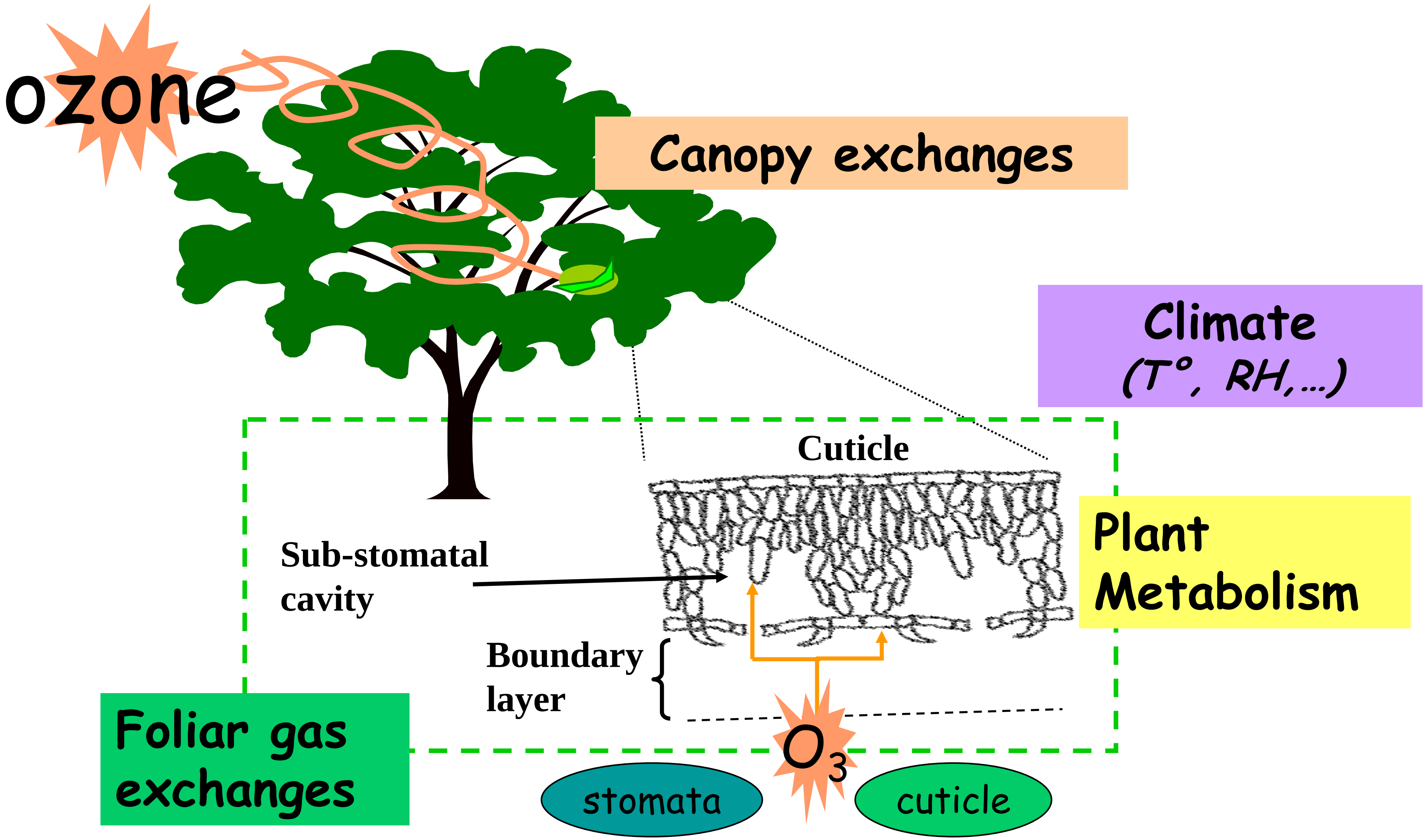


Simulated changes in land carbon storage due to O_3 increase Sitch et al., 2007 **Nature, *448*, 791-95**

Increasing ozone concentration in the atmosphere diminishes plant productivity

Ozone will lower the capacity of plants to sequester the increasing level of carbon (rise in atmospheric CO_2)

Exchanges of ozone with vegetation



French ANR Programme

VULNOZ

Objectives :

Interlocking / integration of different models to assess risks for the coming 20 years

Needing more knowledge on :

- ozone deposit
- cellular and foliar impact of ozone
- impact on the yield in crop field and forest stand
- scenarii of evolution over 20 years
- socioeconomic recommendation

Indices of ozone exposure

AOT40 (accumulated exposure over 40 ppb):

$$\mathrm{AOT40} = \Sigma_d . \Sigma_h \left([O_3]_h - 40 \right)$$

Critical level over a vegetation period: *-forest trees: 10 ppm.h*
-crops: 3 ppm h

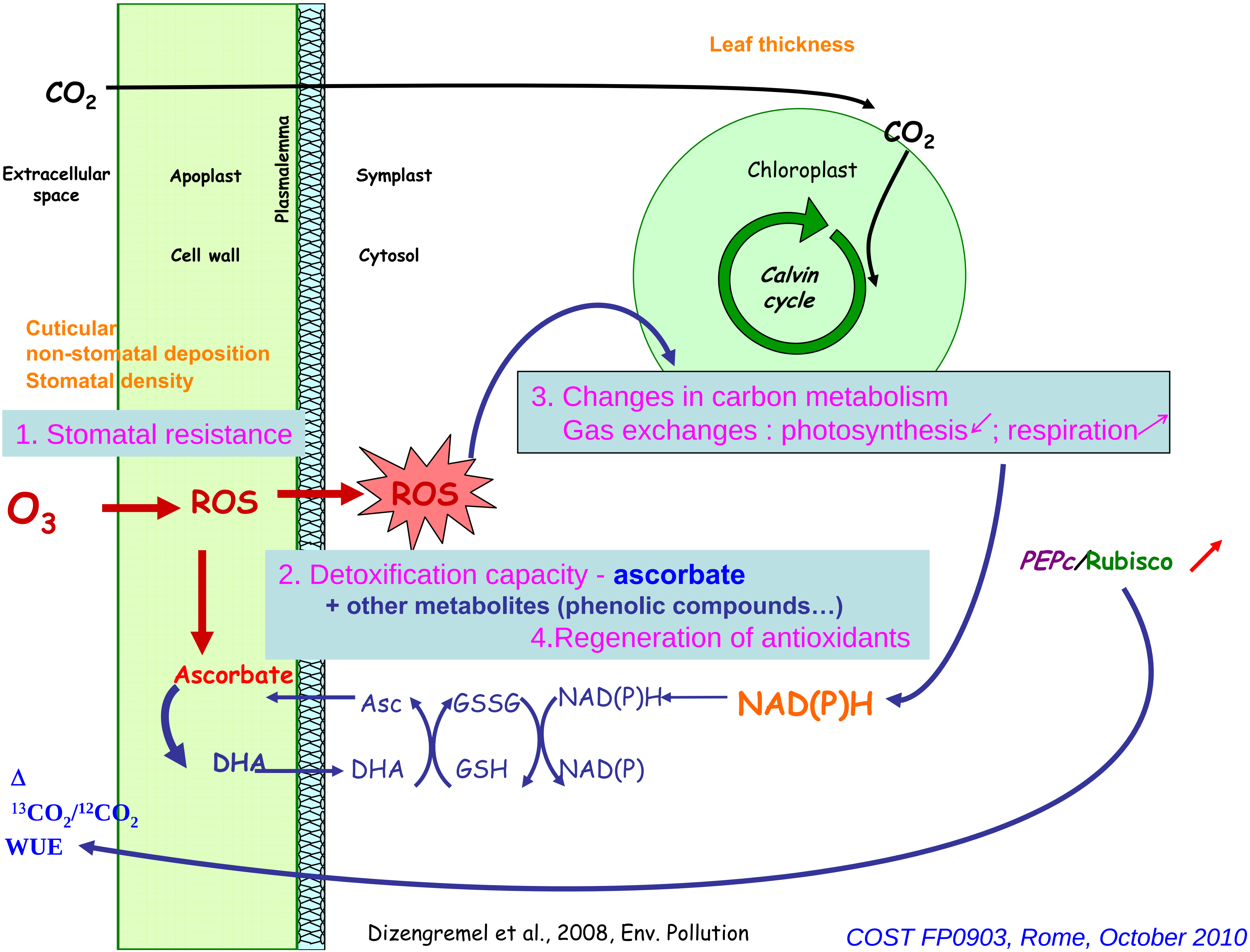
CUO (cumulative uptake of ozone) or POD

$$\mathrm{CUO} = \sum_d . \sum_h \left(F_{O_3} \times 3600 \right)$$

Karlsson *et al.* (2007)
Pleijel *et al.* (2007)

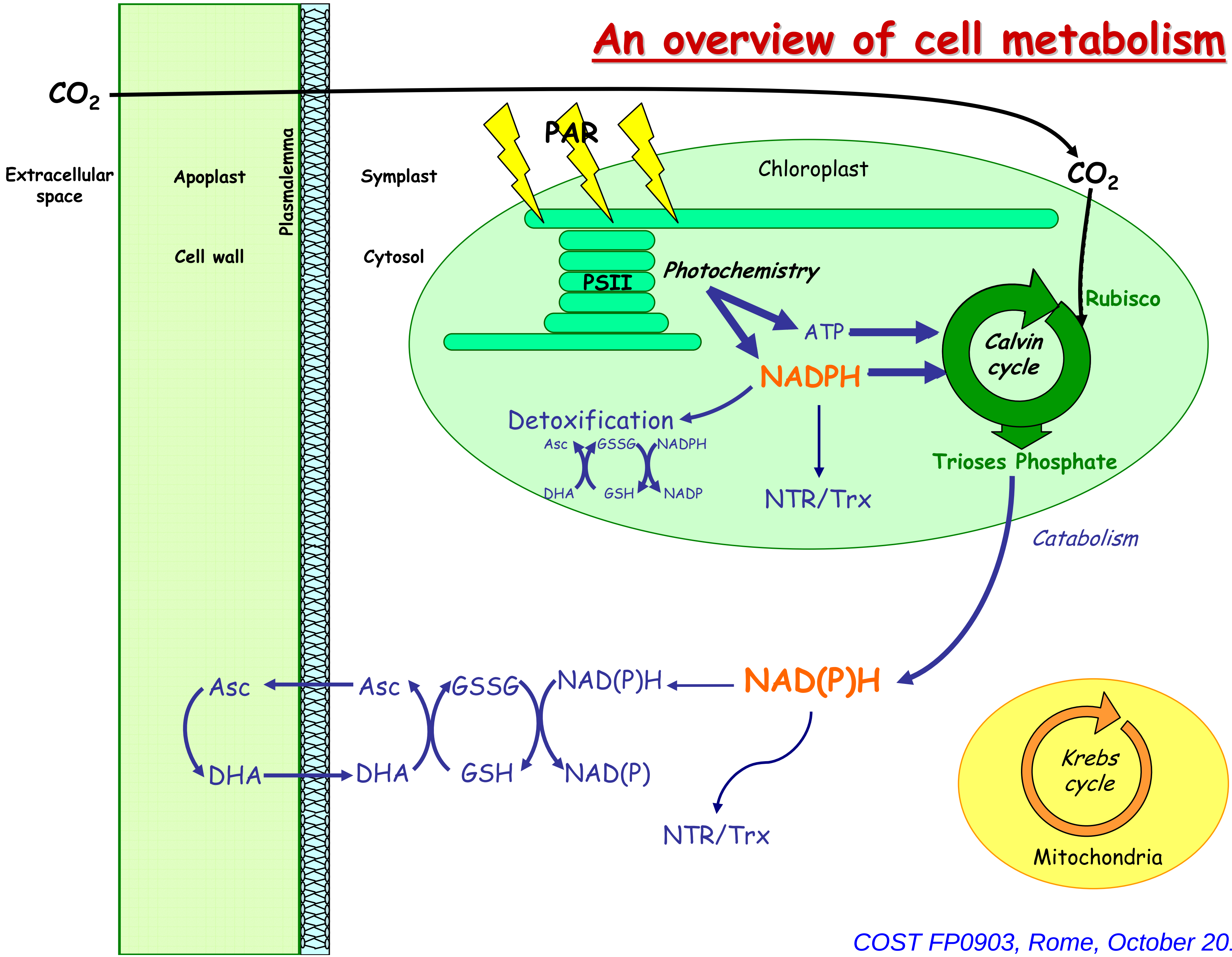
Instantaneous ozone flux: $F_{O_3} = [O_3] \times \frac{g_s}{1.6}$

- Towards an effective ozone flux
- Detoxification/Metabolism must be taken into account

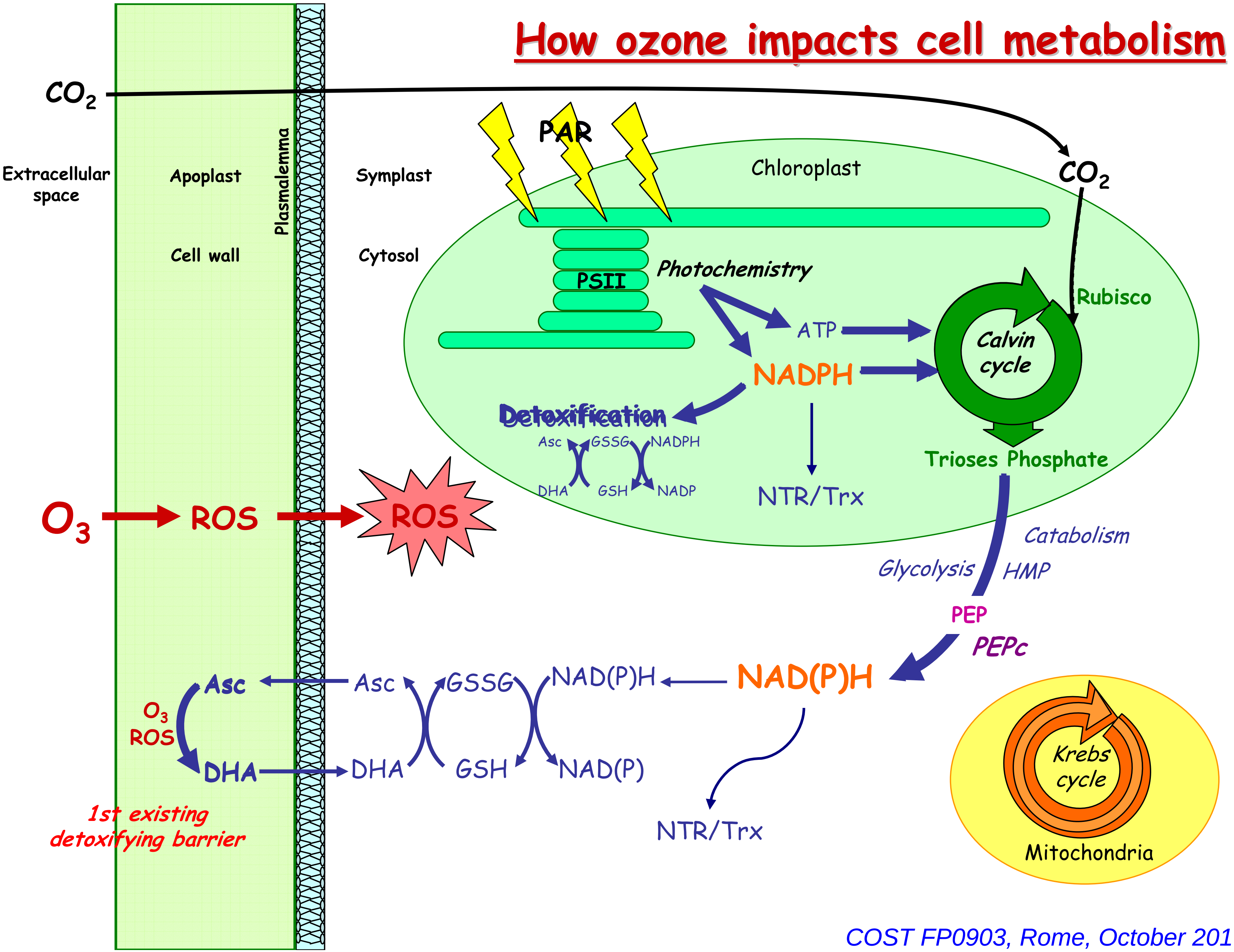


Dizengremel et al., 2008, Env. Pollution

An overview of cell metabolism



How ozone impacts cell metabolism



Effect of ozone on carboxylation

Rubisco & PEPc

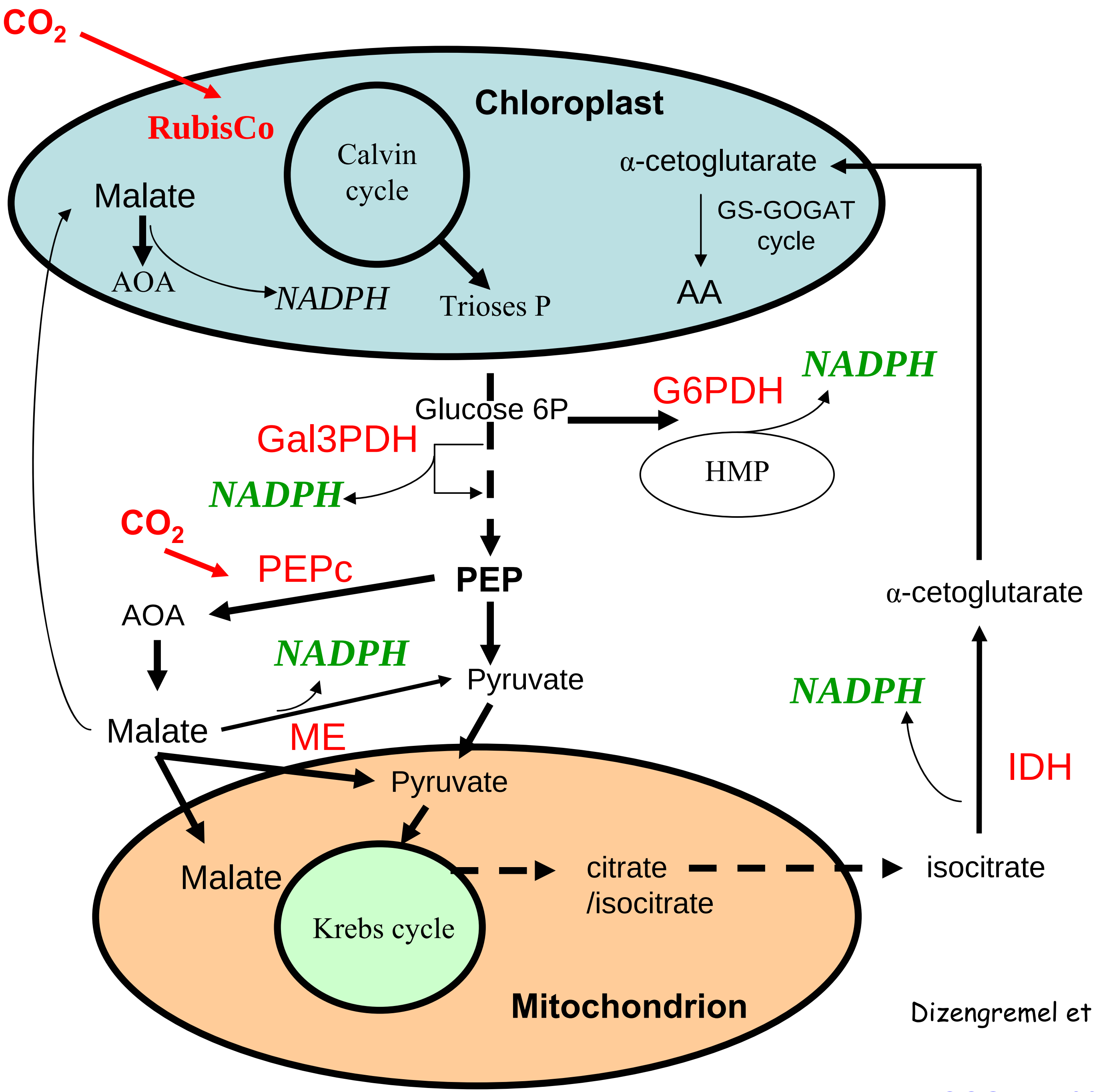
Specific activity ($nkat.mg_{prot}^{-1}$)	Rubisco		PEPc		Rubisco / PEPc ratio	
	C	O_3	C	O_3	C	O_3
Poplar 60 ppb O_3, 2 weeks	17.0	9.1	0.36	1.35	47.2	6.74
Norway spruce 200 ppb O_3, 12 weeks	13.2	6.2	0.45	2.55	29.3	2.43
Aleppo pine 200 ppb O_3, 5 weeks	13.6	7.5	0.55	1.96	24.7	3.82

Fontaine *et al.* (1999), Physiologia Plantarum

Pelloux *et al.* (2001), Plant, Cell & Environment

Dizengremel (2001), Plant Physiol. Biochem.

Fontaine *et al.* (2003), Physiologia Plantarum

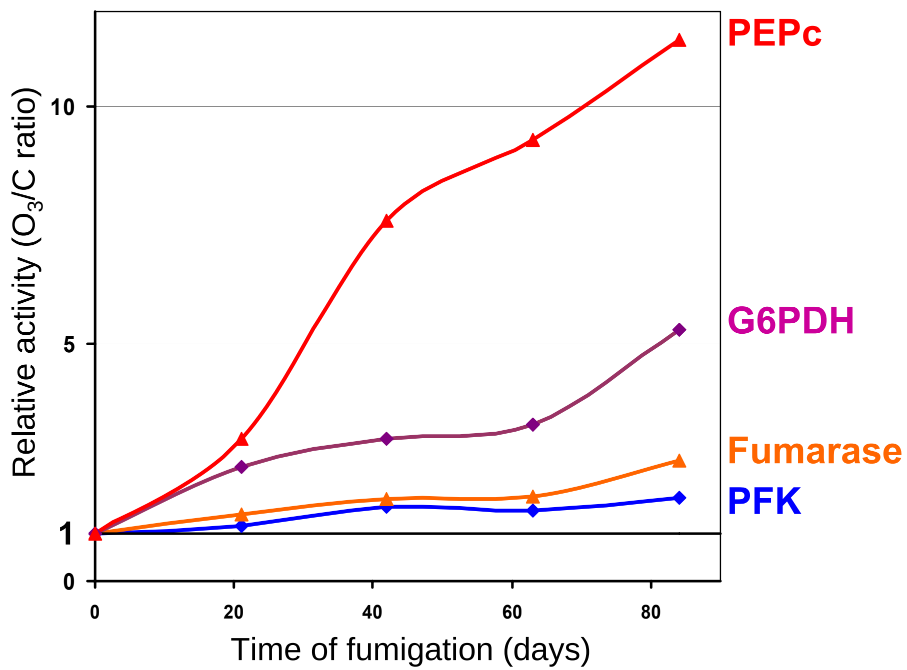


Dizengremel et al., 2009, Plant Biology

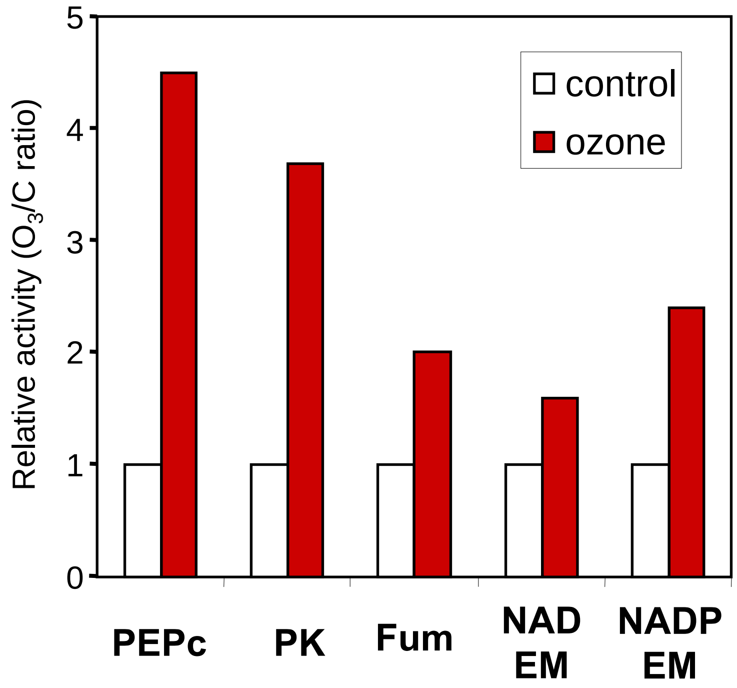
Effect of ozone on enzymes linked to catabolism

Glycolysis, Krebs cycle and anaplerotic pathway

Norway spruce
200 ppb O_3, 12 weeks



Poplar
100 ppb O_3, 24 days



Sehmer *et al.* (1998), Physiologia Plantarum; Dizengremel *et al.* (2009), Plant Biology

Which metabolites/enzymes are relevant to measure?

Ascorbate
Ascorbate peroxydases

- total Asc (symplastic + apoplastic) could be a good indicator of ozone resistance.

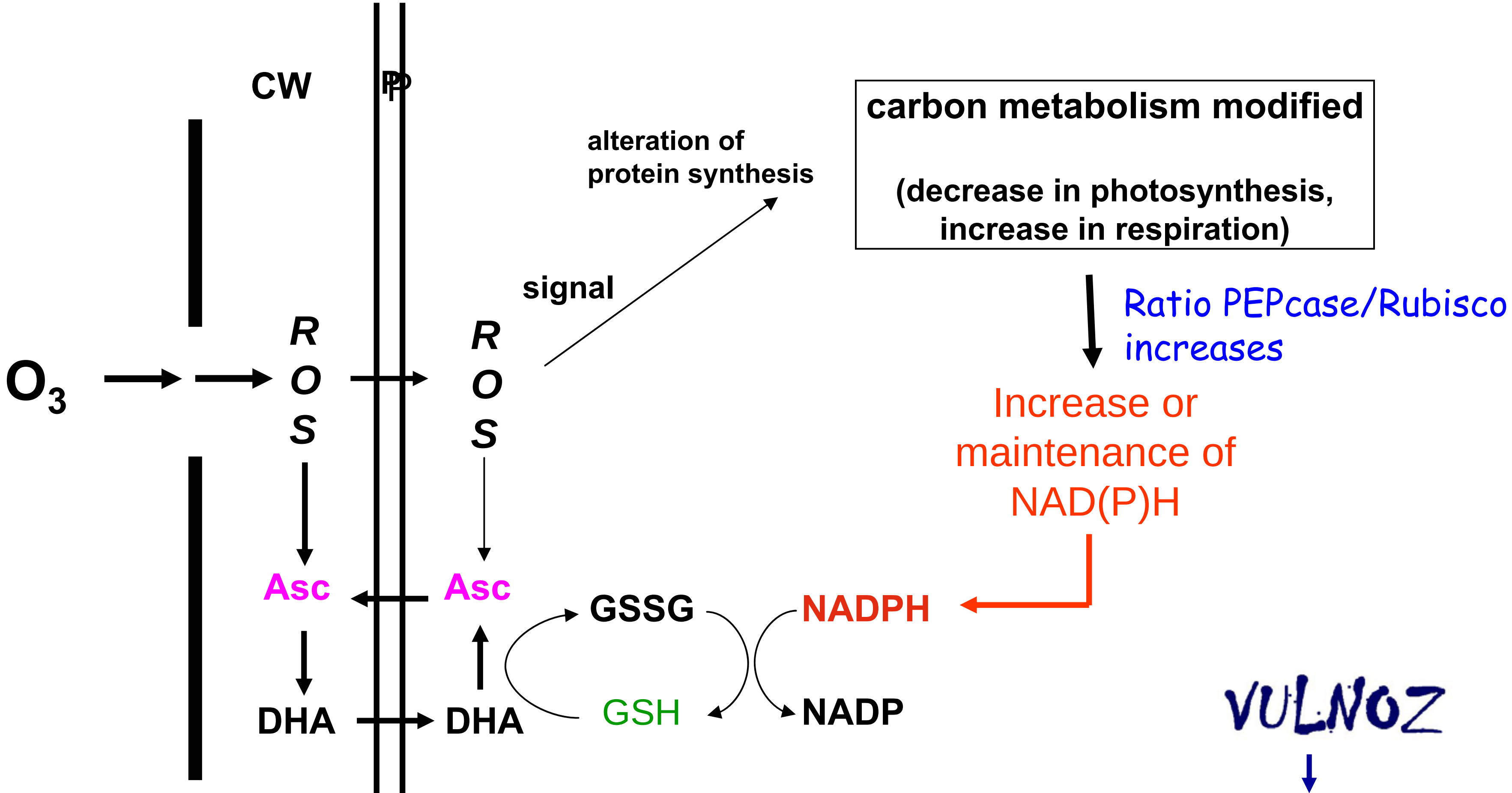
Glutathione
Glutathione Reductase

- this metabolite acts to reduce Asc through glutathione reductase activity.

Reducing power
(NADPH & NADH)

- these nucleotides are the driving force of the detoxifying system (for regeneration of ascorbate pool).

It is necessary to confirm that ozone stimulates the activity of the cytosolic enzymes providing NADPH.



Sub model « detoxification » to be finalised : SPAC model (Tuzet *et al.* 2007, 2010) combined to SODA model (Plöchl *et al*. 2000)

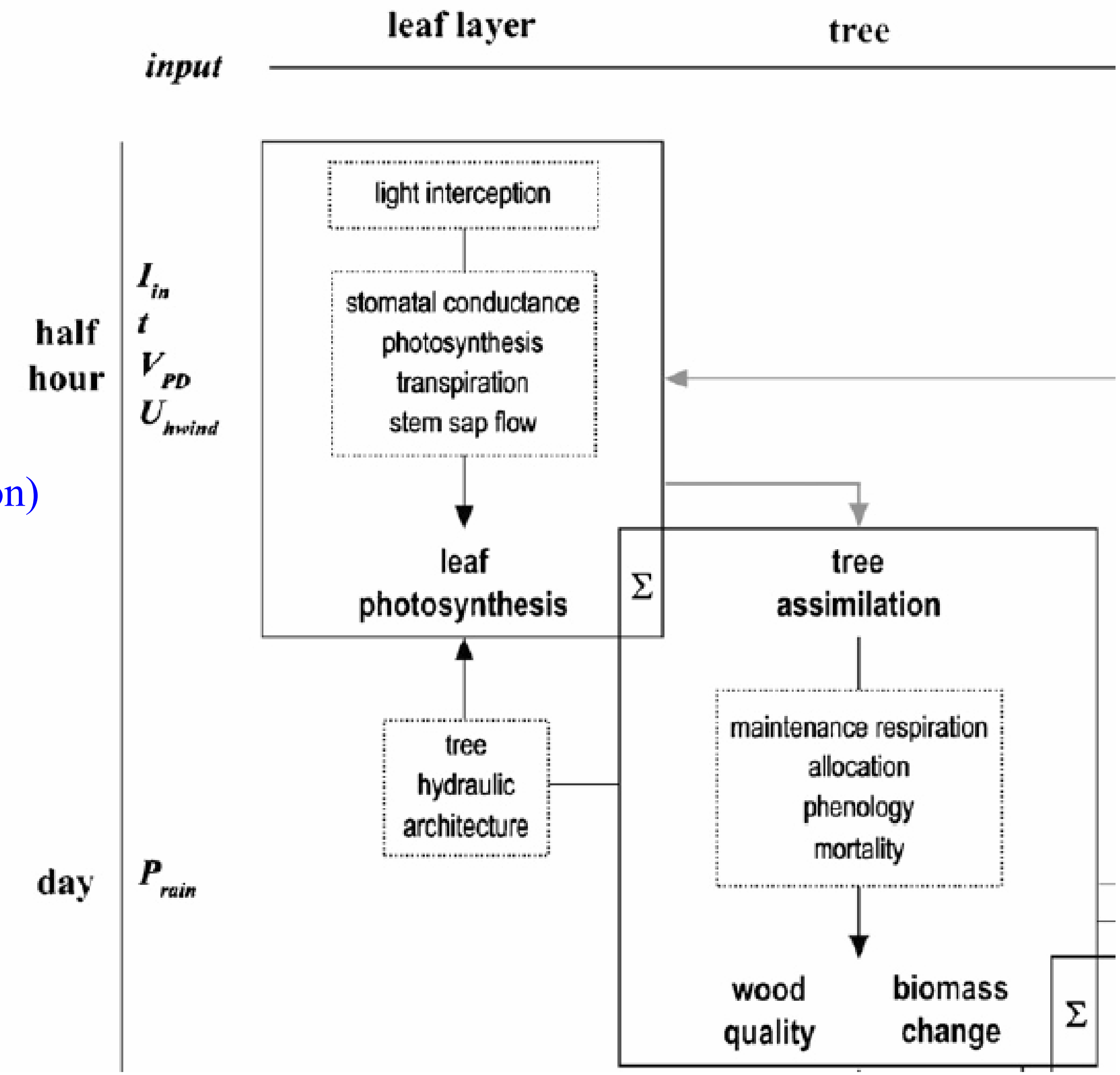
This sub-model will be used in different models :

CERES (yield/field) – ORCHIDEE (regional scale) and AROPAj (economic risks)

MODELING AND RISK UPSCALING
which models are available? example…

ANAFORE

Deckmyn *et al.*, 2008

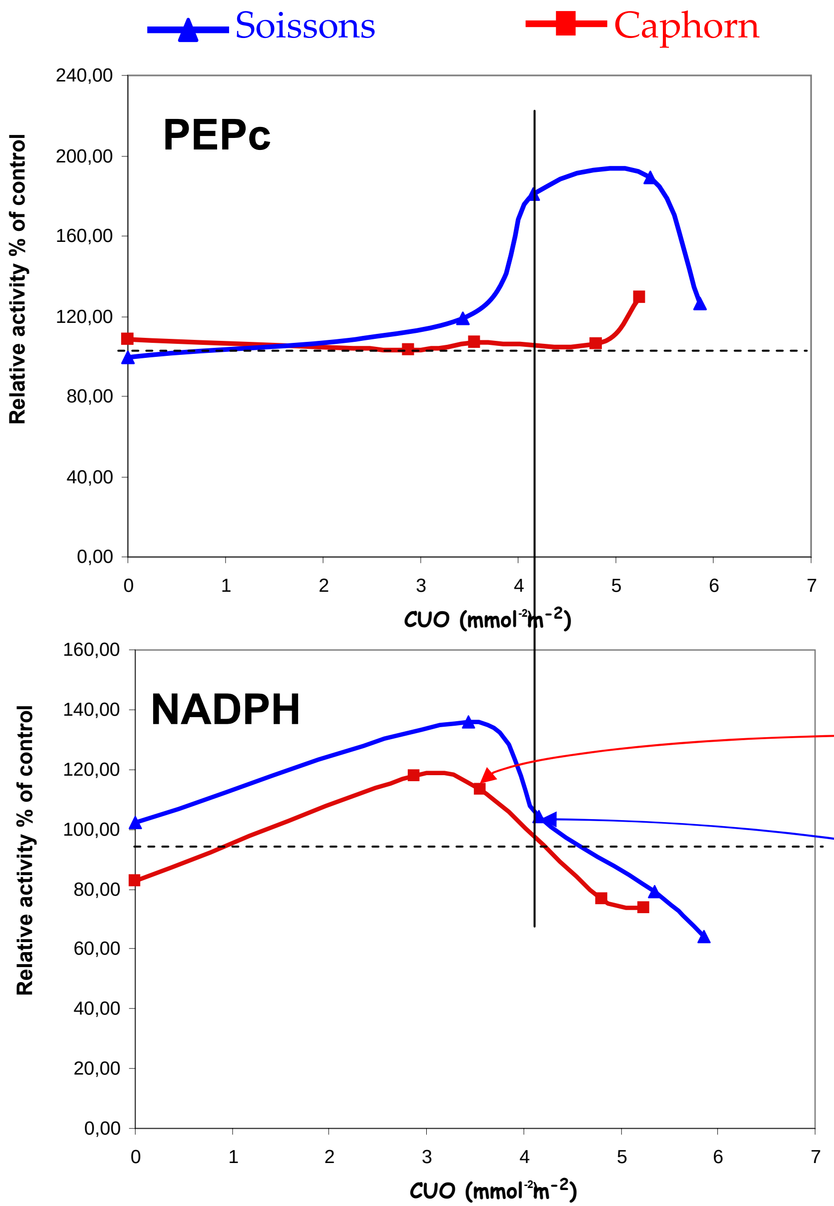


• New modules? (detoxification)

• New parameters :
- PEPc/RubisCo
- ascorbate , NADPH, …
- stomatal density…

• new parameterization

..... Some preliminary results



Winter wheat (*Triticum aestivum*) cultivars :

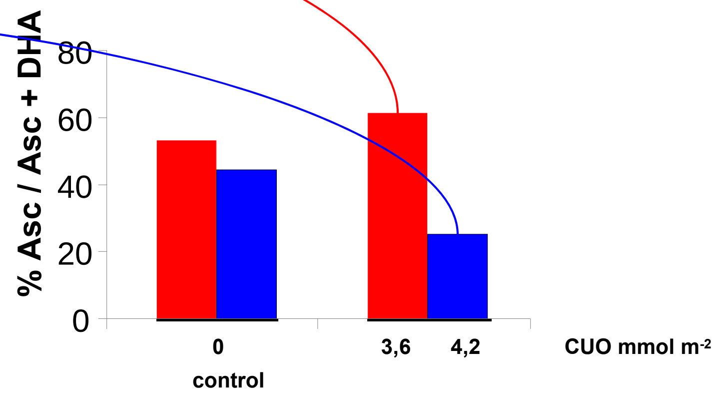
Soissons (sensitive)

Caphorn (tolerant)

Higher stimulation of PEPc activity in the sensitive cultivar

Higher content of NADPH as a response to ozone fumigation

Partial uncoupling between NADPH content and PEPc activity changes :
Role of NADPH generating enzymes ?

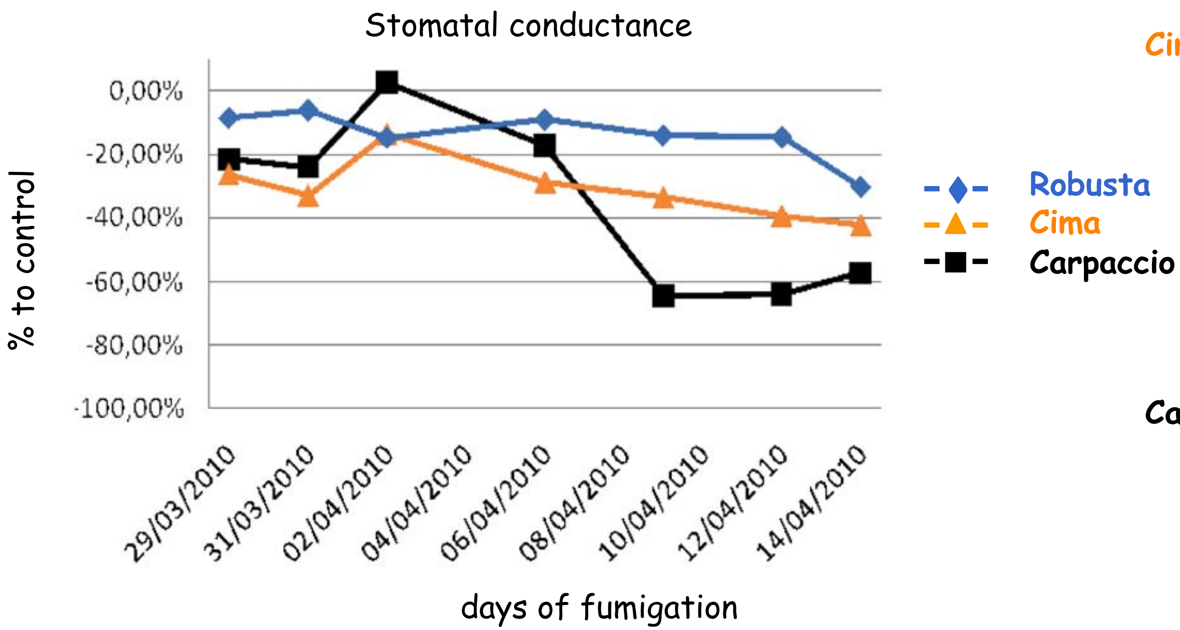


Poplar Experiment in phytotronic chambers

- 3 genotypes *Pop. deltoïdes* × *Pop. nigra* (Carpaccio, Cima, Robusta).
- Ozone 120ppb

Parameters :

Gas exchanges + Chlorophyll
Growth + Leaf surface
Ascorbate + glutathione
NADP(H) + NADPH generating enzymes
PEPc + RubisCo
Guard cells transcriptomic
Metabolomic



Robusta (sensitive +++)

Cima (tolerant)

Carpaccio (tolerant)

The ozone group at Nancy University

- Primary carbon metabolism

Photosynthesis
Assimilation / Stomatal conductance
Leaf anatomy

Didier Le Thiec, INRA Nancy
Jennifer Dumont, PhD

Nancy-Université
Université Henri Poincaré

INRA

Photochemistry / Calvin cycle: Sacha Bohler PhD

Respiration

Respiratory chain — Yves Jolivet

Photorespiration — Marie-Paule Sauder, Marie-Noëlle Vaultier

Detoxification — Ata Dghim,PhD

Comparison of C3/C4 plants

JOENSUUN YLIOPISTO — E. Oksanen

Centre de Recherche Public Gabriel Lippmann — J.-F.Hausman, J. Renaud

Umeå Plant Science Centre — P. Gardeström

- Cell wall

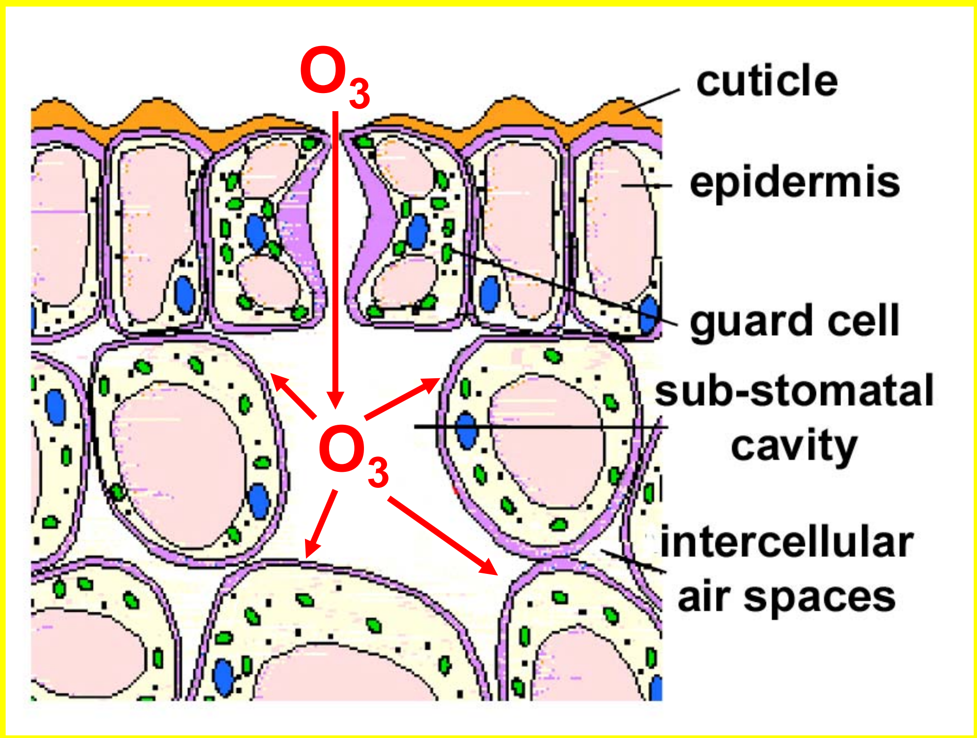
Cellulose: Dany Afif

Lignins: Mireille Cabané, Nicolas Richet, PhD

INRA

Catherine Lapierre, INRA Grignon

Towards a model for Ozone Risk Assessment : from atmosphere to cell



The sensitivity of a leaf to ozone lies on the ***stomatal conductance***, on the ***existing scavenging system*** and the ***detoxifying capacity (regeneration)***.

The aim is to determine a sub-cellular model taking into account these aspects.

This model will have to be further integrated into a plant model and in a stand model.

The ultimate aim is to improve ozone risk assessment by the determination of more accurate critical levels of exposure than those currently used by EU.

Increasing tolerance to ozone by elevating foliar ascorbic acid confers greater protection against ozone that increasing avoidance (Chen and Gallie, 2005, Plant Physiology, 138, 1673-1689)
Increasing the level of ascorbate through enhanced ascorbate recycling provided greater protection against oxidative damage than reducing stomatal area aperture.

Our data lead to hypothesize that O3-tolerance in bean depends more on a superior potential cultivar-specific ability more than stress-induced physiological and biochemical adjustments to avoiding and countering stress-induced oxidative damage. (Guidi et al., Environmental pollution, 2010, 158, 3164-3171)

Good correlation between tolerance to ozone and high endogenous levels of antioxidant metabolites such as AA and GSH in tobacco (Pasqualini et al., Plant cell Environment, 2001, 24, 245-252)

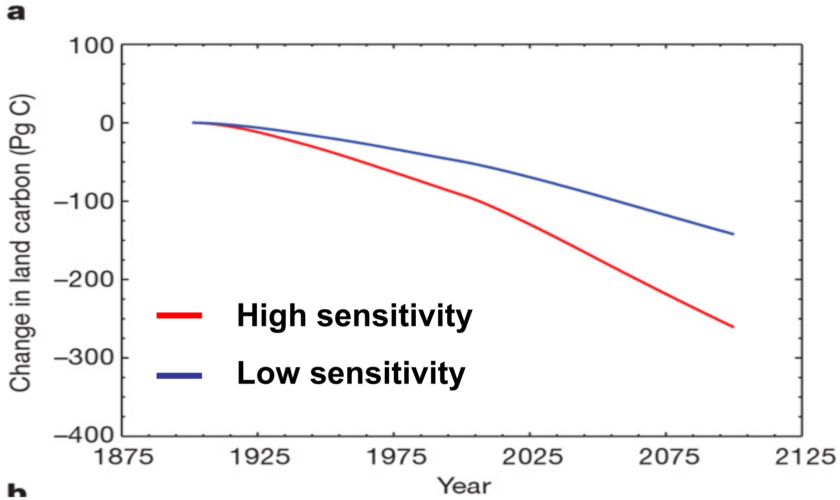
Ozone flux was best explained by stomatal conductance and symplastic (rather than apoplastic) ascorbate explaining 66% of the total variation in leaf flux. It is important to measure processes other than stomatal conductance to explain steady-state leaf-level fluxes of pollutant gases. (Eller and Parks, 2006, Plant Cell Environment, 29, 1742-1750).

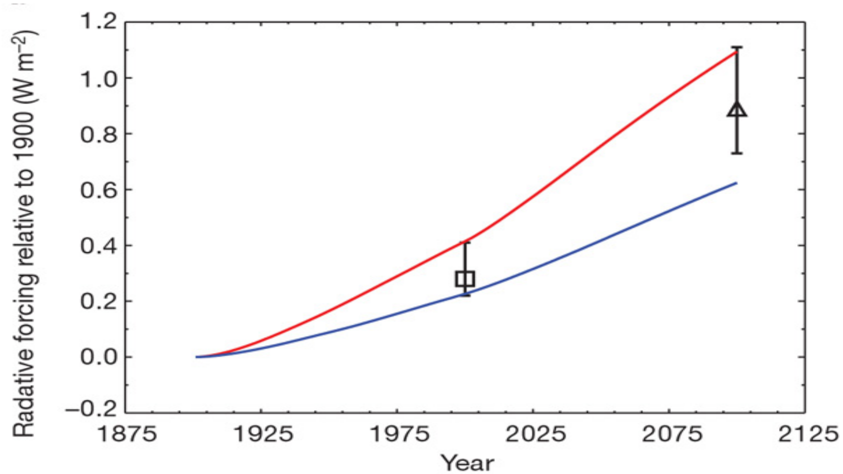
Other factors than constitutive total apoplastic ascorbate contribute to the differential ozone tolerance of two clones of trifolium repens. The symplastic redox status could play a role (D'Haese et al., 2005, Plant Cell Environment, 28, 623-632).

The increase in foliar ascorbate under ozone stress in the sensitive poplar clone Eridano, with a greater stomatal conductance, was insufficient to counteract ROS accumulation and the consequent oxidative stress. The higher influx of ozone into Eridano leaves compared with the tolerant clone I-214 resulted in a lower *potential detoxification capacity per unit of ozone influx*. (Di Baccio et al., 2008, Tree Physiol., 28, 1761-1772).

Recent developments highlight the complexity of redox-dependent defence reactions and the importance of interactions between the reduction state of soluble redox couples and their concentration in mediating dynamic signalling in response to stress – i.e. reactive oxygen species (Noctor, 2006, Plant cell Environment, 29, 409-425).

Temporal changes in land carbon storage and radiative forcing due to ozone





Simulated changes in land carbon storage due to O_3 increase

Sitch et al., 2007 **Nature, *448*, 791-95**

Simulated changes in indirect radiative forcing due to O_3 increase (part of non-assimilated CO_2)

For comparison, estimates of the ***direct*** radiative forcing due to O3 increase only are shown by the square and the triangle

Increasing ozone concentration in the atmosphere decreases plant productivity

Ozone will lower the capacity of plants to sequester the increasing level of carbon (rise in atmospheric CO_2)

Consequence: indirect radiative forcing of ozone, due to additional level of CO_2 in the atmosphere, which is added to the direct radiative forcing

Which links could exist between metabolism and ozone flux related to stomatal conductance?

WUE = Water Use Efficiency

$$WUE = A / g_s \quad (1)$$

$A = CO_2$ assimilation $= g_{CO_2} (Ca - Ci)$ (2)

g_s = stomatal conductance to water $= 1.6 . g_{CO_2}$ (3)

Ca = atmospheric $[CO_2]$

Ci = internal $[CO_2]$

From (1), (2) and (3):

$$WUE = (Ca - Ci) / 1.6 \quad (4)$$

According to Farquhar *et al.* (1982) :

$$\Delta = a + (b - a) (Ci / Ca) \quad (5)$$

Δ = discrimination between ^{12}C and ^{13}C isotopes

a = stomatal diffusion

b = isotopic discrimination due to carboxylation

From (4) and (5) :

$$WUE = Ca / 1.6 \times (b - \Delta / b - a) \quad (6)$$

According to Farquhar & Richards (1984) :

$$b = \beta . b_1 + (1-\beta) b_2$$

β = PEPc activity / (PEPc activity + Rubisco activity)

b_1 = isotopic discrimination due to PEPc

b_2 = isotopic discrimination due to Rubisco

- As ozone induces a decrease in the Rubisco/PEPc ratio, β, b and consequently WUE should be modified under ozone treatment.