Intégration d'un module de détoxication métabolisme-dépendant dans un modèle d'évaluation des risques encourus par les arbres soumis à l'ozone

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- **Protéomique :**

  Sacha Bohler, Jenny Renaut, Jean-François Hausman

Centre de Recherche Public Gabriel Lippmann

- **Transcriptomique :**

  Per Gardeström, Vaughan Hurry, Catherine Benedict

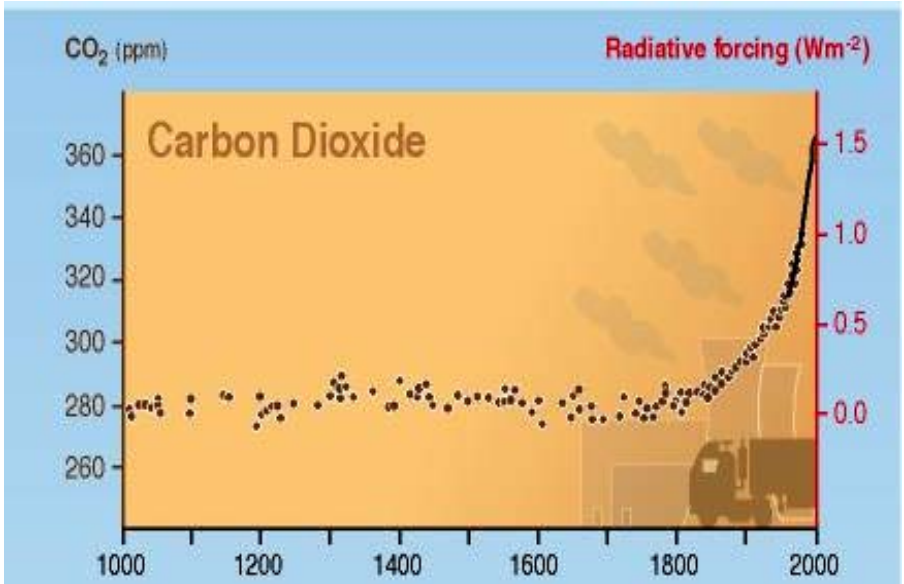
Umeå Plant Science Centre

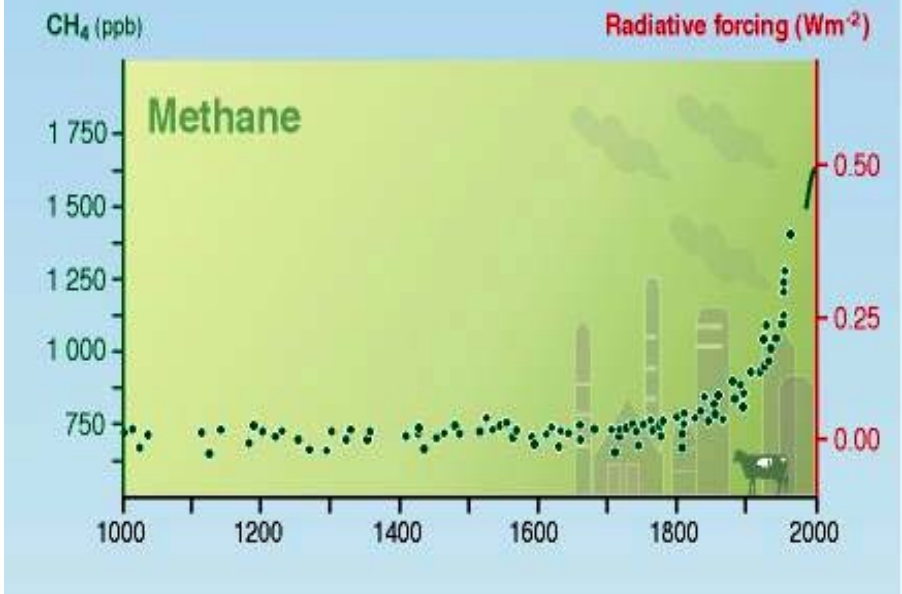
Wood fuel is the only source of fuel for one third of the world's population

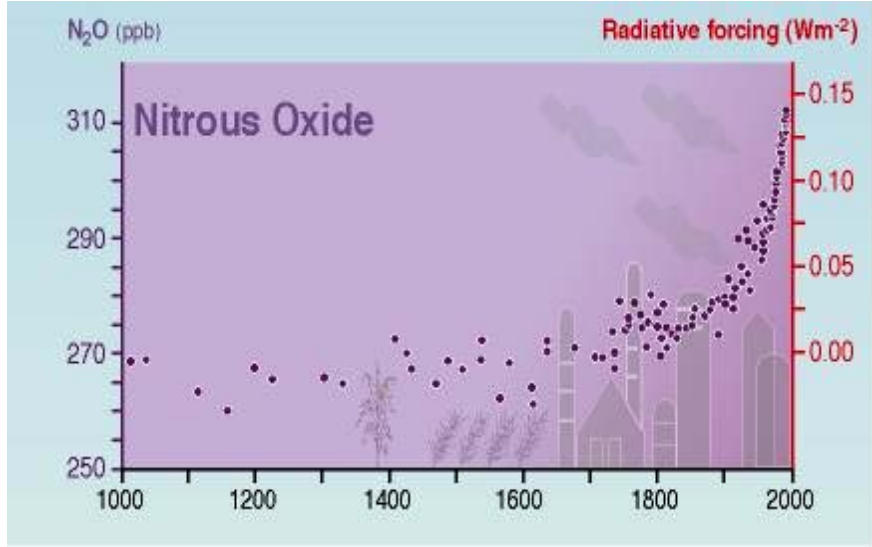
Wood demand will double in next 50 years

Climate change is projected to increase forest productivity, but forest management will become more difficult, due to an increase in pests and fires

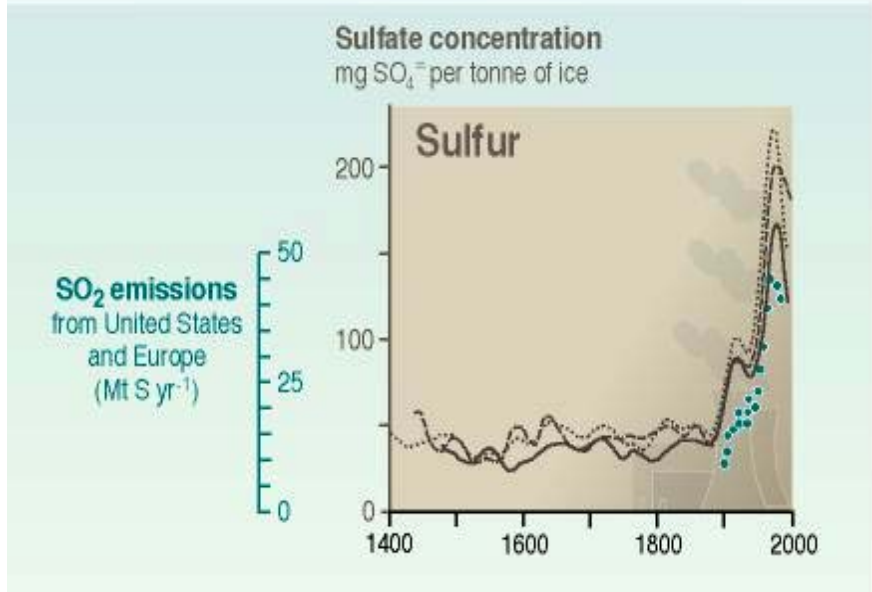
Human activities have changed the composition of the atmosphere since the pre- industrial era





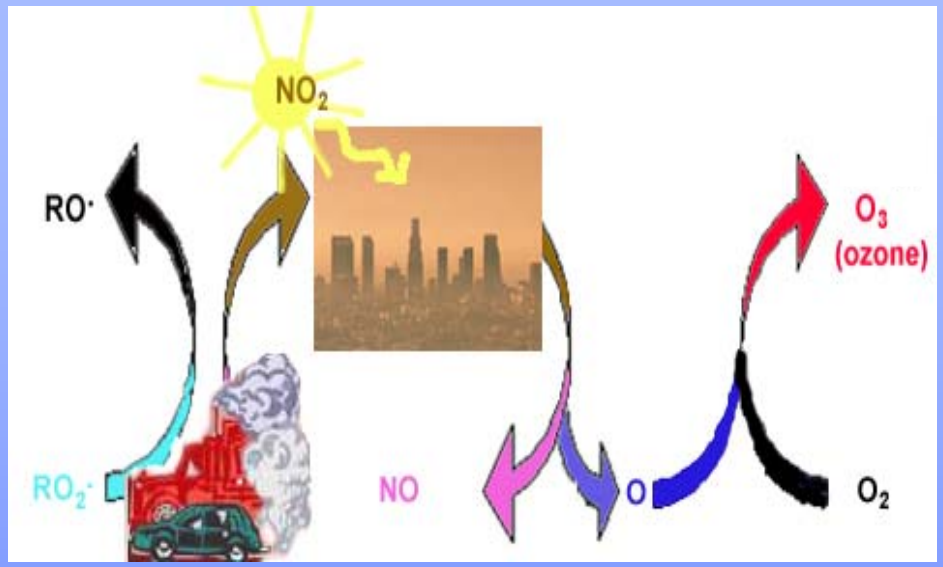


Sulfate aerosols deposited in Greenland ice



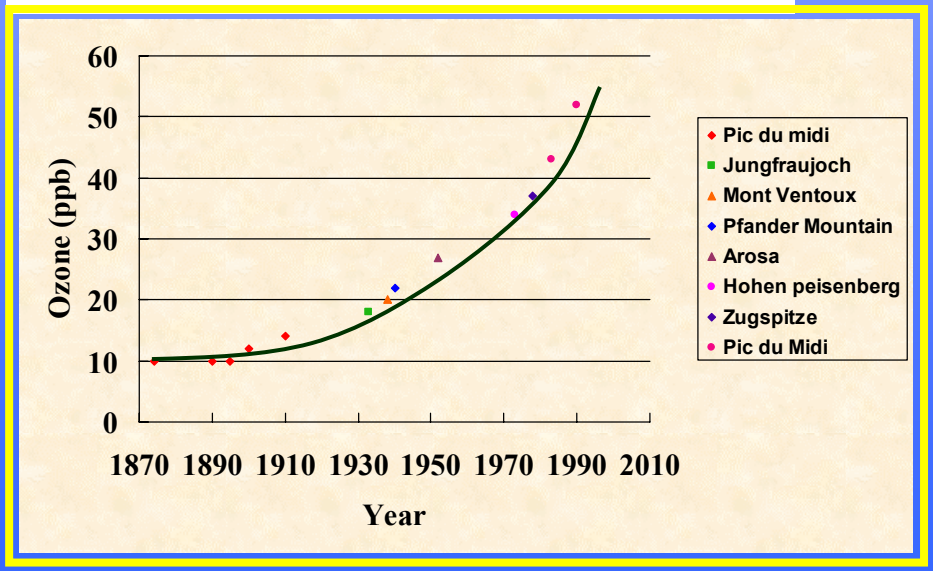
Ozone formation

Evolution of ozone level



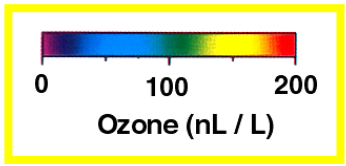
(C .Herman & R. Frey, St-Louis, USA, 2001)

Ozone concentrations 1870-2000



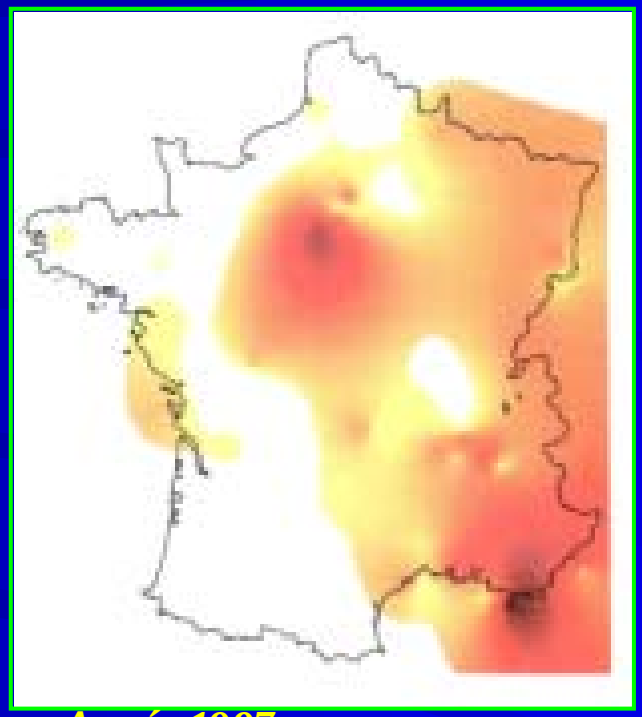
Pollution climate at L.A :

August 27, 1987

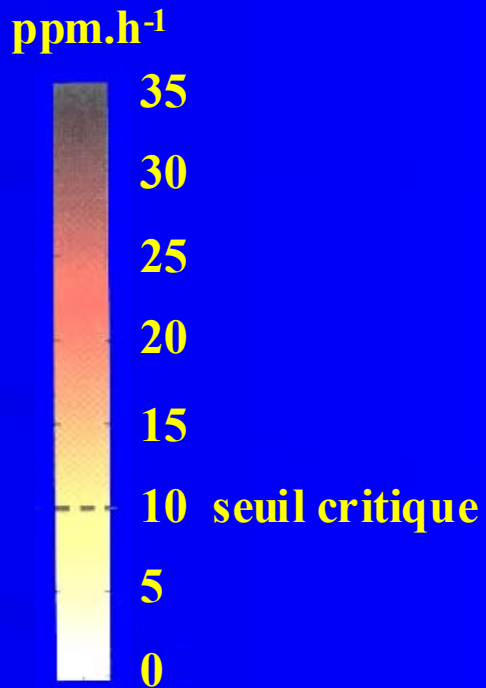


Réponse des plantes à l'ozone

Ozone : polluant atmosphérique



Année 1997

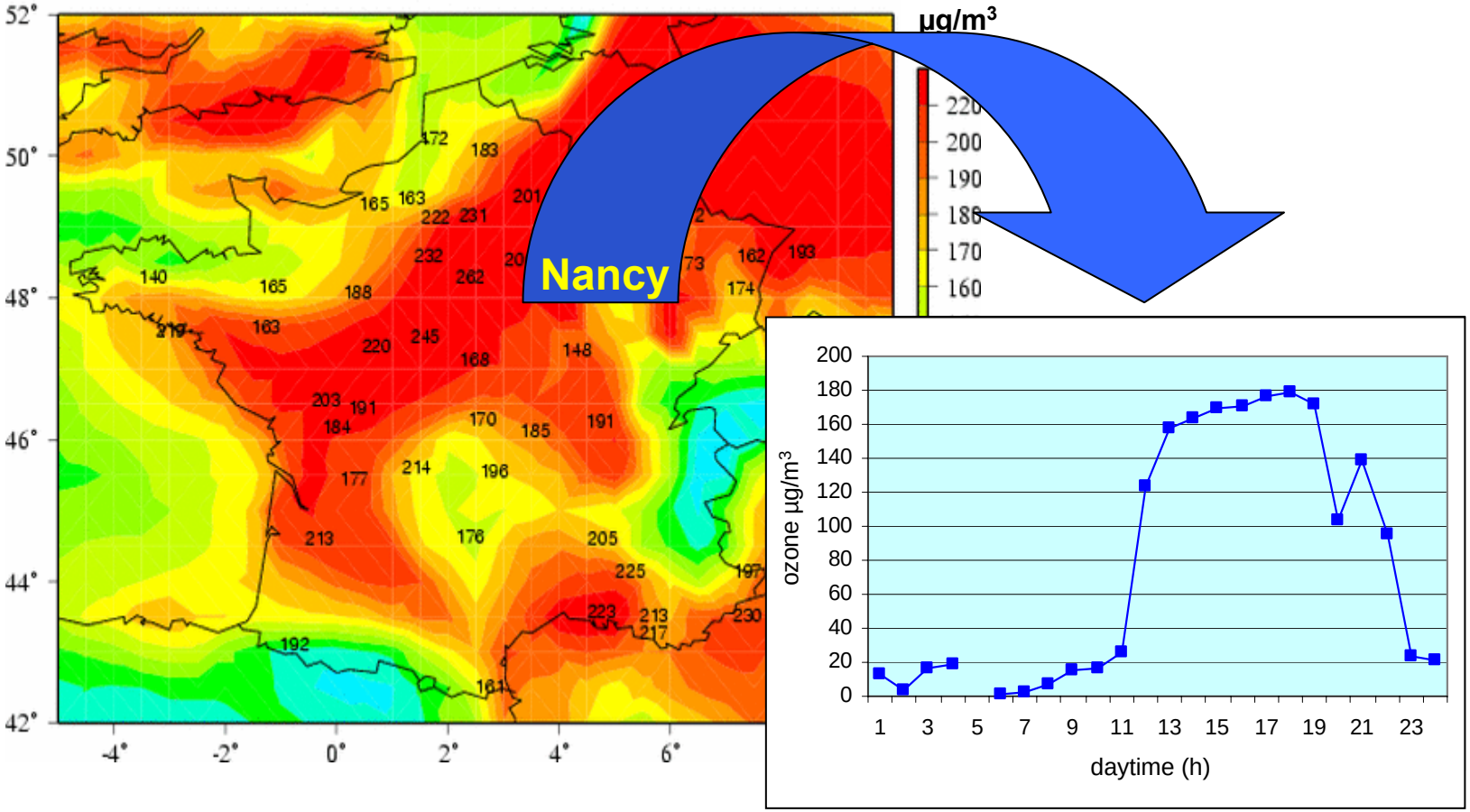


Pour les plantes, un seuil critique (AOT 40) de 10 ppm.h^{-1} (6 mois) est fréquemment dépassé dans les pays industrialisés

(Source : Cahiers du Département de la Santé des Forêts, 1999)

Ozone level in France

August 8, 2003



Peaks up to 180 µg/m³ 100 ppb

Smog over :

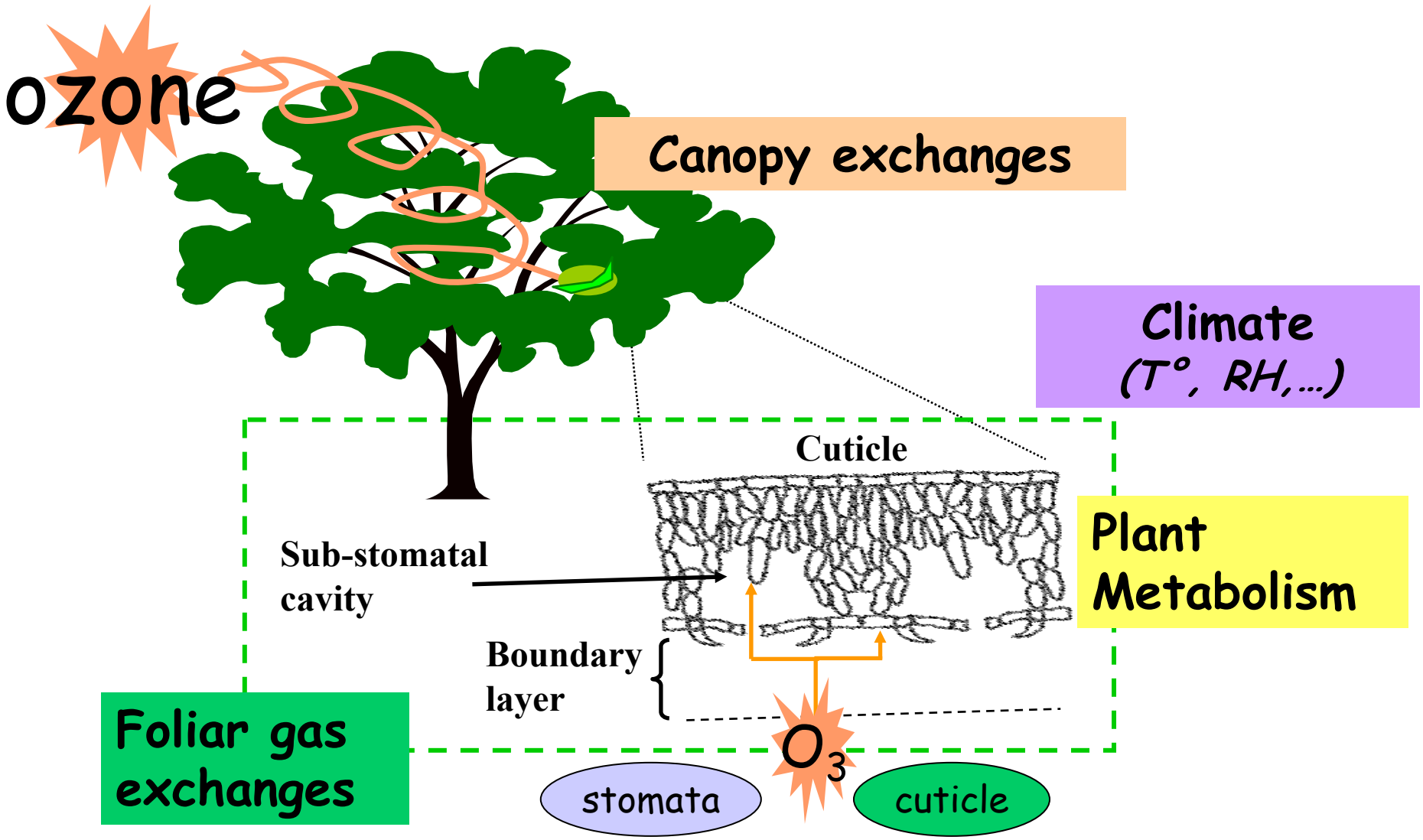
Los Angeles

Athens

Ponderosa pine

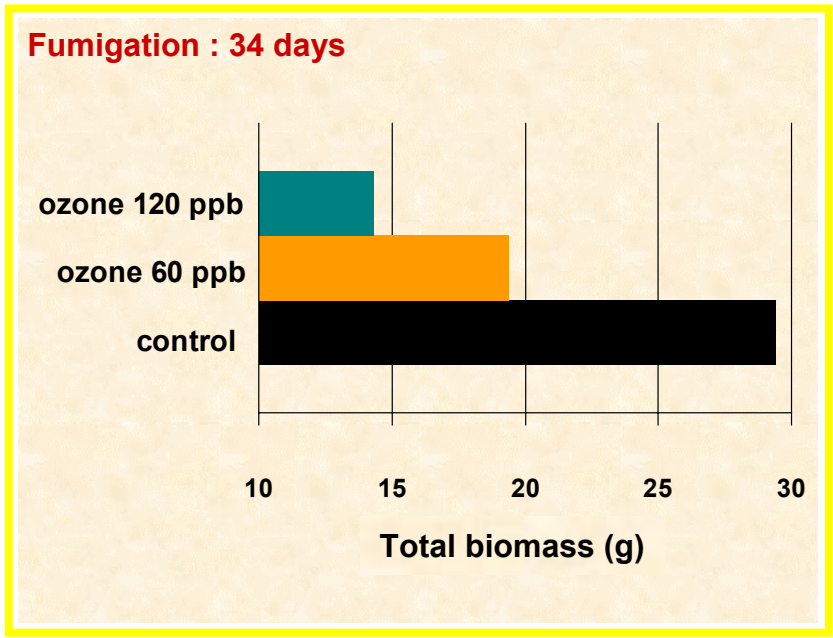
Aleppo pine

Exchanges of ozone with vegetation

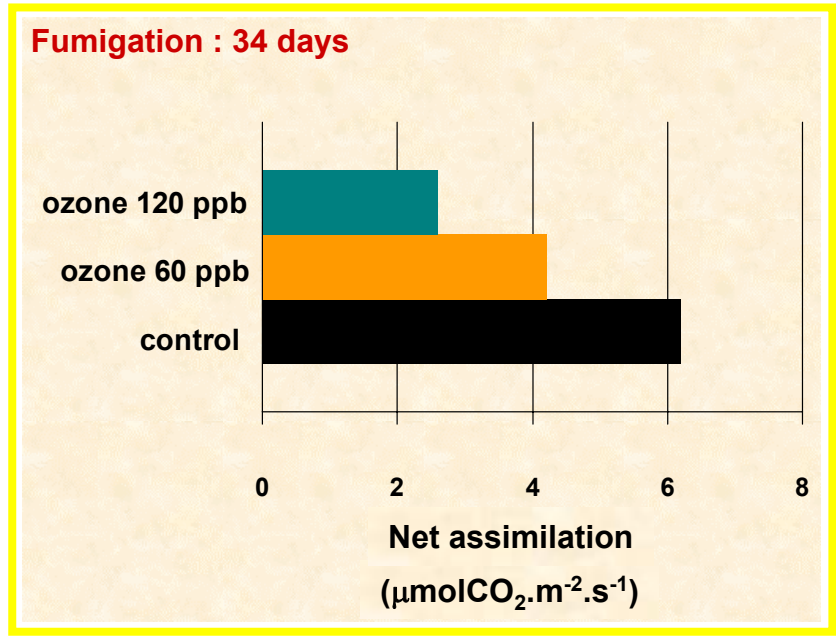


Effect of ozone on growth of poplar

Biomass



Photosynthesis



Parallel decrease of biomass and photosynthesis

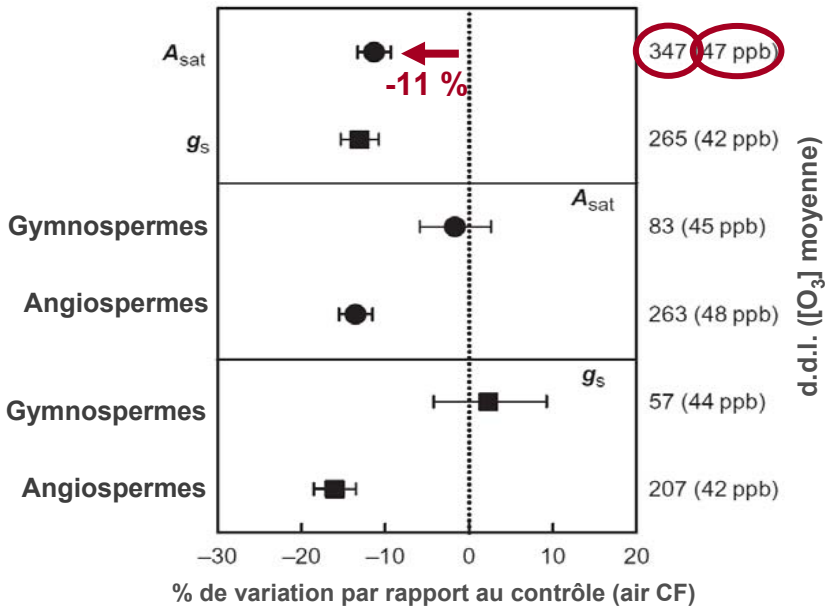
Impact de l'ozone sur les arbres forestiers

Assimilation de CO_2 et conductance stomatique

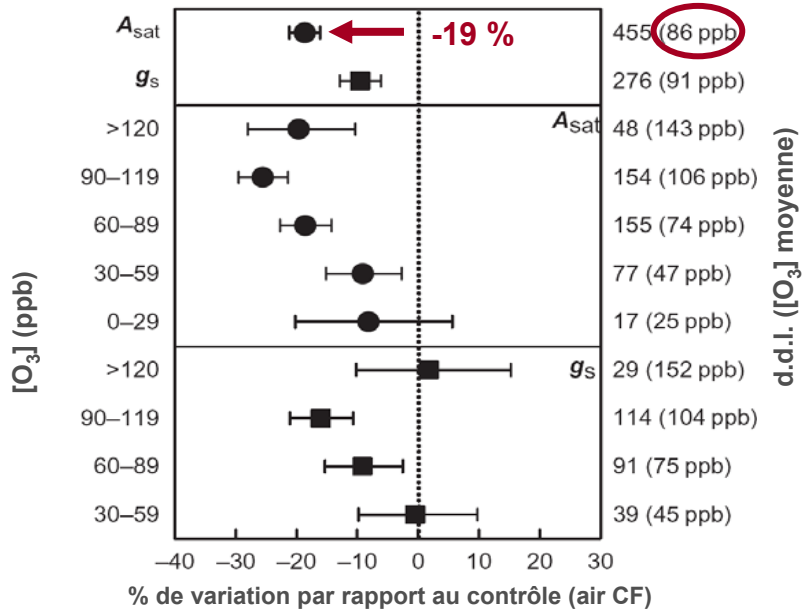
Méta-analyse de 61 (A_{sat}) et 55 (g_s) études publiées

Wittig *et al.* (2007) *Plant, Cell and Environment*, **30** (9) : 1150-1162.

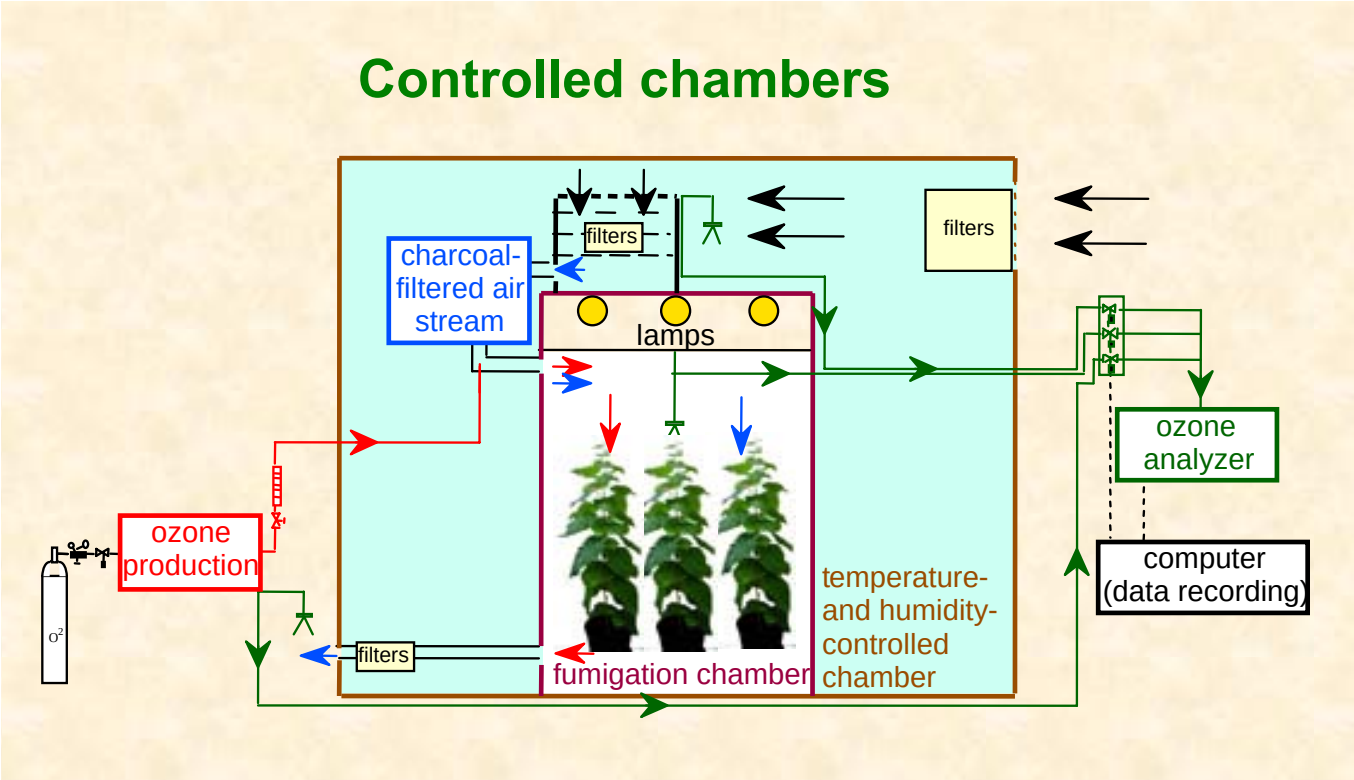
Concentration ambiante en O_3



Concentration élevée en O_3



Experimental design at Nancy



ozone 60-200 ppb
light 200-300 µmol.m^{-2}.s^{-1}
temperature 22/18°C (day/night)
humidity 75/85 % (day/night)

Symptômes foliaires observés

Mûrier blanc

Peuplier noir

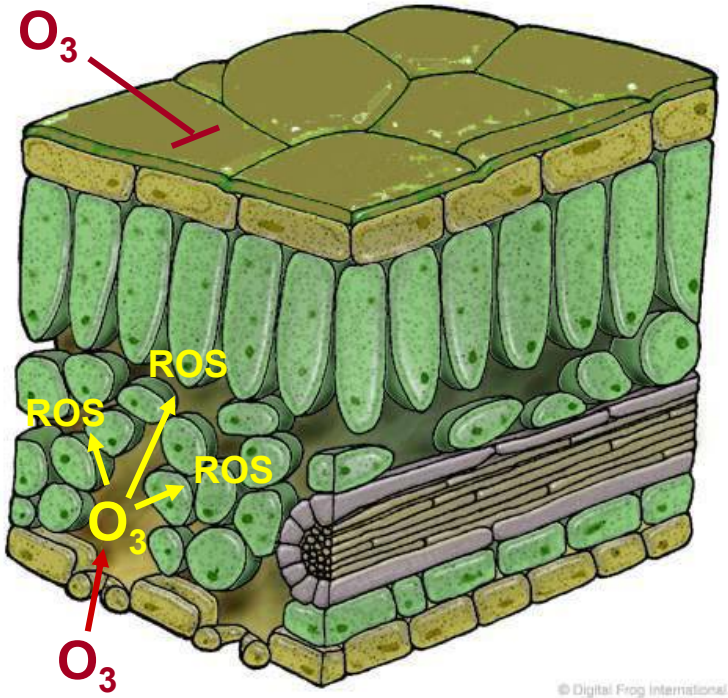
Populus tremula x alba

Chambres phytotroniques

35 jours, ozone 120 ppb

Source : Field Exercise of the 4th UN/ECE ICP-Forests Ozone Training Course.
Italie/Suisse, 25-27 août 2003. http://www.wsl.ch/ozone

Action de l'ozone sur les végétaux



► **Pénétration par les stomates**

► **Décomposition rapide**

► **Génération de ROS**

(espèces oxygénées réactives)

$O_2^{-\cdot}$; $HO^{\cdot}$; H_2O_2 ; etc.

► **Stress oxydant**

Exposition chronique
Concentration en ozone modérée

→ Perturbation du métabolisme

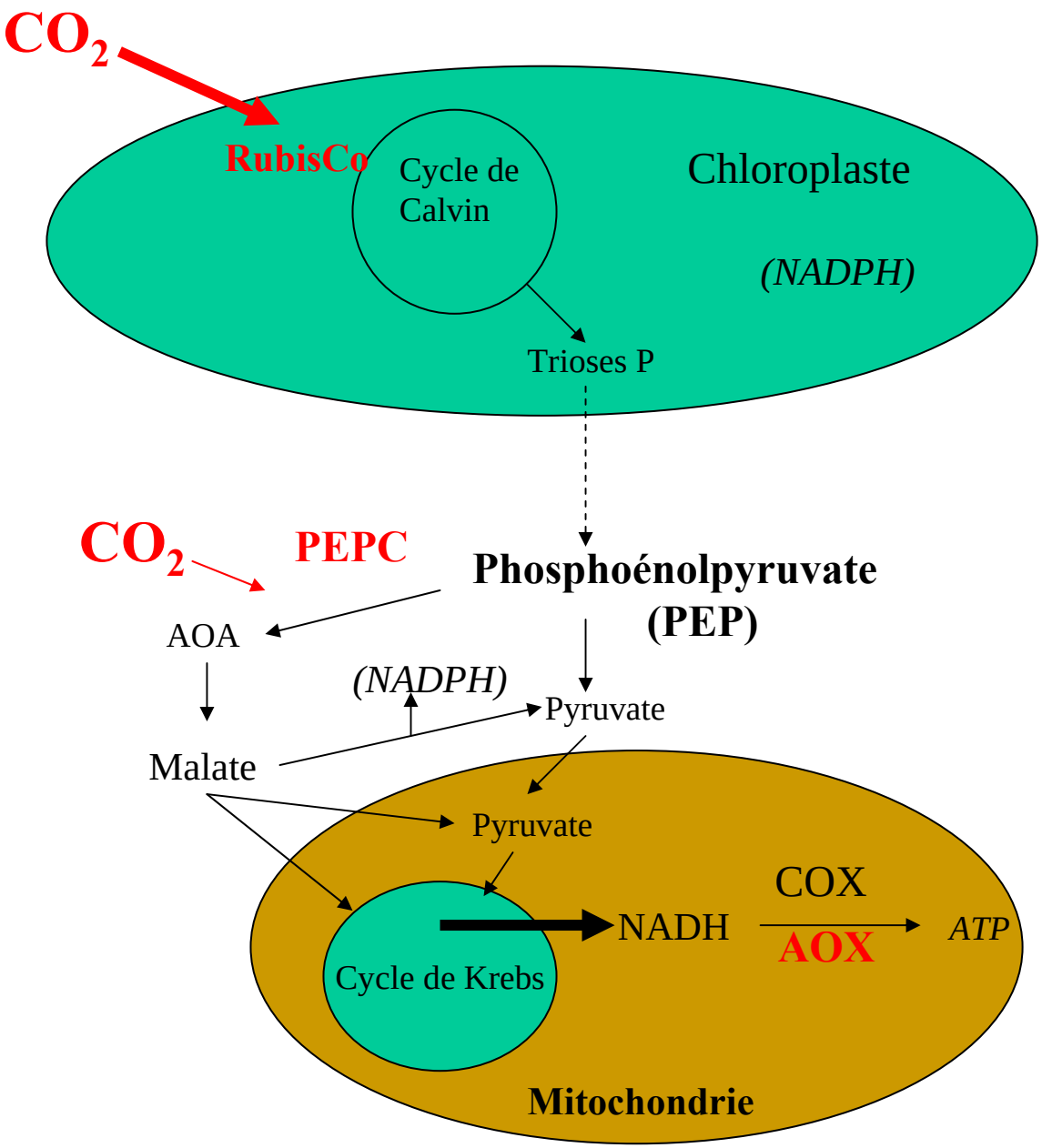
Photosynthèse réduite
Coûts de détoxication et de réparation

→ Réduction de croissance.

→ Sénescence accélérée.

Exposition ponctuelle
Pics de concentration en ozone

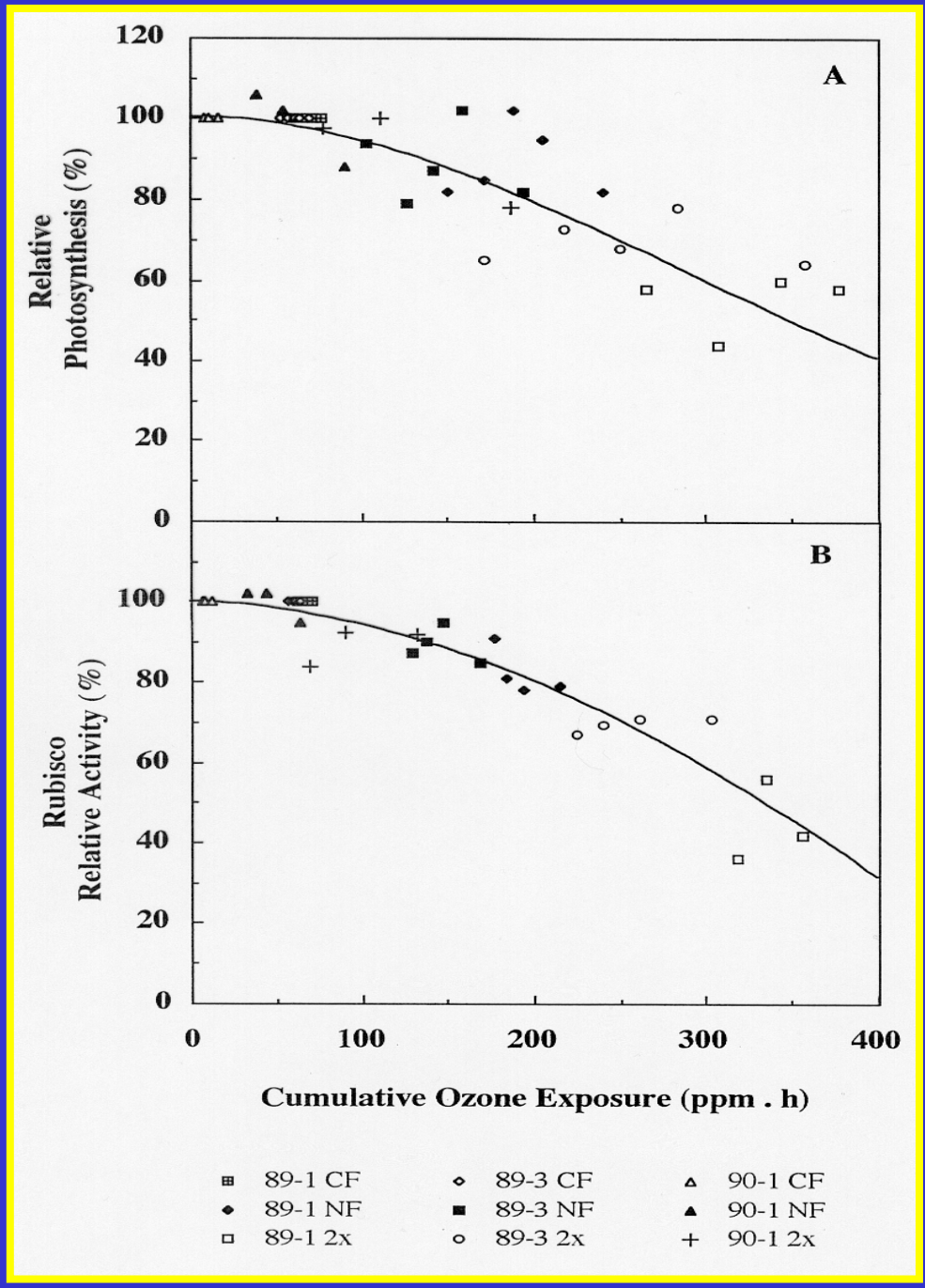
→ Symptômes foliaires
(chlorose, brunissement, nécrose)


CO_2
RubisCo
Cycle de Calvin
Chloroplaste
(NADPH)
Trioses P
CO_2
PEPC
Phosphoénolpyruvate (PEP)
AOA
(NADPH)
Pyruvate
Malate
Pyruvate
COX
NADH
AOX
ATP
Cycle de Krebs
Mitochondrie

Comparaison de l'effet de l'ozone
sur
la Photosynthèse (A)
et
l'activité RubisCO (B)

d'aiguilles de Pin loblolly
(*Pinus taeda*) âgés de 3 ans

Dizengremel et al., 1994

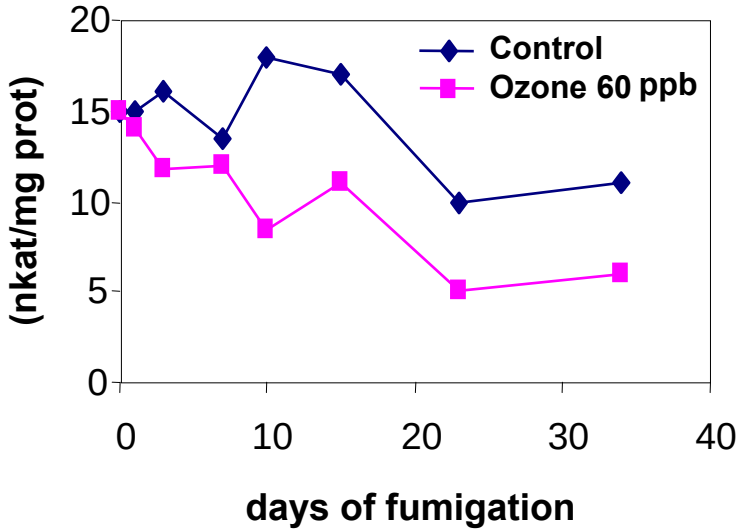


Effect of ozone on carboxylation:

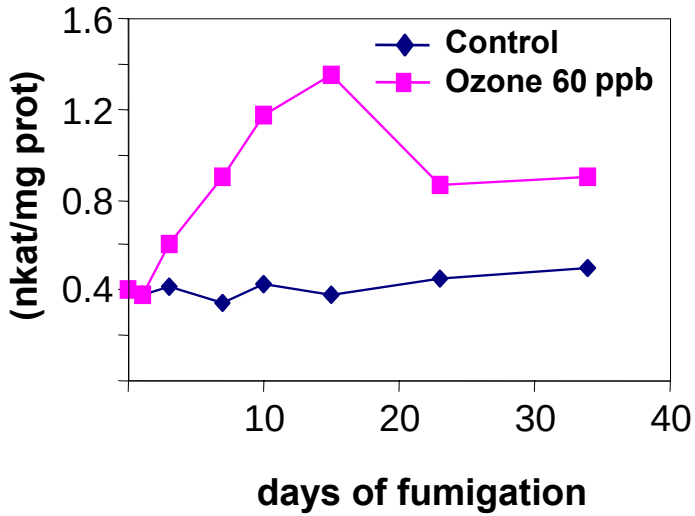
Rubisco & Pepc

Poplar, 60 ppb O_3

Rubisco



PEPc



In ozone-treated plants :

inhibition of Rubisco activity

and huge stimulation of Pepc activity

Indices of ozone exposure

$$AOT40 = \sum_{i}^{j}([O_3]_h - 40)$$

AOT40 (accumulated exposure over a threshold of 40 ppb)

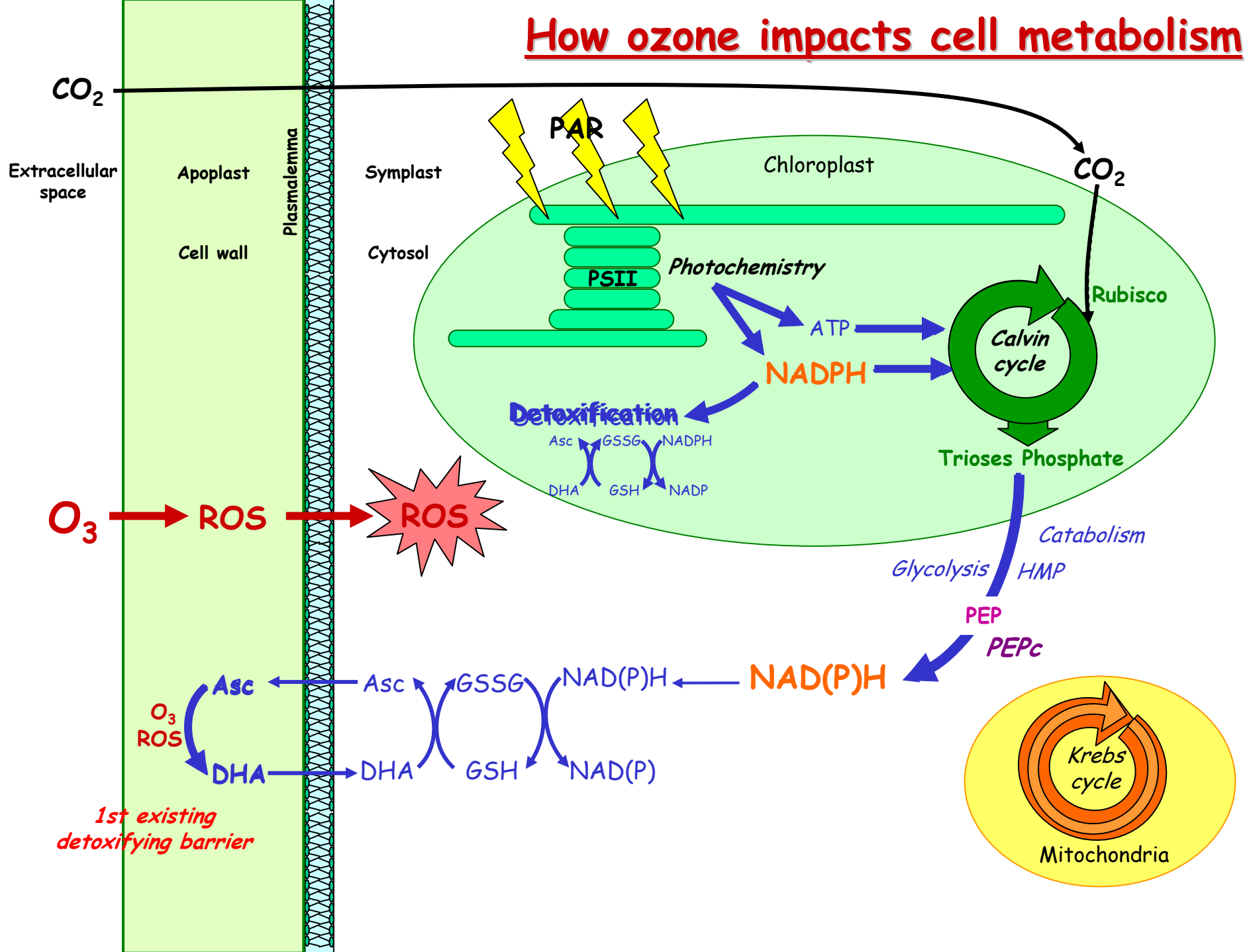
$$CUO = \sum_{i}^{j}(FO_3)_h$$

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

CUO (cumulative uptake ozone)

- Differences exist according to species, climate, etc…
- Effective ozone flux
- Detoxification/Metabolism

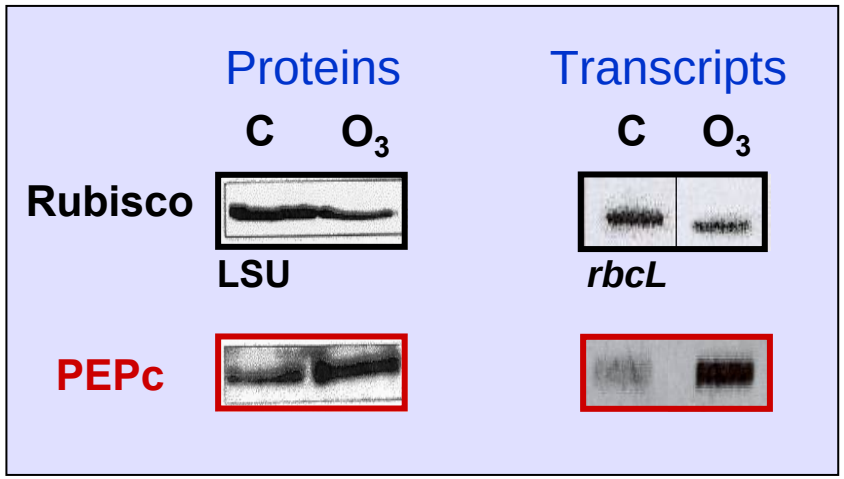
How ozone impacts cell metabolism



Effect of ozone on carboxylation

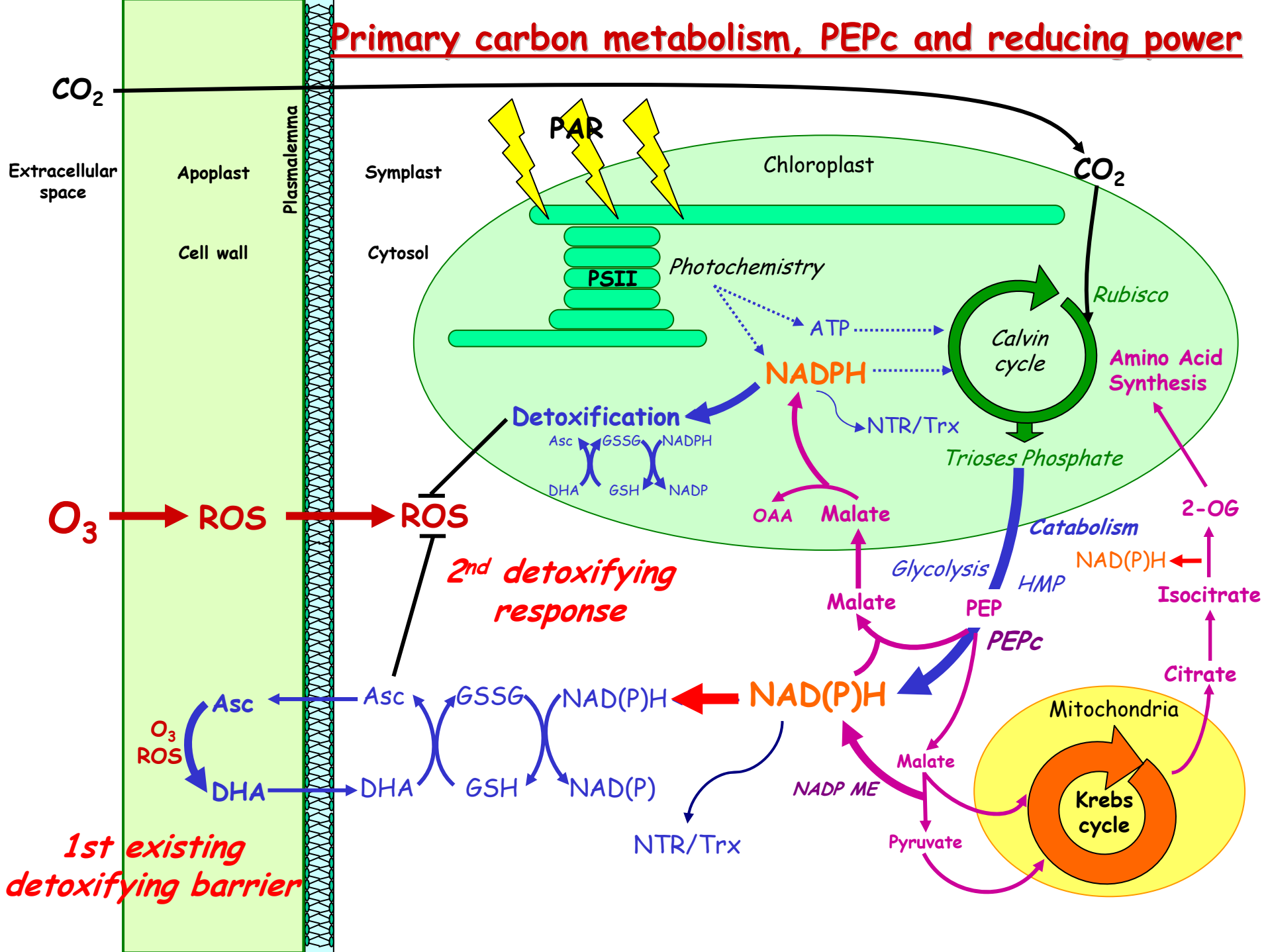
Rubisco & PEPc

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

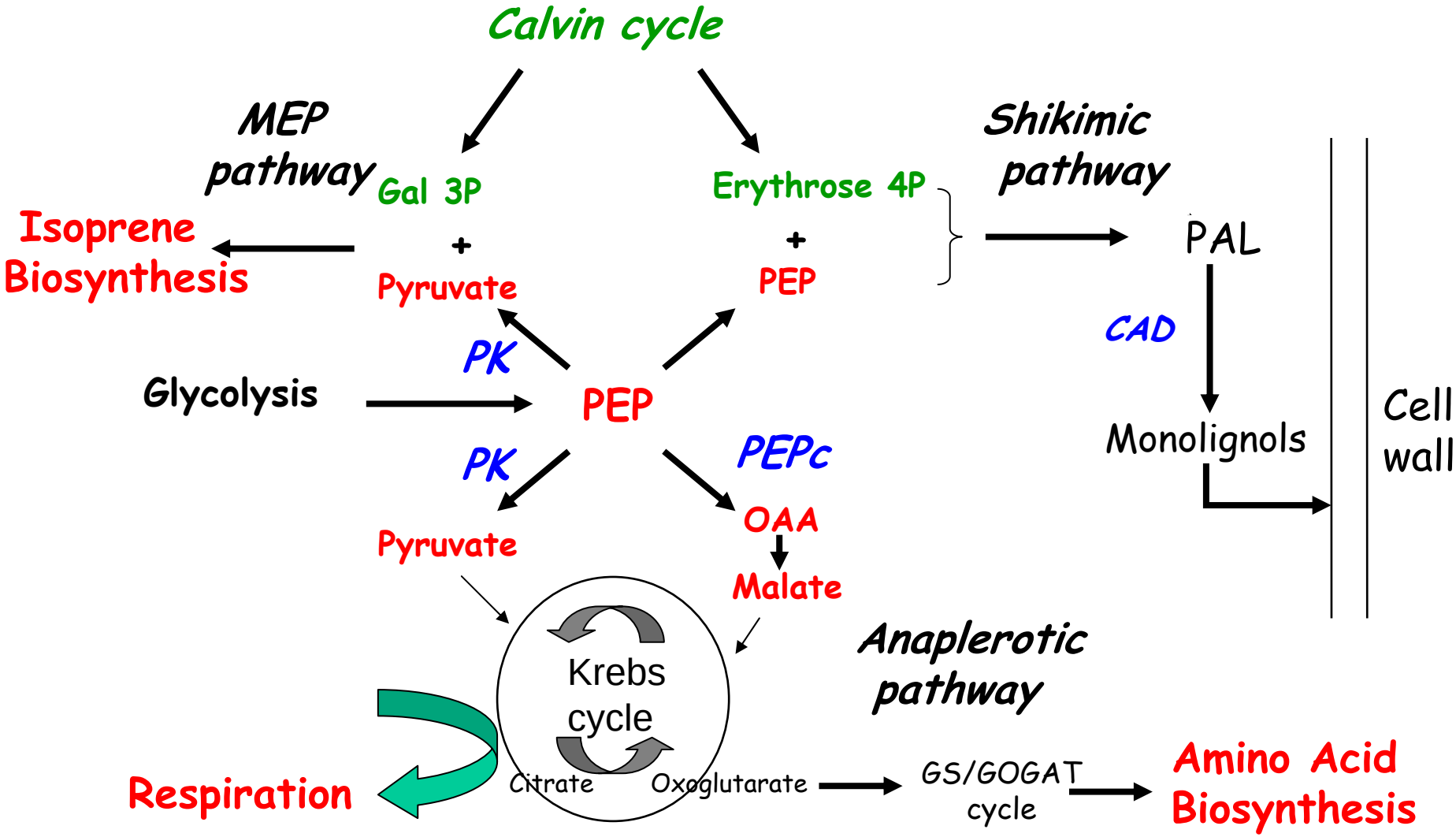


- In ozone-treated plants : higher contribution of PEPc to carboxylation activity
- putative transcriptional regulation of Rubisco and Pepc corresponding genes

Fontaine *et al.*, 1999, 2003; Pelloux *et al.*, 2001; Dizengremel, 2001


Primary carbon metabolism, PEPc and reducing power
CO_2
Extracellular space
Apoplast
Cell wall
Plasmalemma
Symplast
Cytosol
PAR
Chloroplast
CO_2
PSII
Photochemistry
ATP
NADPH
Calvin cycle
Rubisco
Amino Acid Synthesis
NTR/Trx
Detoxification
Asc
GSSG
NADPH
DHA
GSH
NADP
Trioses Phosphate
OAA
Malate
O_3
ROS
ROS
2nd detoxifying response
Catabolism
2-OG
NAD(P)H
Glycolysis
HMP
Isocitrate
Malate
PEP
PEPc
Citrate
Asc
Asc
GSSG
NAD(P)H
NAD(P)H
Mitochondria
O_3
ROS
DHA
DHA
GSH
NAD(P)
Malate
NADP ME
Krebs cycle
NTR/Trx
Pyruvate
1st existing detoxifying barrier

PEP as a key metabolite at crossroads for many purposes?



Which links could exist between PEPc increase, discrimination against $^{13}CO_2$ and WUE

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)

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

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

Ca = atmospheric $[CO_2]$

Ci = internal $[CO_2]$

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

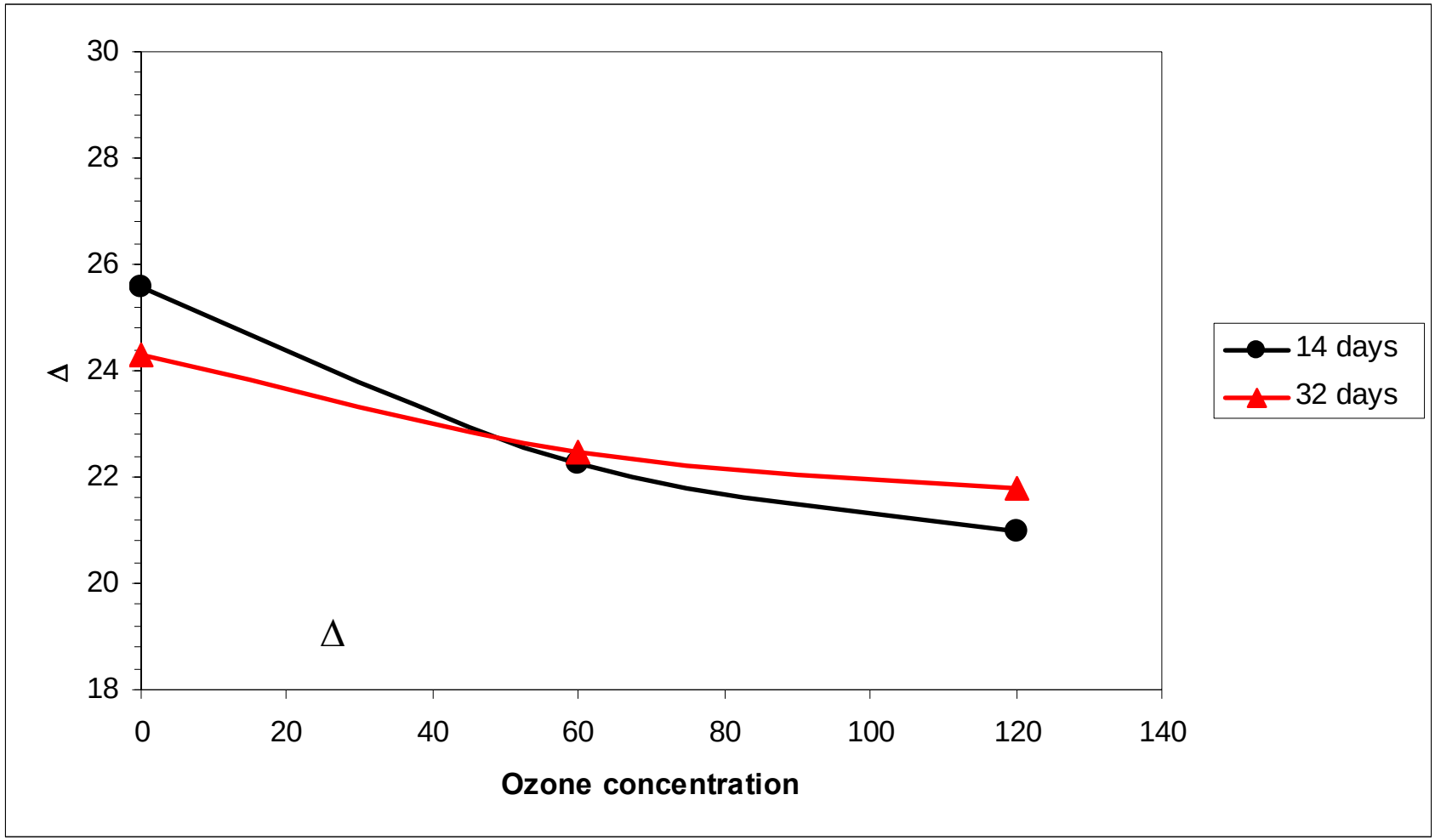
b_2 = isotopic discrimination due to Rubisco

As ozone induces an increase in the PEPc/Rubisco ratio,

-β and b increase,

-and consequently Δ and WUE should be modified.

Δ (calculated) changes in poplar leaves submitted to two external ozone concentrations

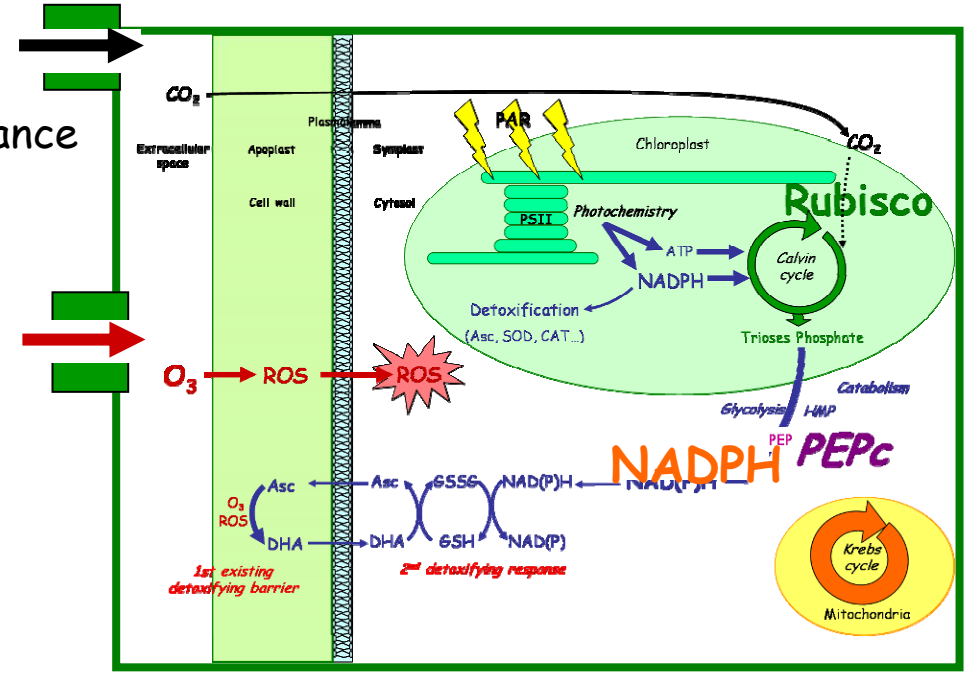


Dizengremel, Le Thiec, Afif, Jolivet, unpublished

Possible links between ozone flux, PEPc increase, isotopic discrimination against $^{13}CO_2$ and WUE

Gas exchanges

- CO_2 assimilation
- Stomatal conductance



Ozone flux

Biomass Productivity ?

Rubisco & PEPc

Δ ?

WUE

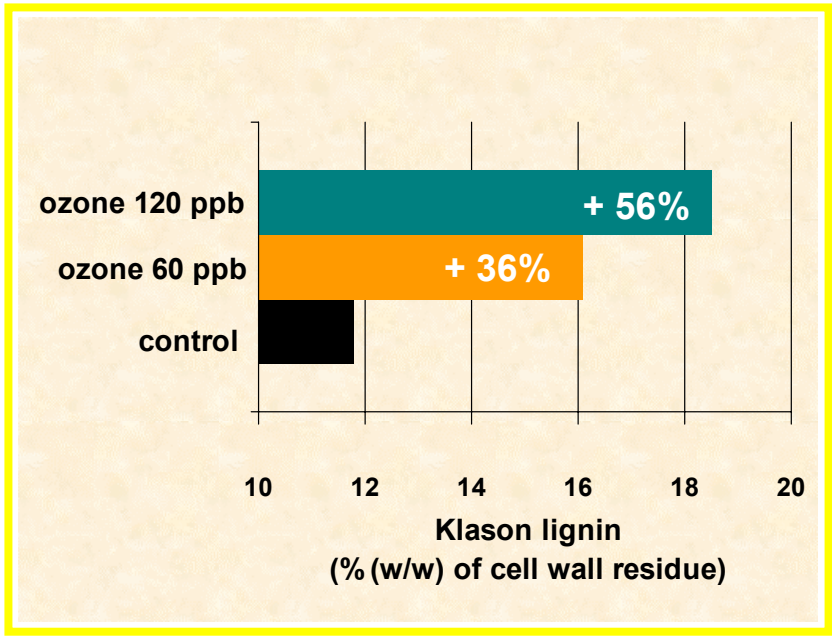
Is there any feedback impact on WUE and/or stomatal conductance ?

(Dizengremel et al., 2008)

Effect of ozone on lignification in poplar leaves:

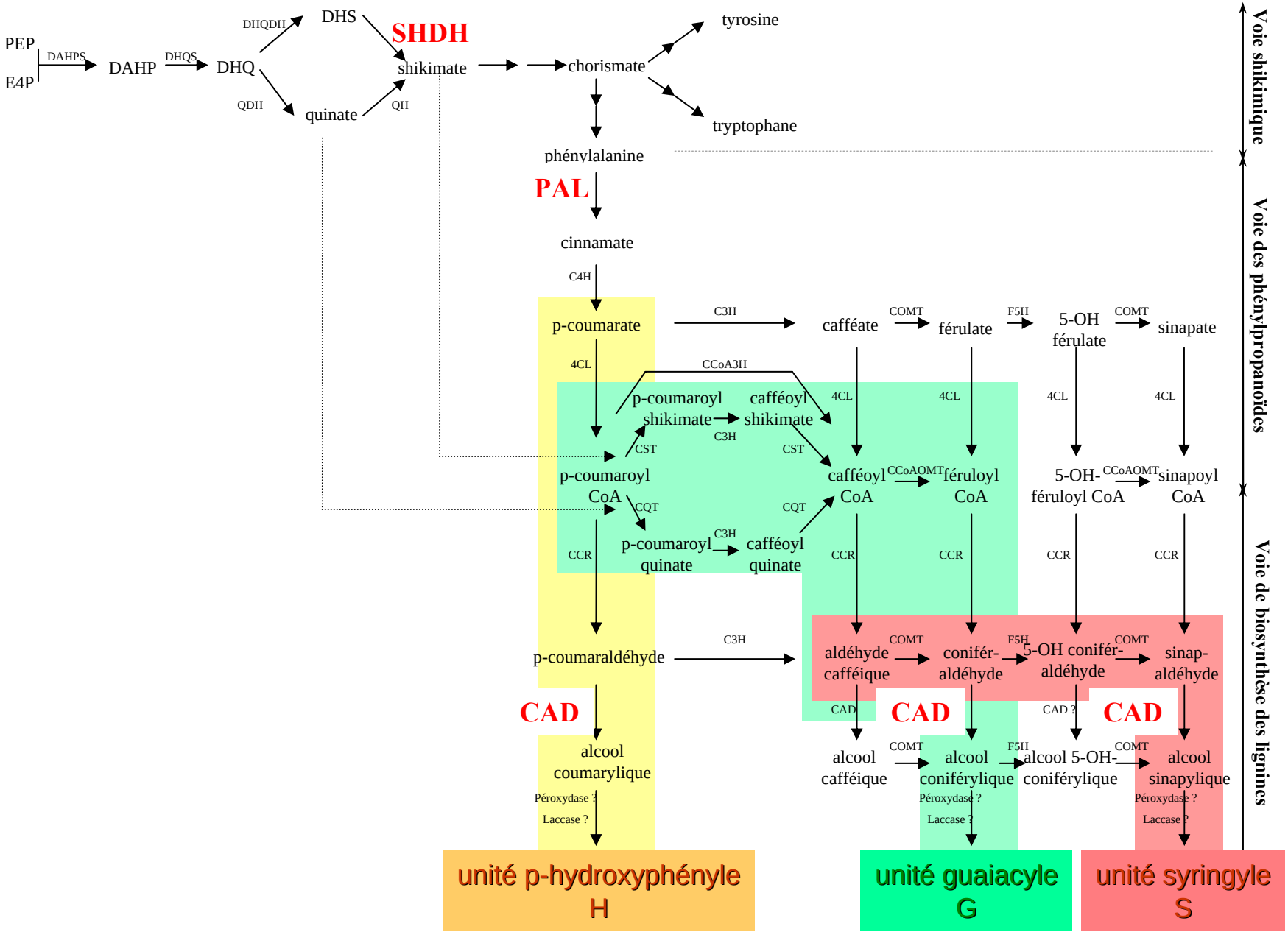
lignin content

Klason lignin



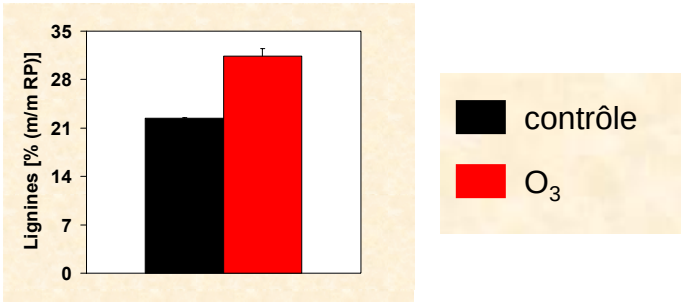
Increase of lignin biosynthesis in leaves under ozone treatment

Voies métaboliques conduisant à la synthèse des lignines



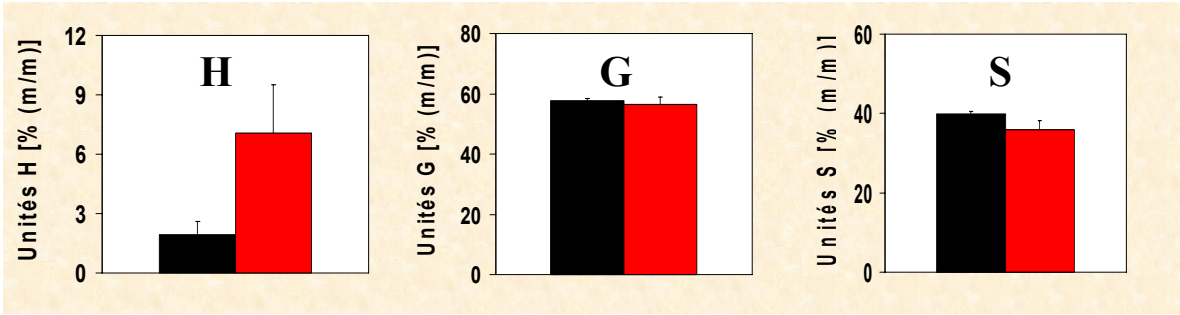
Effet de l'ozone

❖ **teneurs en lignines**



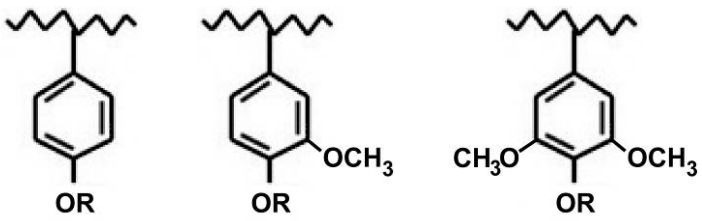
augmentation de 40%

❖ **composition monomérique des lignines**



lignines fortement enrichies en unités H
au détriment des unités S

❖ **deux types de liaisons : C-C et β-O-4**



H **G** **S**

plus de liaisons C-C possibles avec les unités H

plus de liaisons C-C: lignines plus condensées

↳ synthèse de lignines plus condensées sous un stress ozone