OZONE EFFECTS ON TREES

WHERE UPTAKE AND DETOXIFICATION MEET

Ludwig De Temmerman

Introduction

Ozone is the most important air pollutant

- doubled during the past 100 years

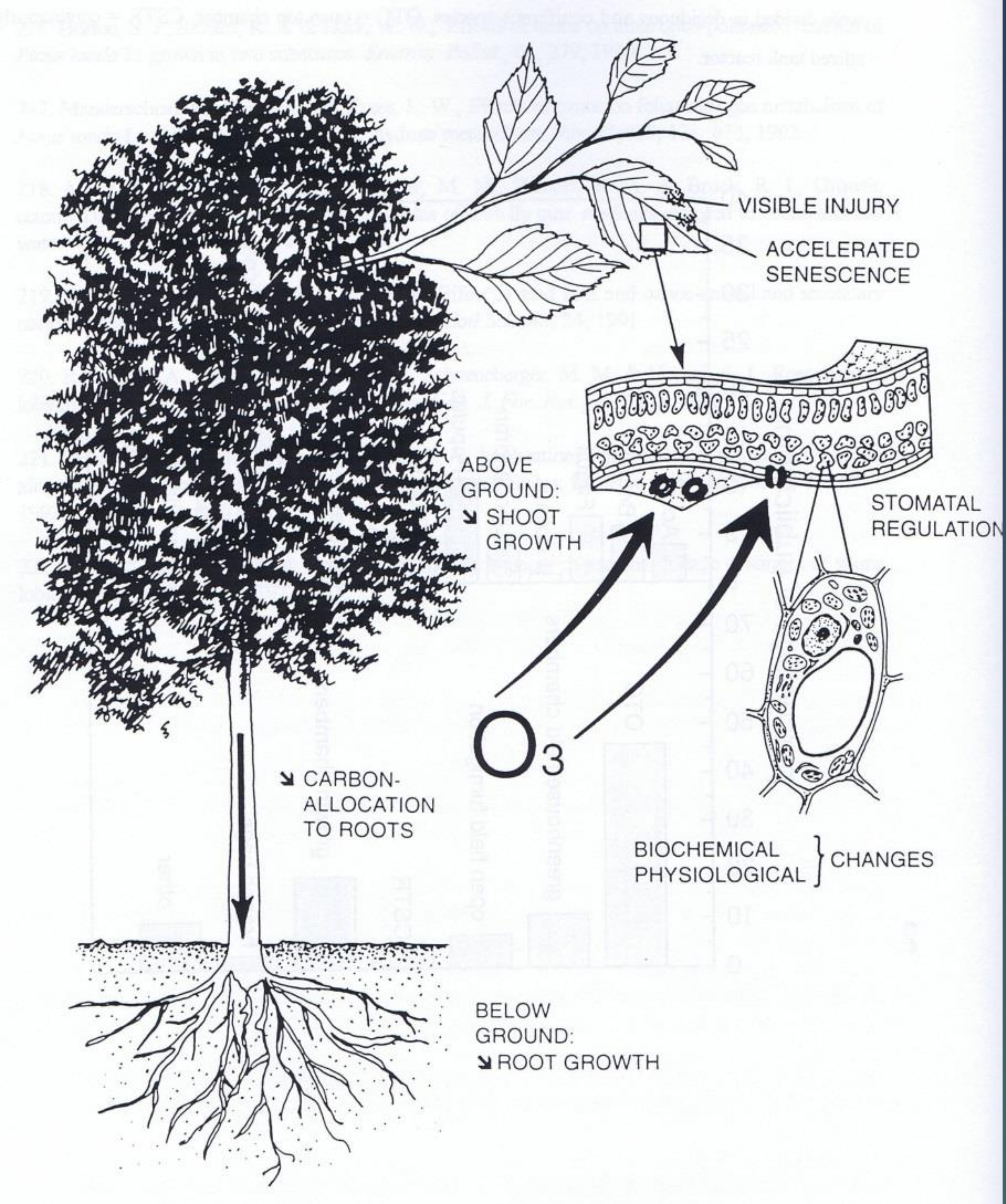
- future annual rate: + 0.8-2.5 %

- proportionally more important

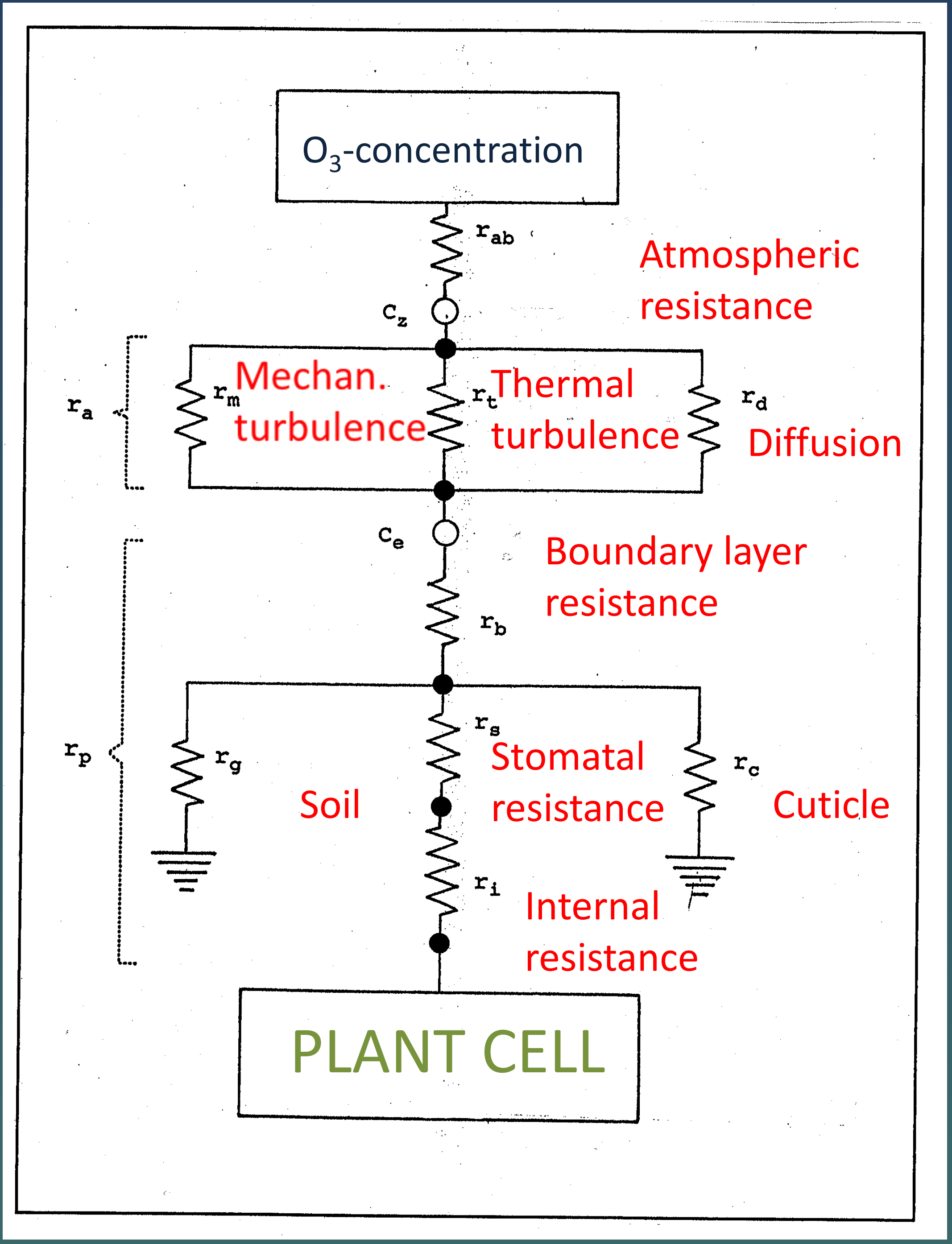
Introduction

- Complex situation in a town environment
  - Abundance of precursors hydrocarbons and NO_x
  - Ozone depletion by NO but synergistic effects with NO_2
  - Multiple stresses for trees
    - Drought stress (dust particles in stomatal opening)
    - Salt induced stress
    - Altered root development
    - Reduced root aeration
    - Soil impaction

Uptake


VISIBLE INJURY
ACCELERATED SENESCENCE
ABOVE GROUND: ↘ SHOOT GROWTH
STOMATAL REGULATION
O_3
↘ CARBON-ALLOCATION TO ROOTS
BIOCHEMICAL
PHYSIOLOGICAL
CHANGES
BELOW GROUND: ↘ ROOT GROWTH

Ozone deposition and plant uptake



Deposition velocity

- Experimentally determined by measurement of ozone at several heights above the ground taking into account micrometeorological parameters.
- When NO is present, there is an overestimation of ozone deposition :

$$O_3 + NO \longrightarrow NO_2$$

- Oxidant deposition ($O_x = O_3 + NO_2$)
- Example (grass): $v_d\, O_3 = 0.6 – 2.3$ cm s^{-1}

  $v_d\, O_x = 0.28 – 1.04$ cm s^{-1}

Duyzer et al. 1983

Deposition velocity for ozone

• Water surface:	0.01-0.05 cm s^{-1}
• Bare soil:	0.1 cm s^{-1}
• Field crops:	
– maize	0.2 – 0.7 cm s^{-1}
– Soybean	0.8 cm s^{-1}
• Mixed forest	0.25 – 1.25 cm s^{-1}
• Pine forest	1 cm s^{-1}

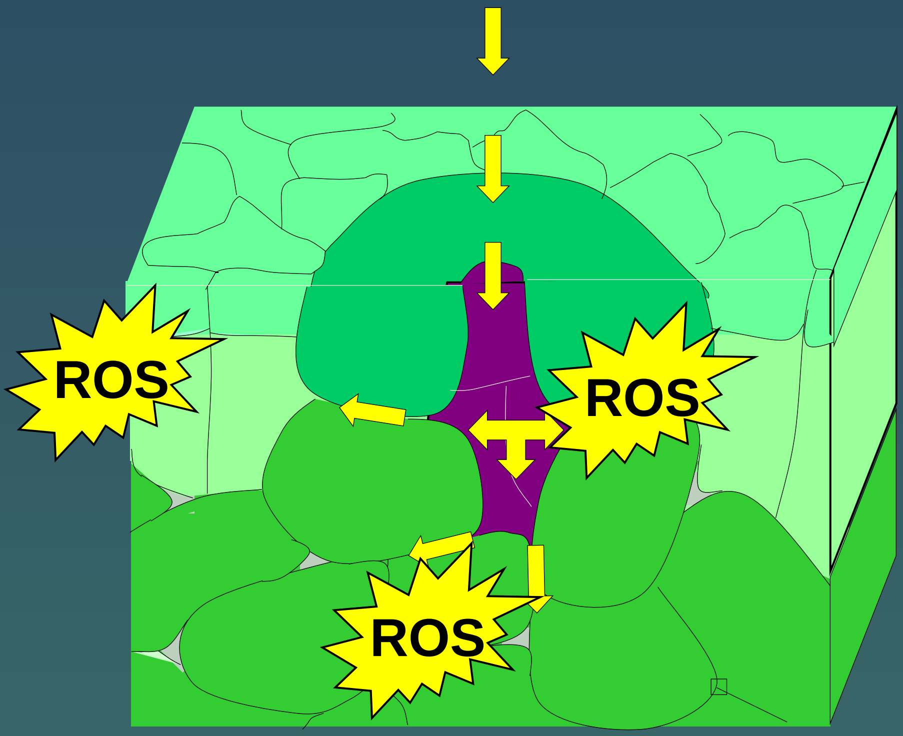
(Lenschow et al. 1982; Weseley et al. 1981; Galbally & Roy, 1980)

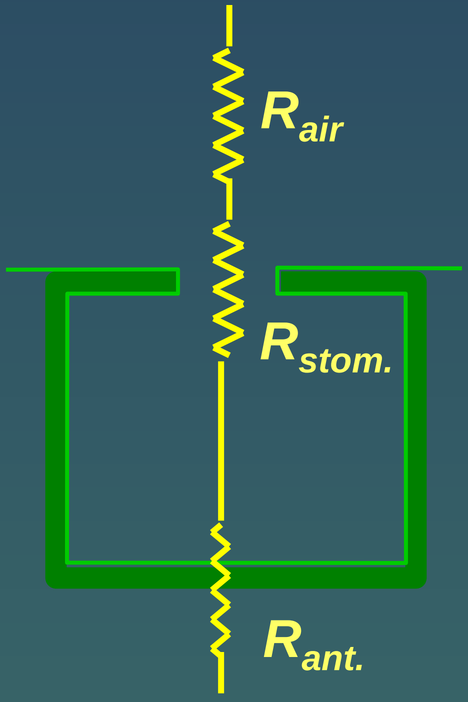
UPTAKE

- Ozone is taken up through the stomata
- Stomatal closure (altering the ozone uptake)
  - Dry soil
  - High wind speed
  - Low air humidity
  - Ozone effects on the guard cells
  - Effects of other pollutants (acidifcation)
- Stomatal opening (promoting ozone uptake)
  - Warm and humid environment (greenhouse)
  - Abundant irrigation
- Ozone flux : quantity per unit surface and time

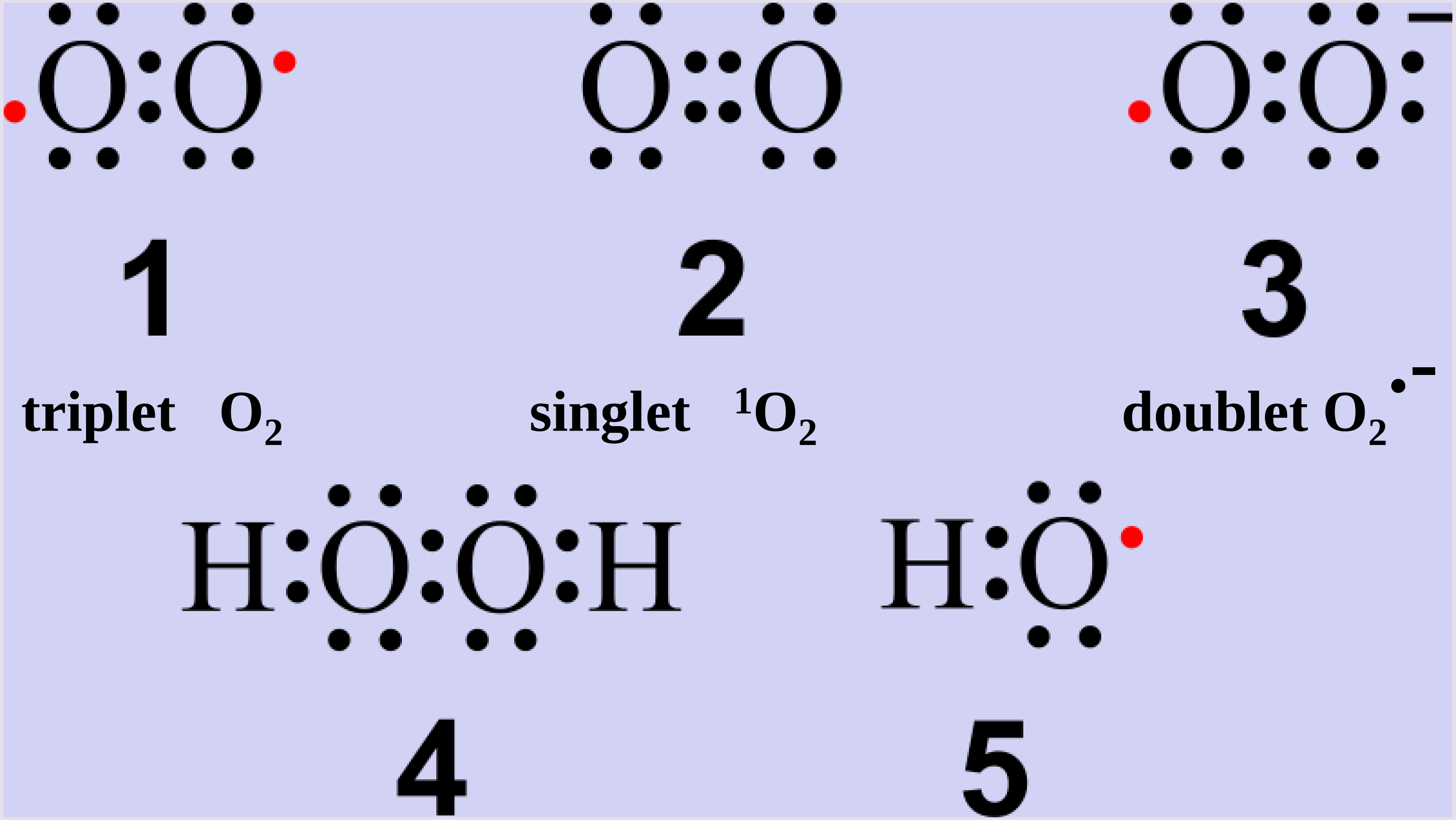
Ozone Produces
Reactive Oxygen Species

Ozone (O_3)



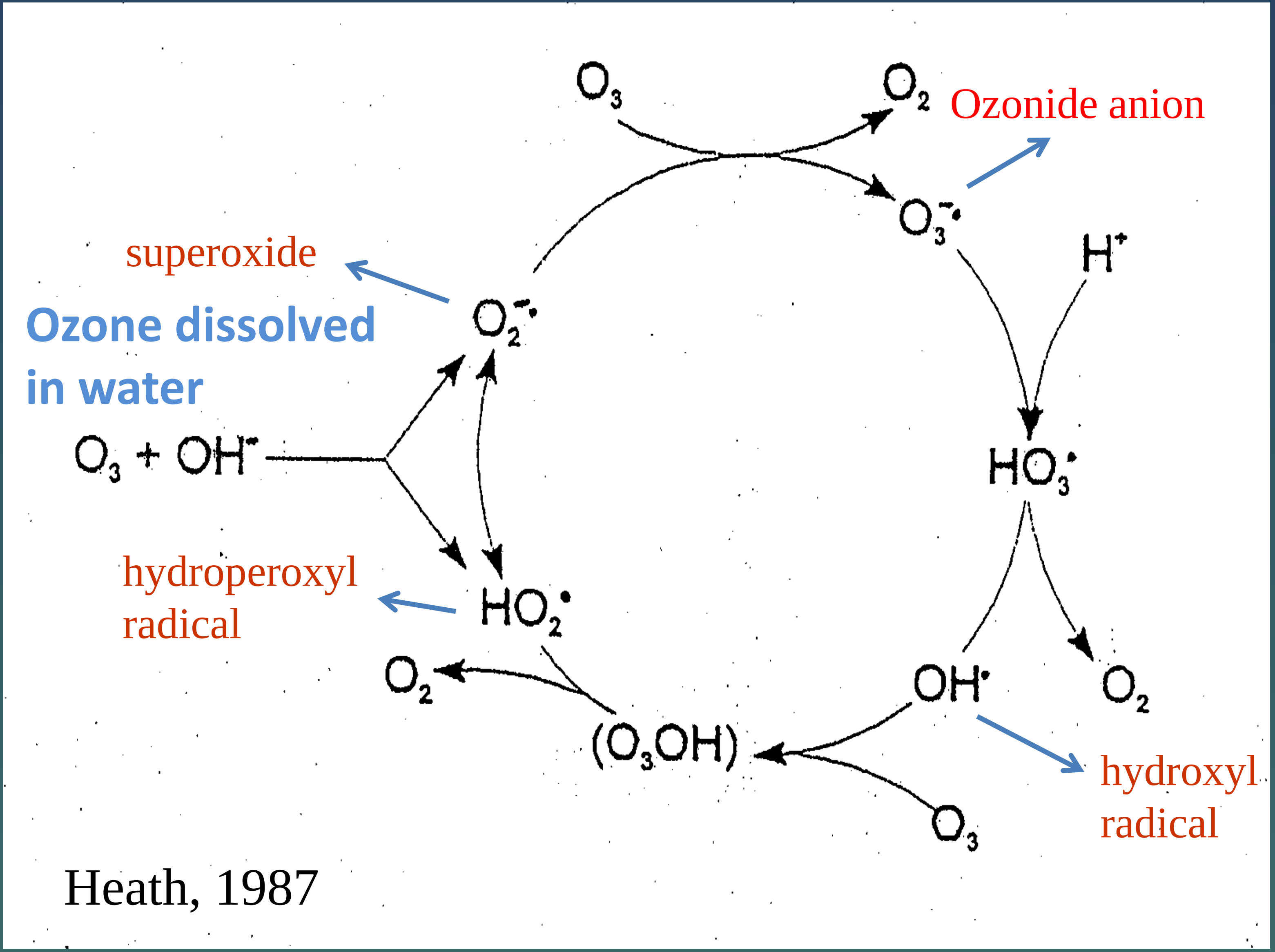


Reactive oxygen species (ROS)

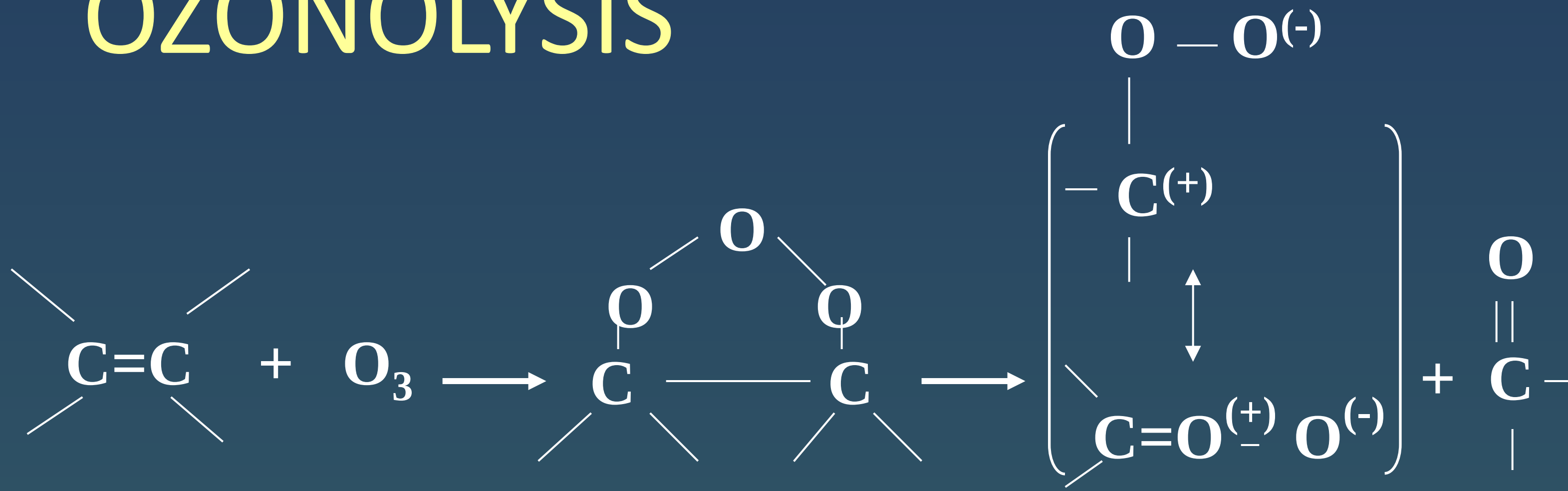


singlet H_2O_2 doublet $OH^{\bullet}$

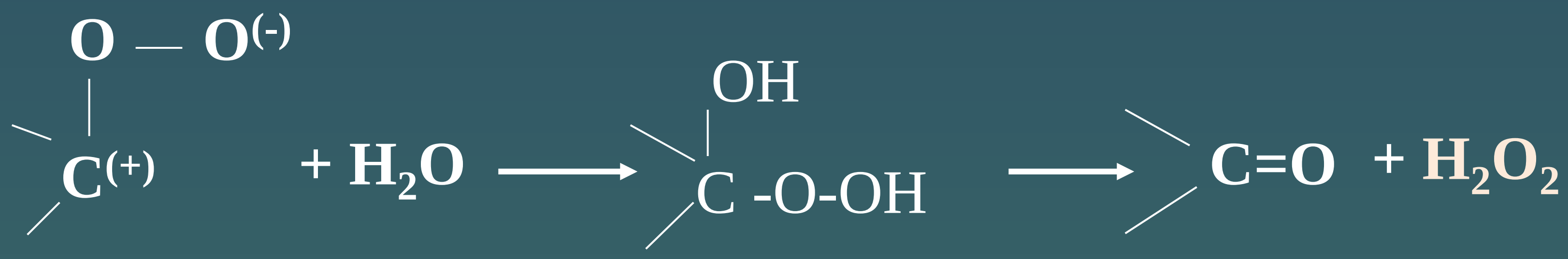
Formation of ROS



OZONOLYSIS



alkene **molozonide** **Criegee zwitterion**



hydroxyhydroperoxide **ketone** **Hydrogen peroxide**

Hippeli & Elstner, 1996

Biogenic alkenes

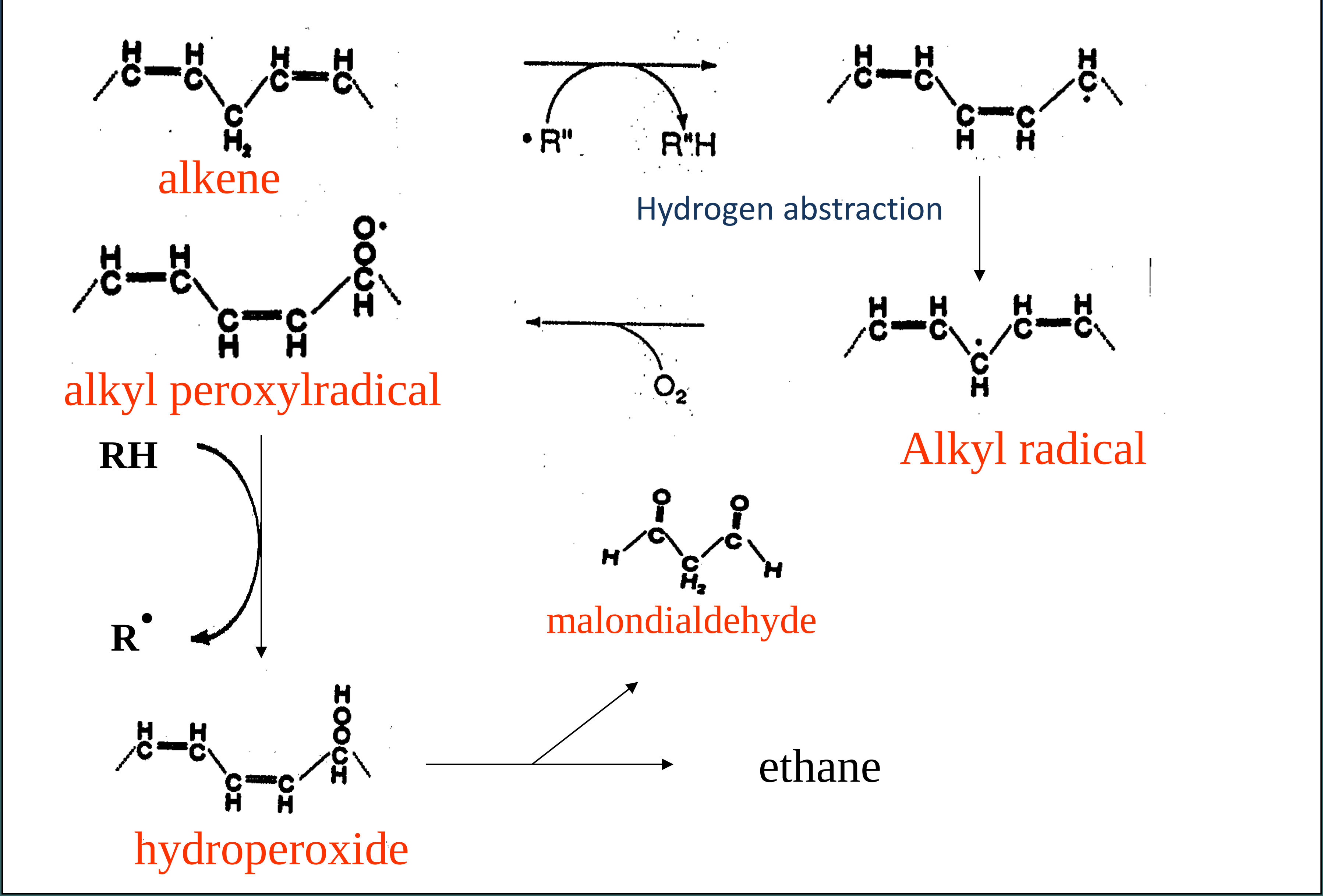
- Alkenes are emitted by plants
  - Ethylene (stress ethylene)
  - Isoprene and monoterpenes (red spruce, Norway spruce and silver fir)

Ozonolysis:

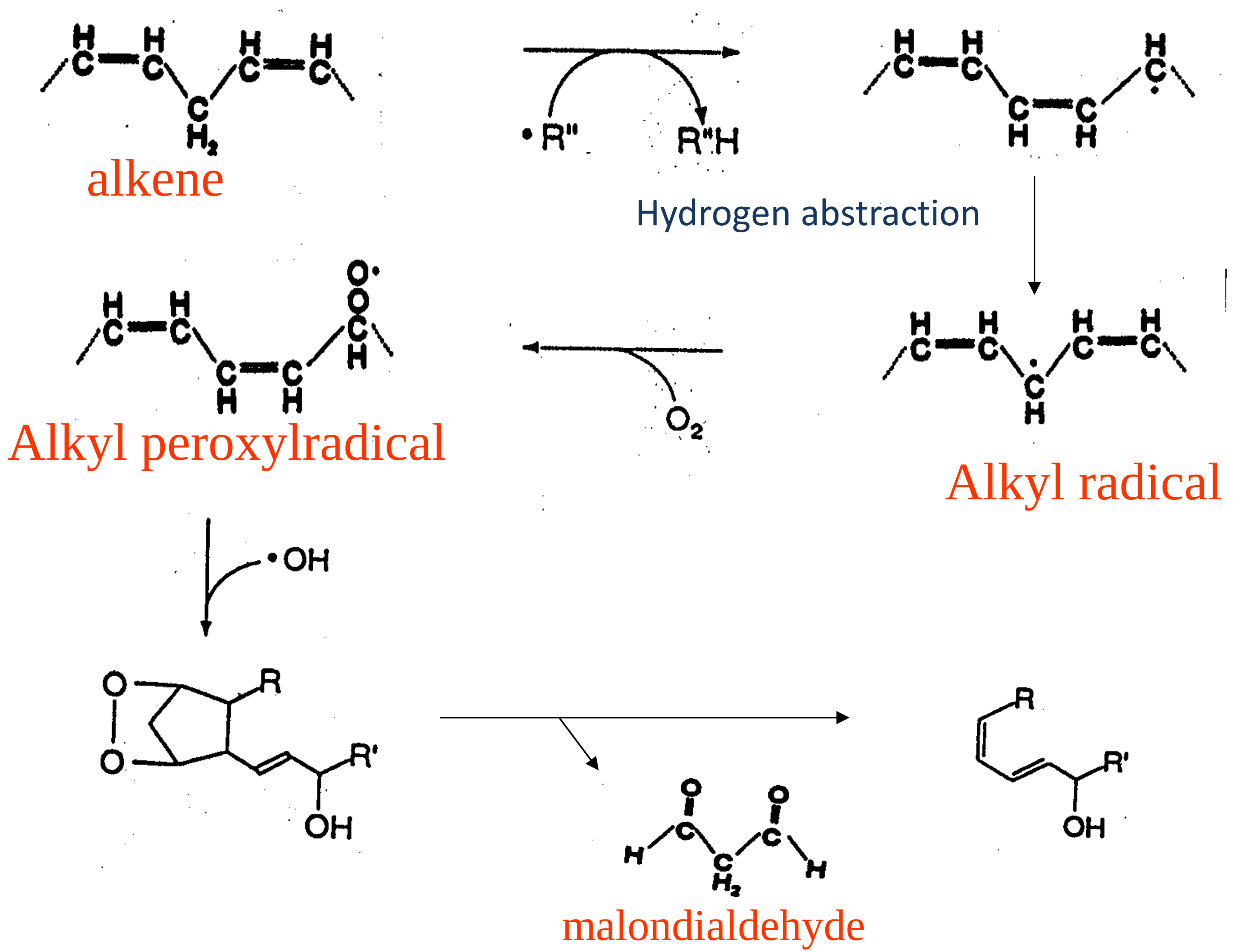
- Less soluble ozone is transferred into a highly soluble ROS !!!

Hewitt et al. 1990

LIPID PEROXIDATION CHAIN REACTION



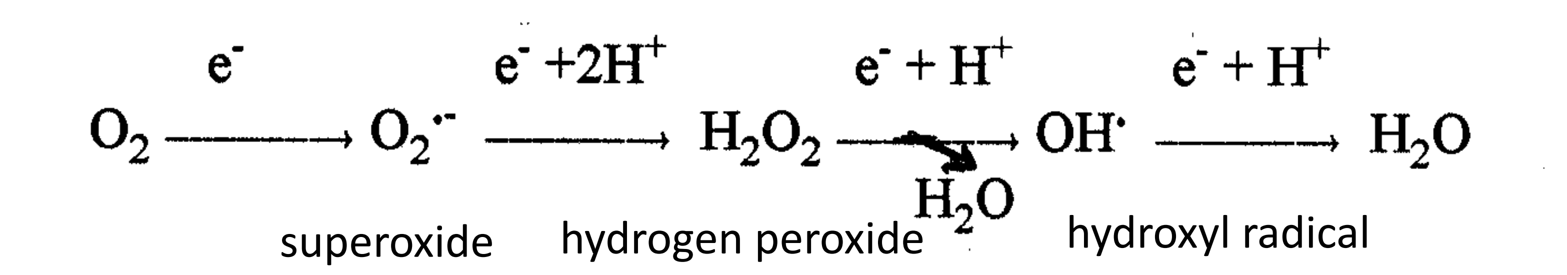
LIPID PEROXIDATION



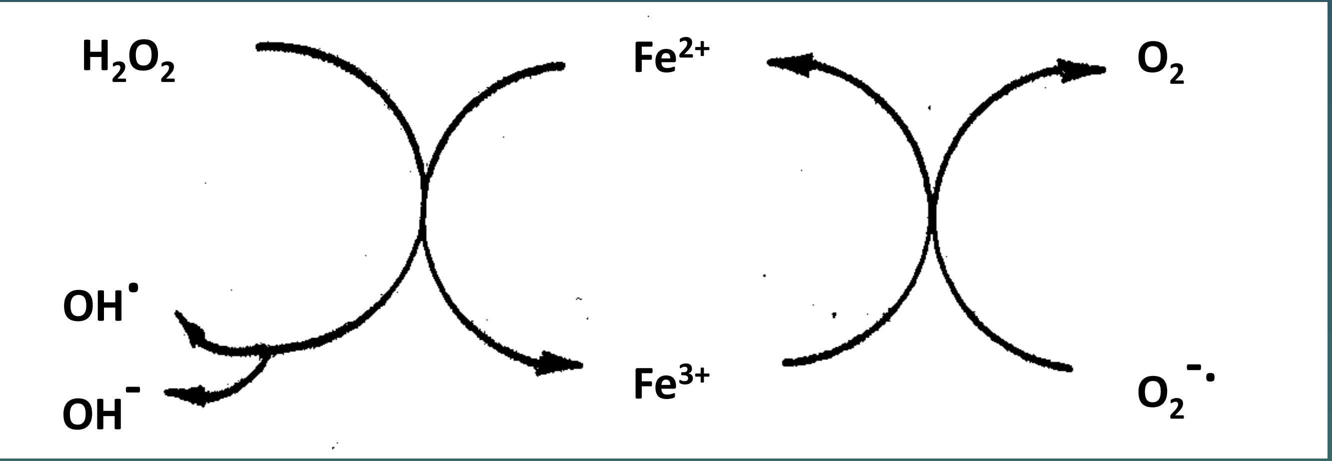
Reactive oxygen species in the symplast

- Stress effects (not specific for ozone)
  - formation of H_2O_2
  - generation of superoxide ($O_2^{-\cdot}$)
  - formation of hydroxyl radicals ($OH^{\cdot}$)
- Antioxidants
  - GSH - Ascorbate - carotenoids - α-tocopherol
- Enzymes
  - SOD (superoxide dismutase)
  - catalase
  - peroxidases

Oxidative stress



Haber-Weiss reaction



Haber & Weiss, 1932

Defence systems

- Antioxidants
  - Ascorbate (Vitamin C)
  - α-tocopherol (Vitamin E)
  - Glutathion (tripeptide composed of cysteine glycine and glutamic acid).
  - Carotenoids
  - Phenolics
- Enzymes
  - Catalase
  - Superoxide dismutase
  - peroxidase

Enzymatic defence

To counteract the Haber-Weiss reaction

- Superoxide dismutase

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

$$HO_2^{\cdot} + HO_2^{\cdot} \longrightarrow H_2O_2 + O_2$$

$$HO_2^{\cdot} + O_2^{\cdot -} + H^+ \longrightarrow H_2O_2 + O_2$$

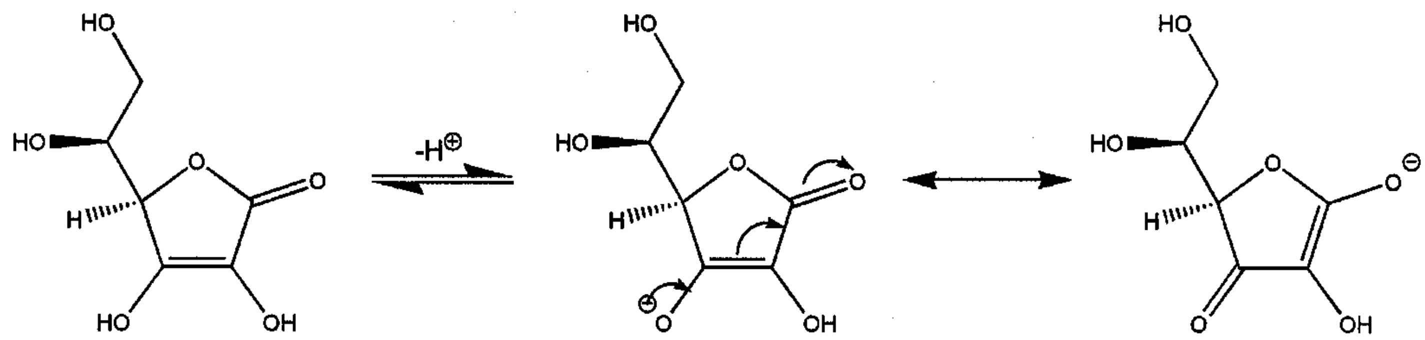
- catalase

$$H_2O_2 + H_2O_2 \longrightarrow 2\,H_2O + O_2$$

- peroxidase

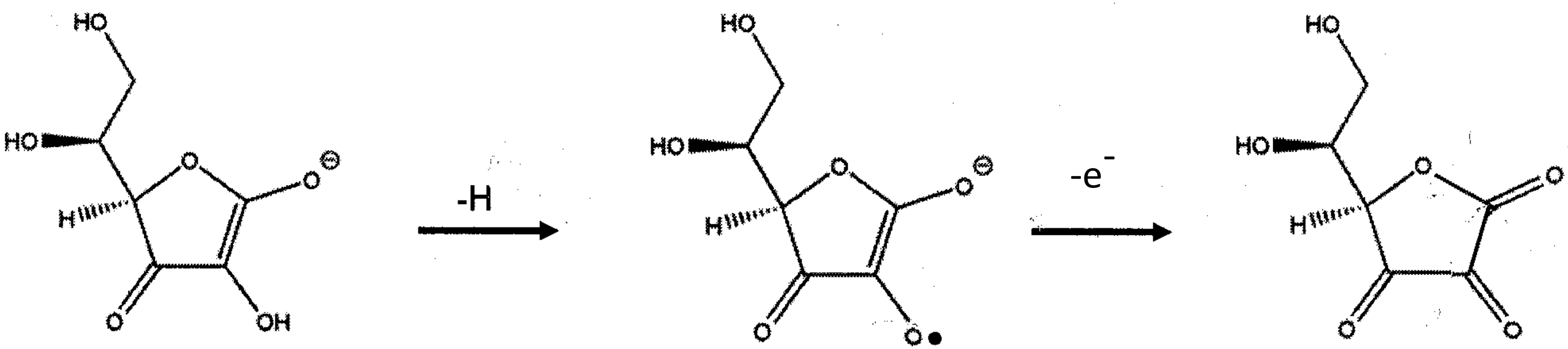
$$H_2O_2 + RH_2 \longrightarrow 2H_2O + R$$

Oxidation of ascorbate



Ascorbic acid

Ascorbate

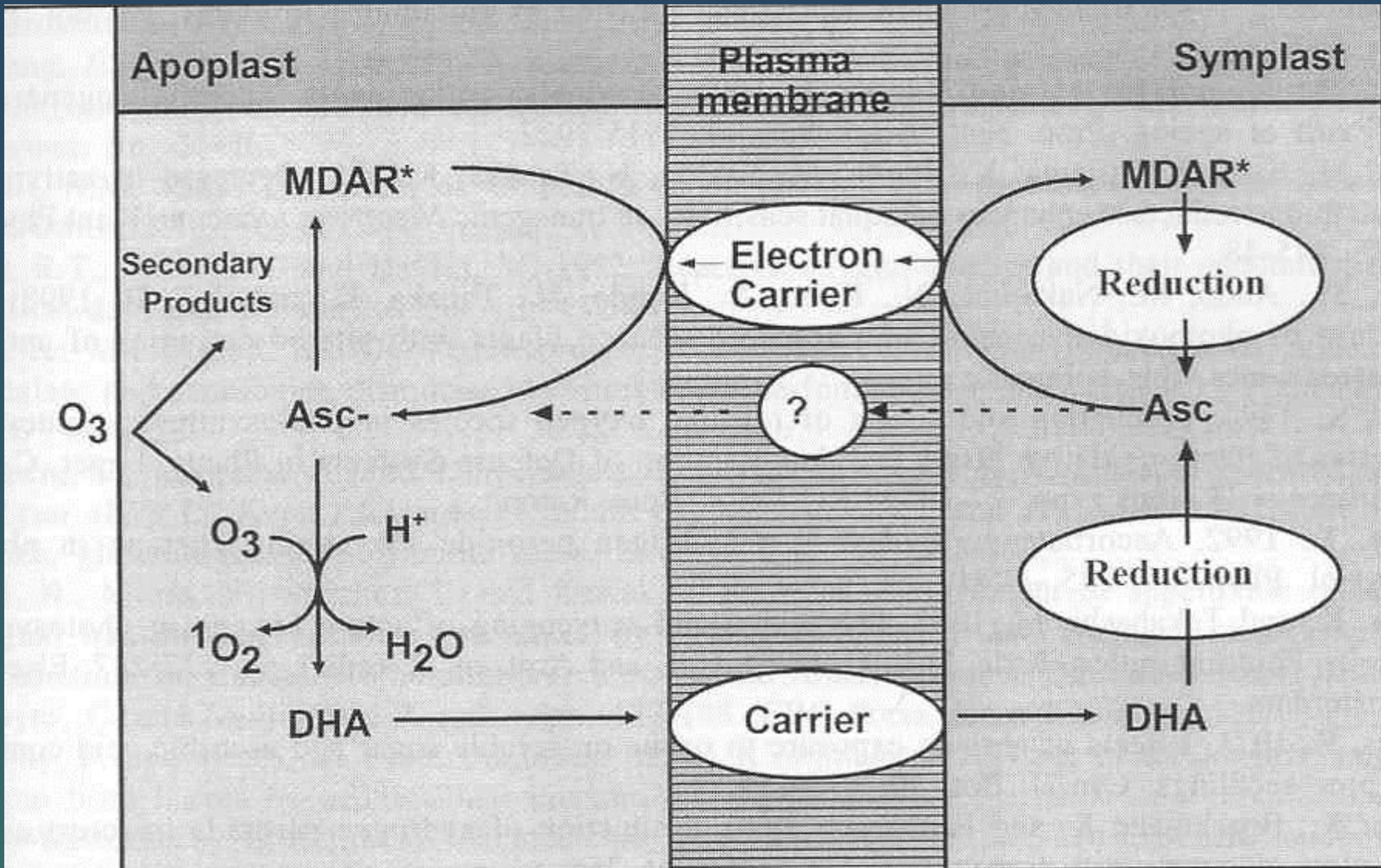


Ascorbate

Monodehydroascorbate

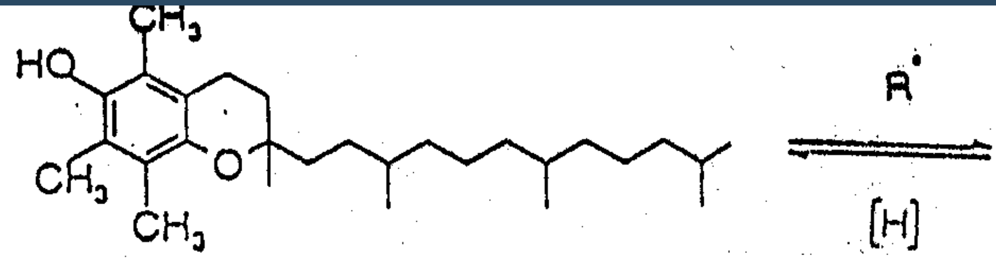
Dehydroascorbate

Regeneration of ascorbate

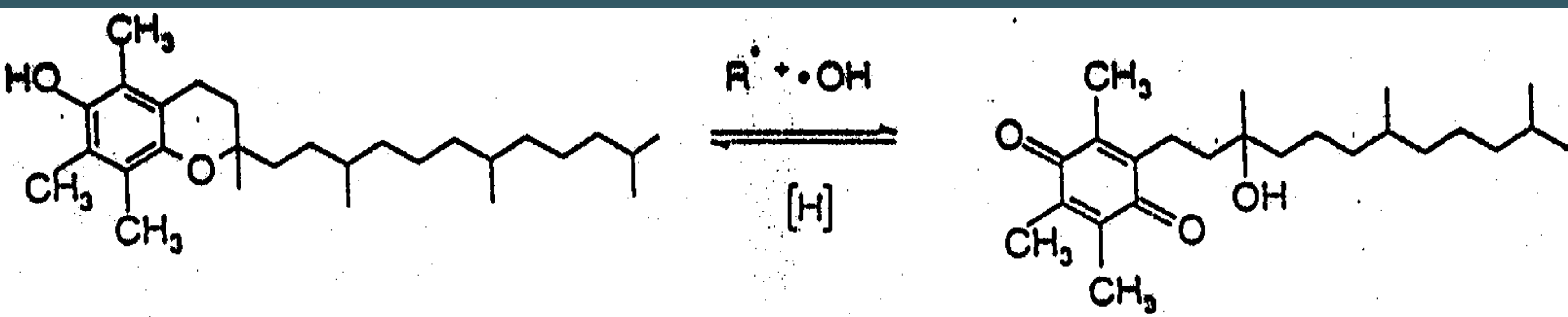


Polle, 1998

The role of tocopherols

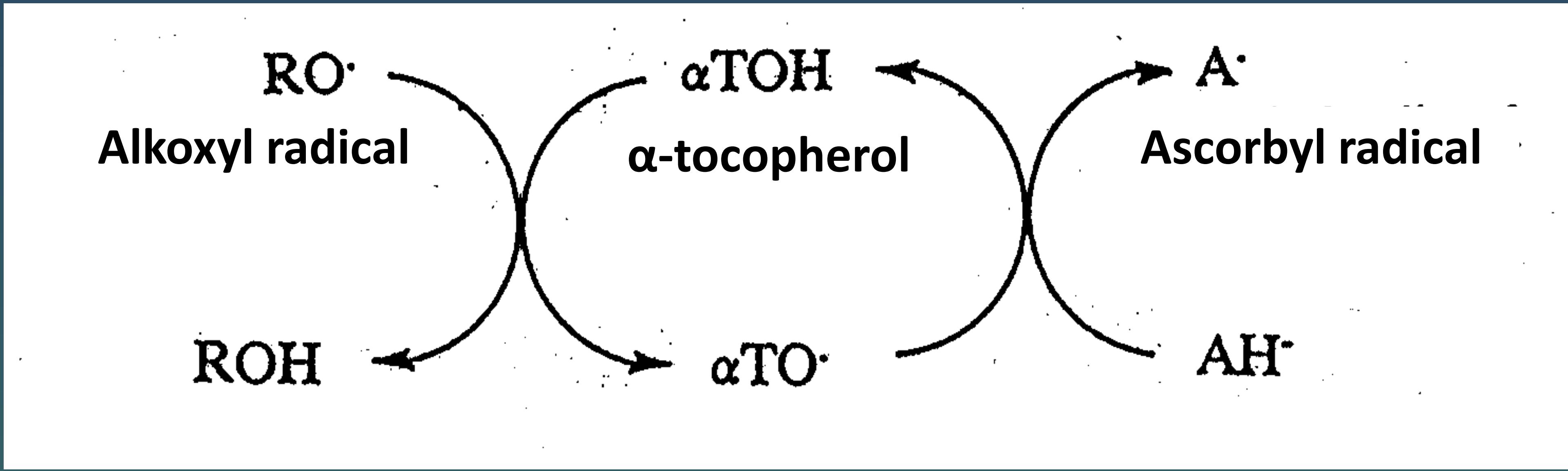


α-tocopherol α-tocopheryl radical



α-tocopherol α-tocopherylquinone

Alkoxyl radical scavenging

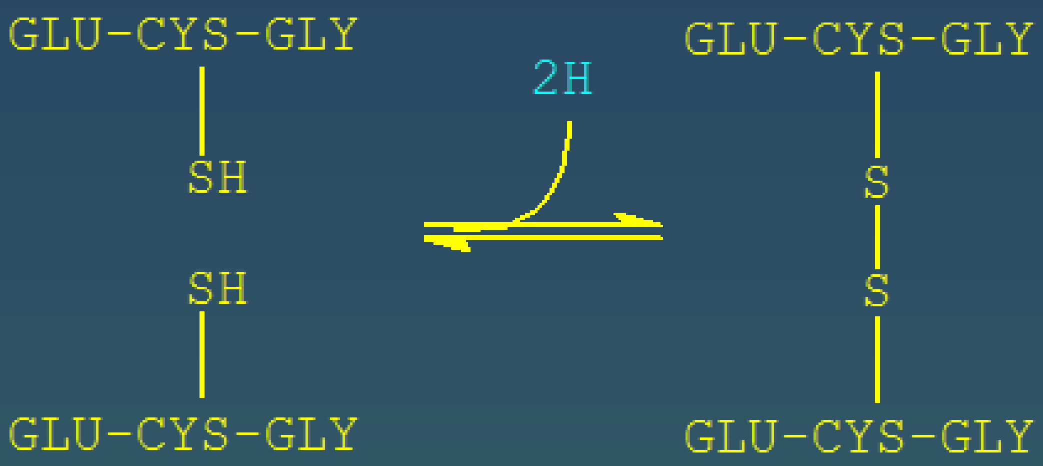


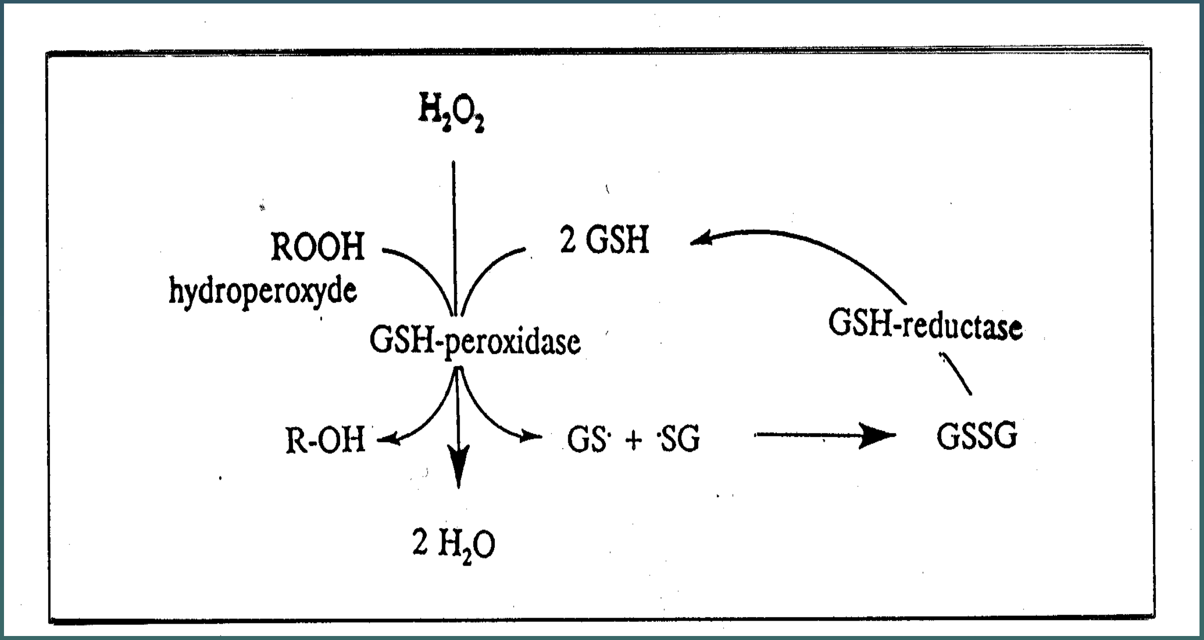
Alcohol

α-tocopheryl radical

Ascorbate

Glutathion





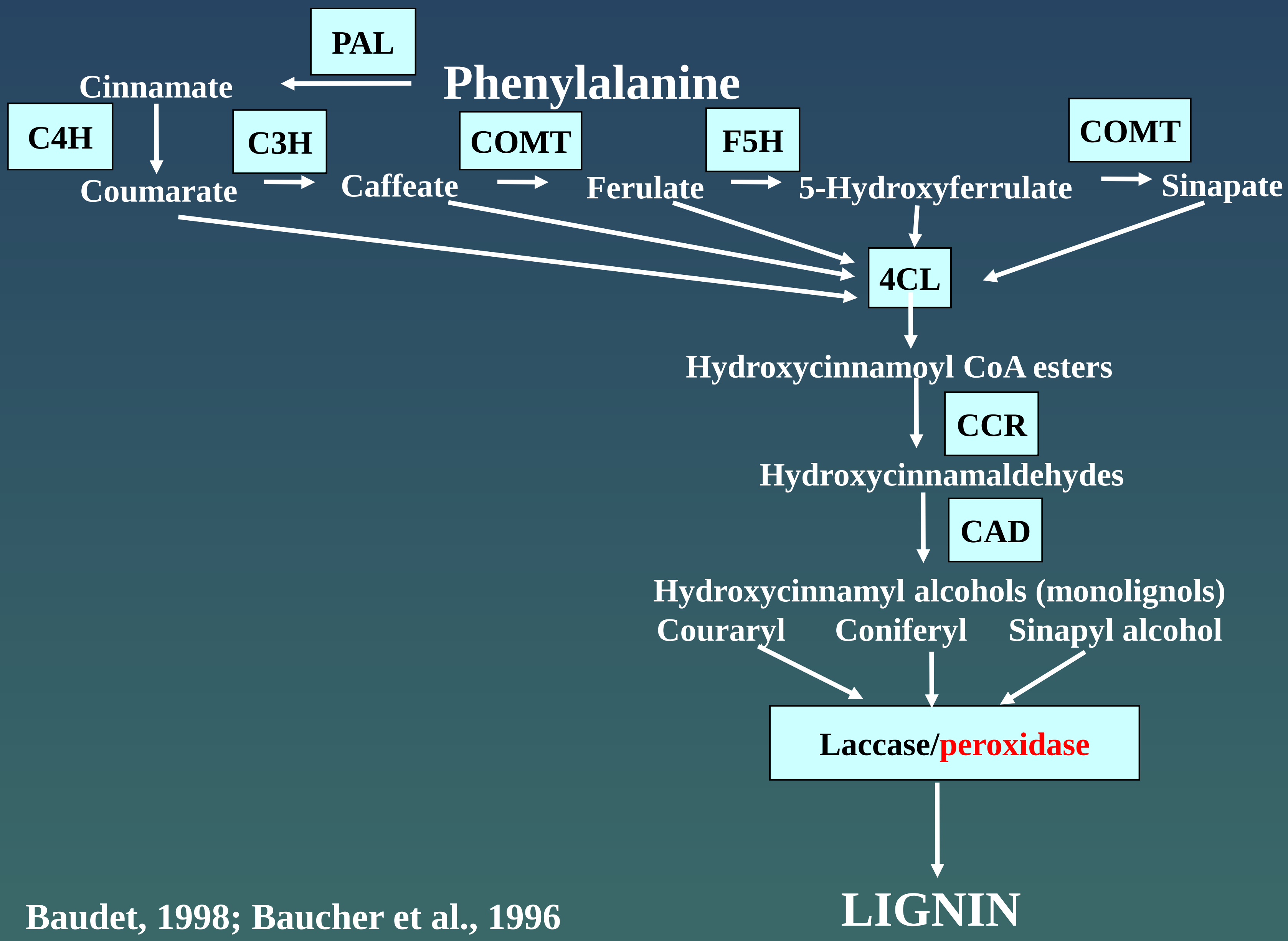
Hagege, 1991

Antioxidative power

What is the role phenolics as antioxidants?

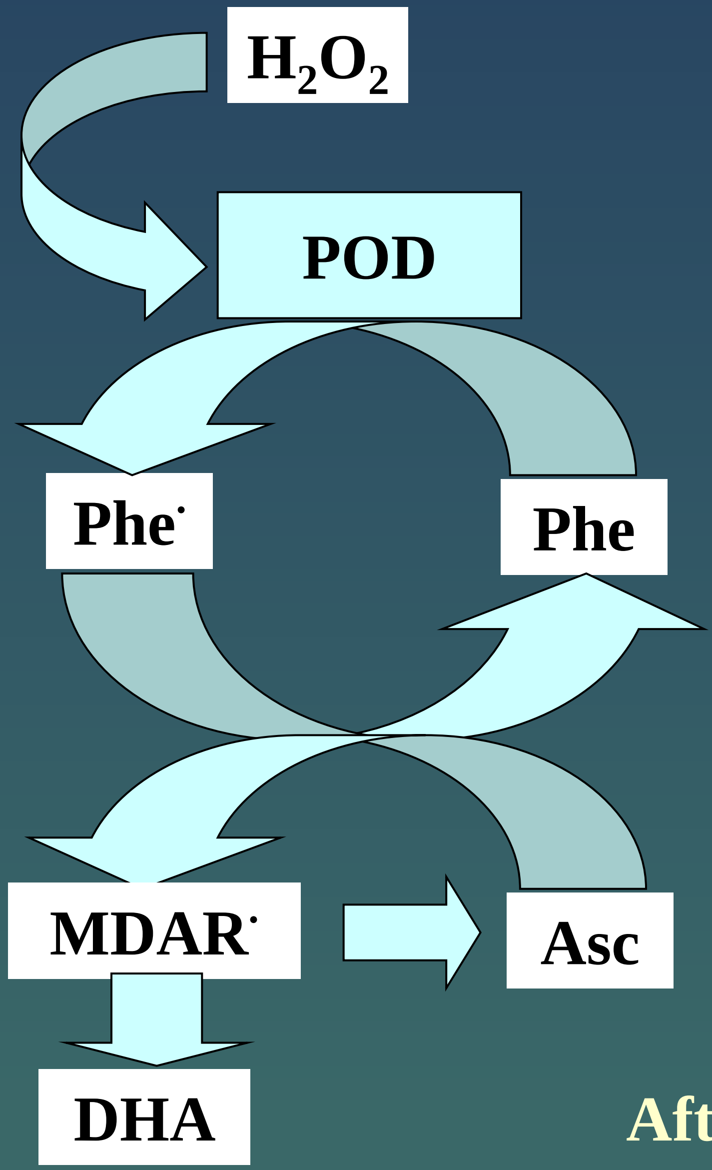
- Phenolic acids
  - ferulic acid
  - caffeic acid
  - catechol
  - syringic acid
  - p-coumaric acid
  - …..
- Enzymes: peroxydases

Lignin biosynthese



Baudet, 1998; Baucher et al., 1996

Apoplastic phenolics

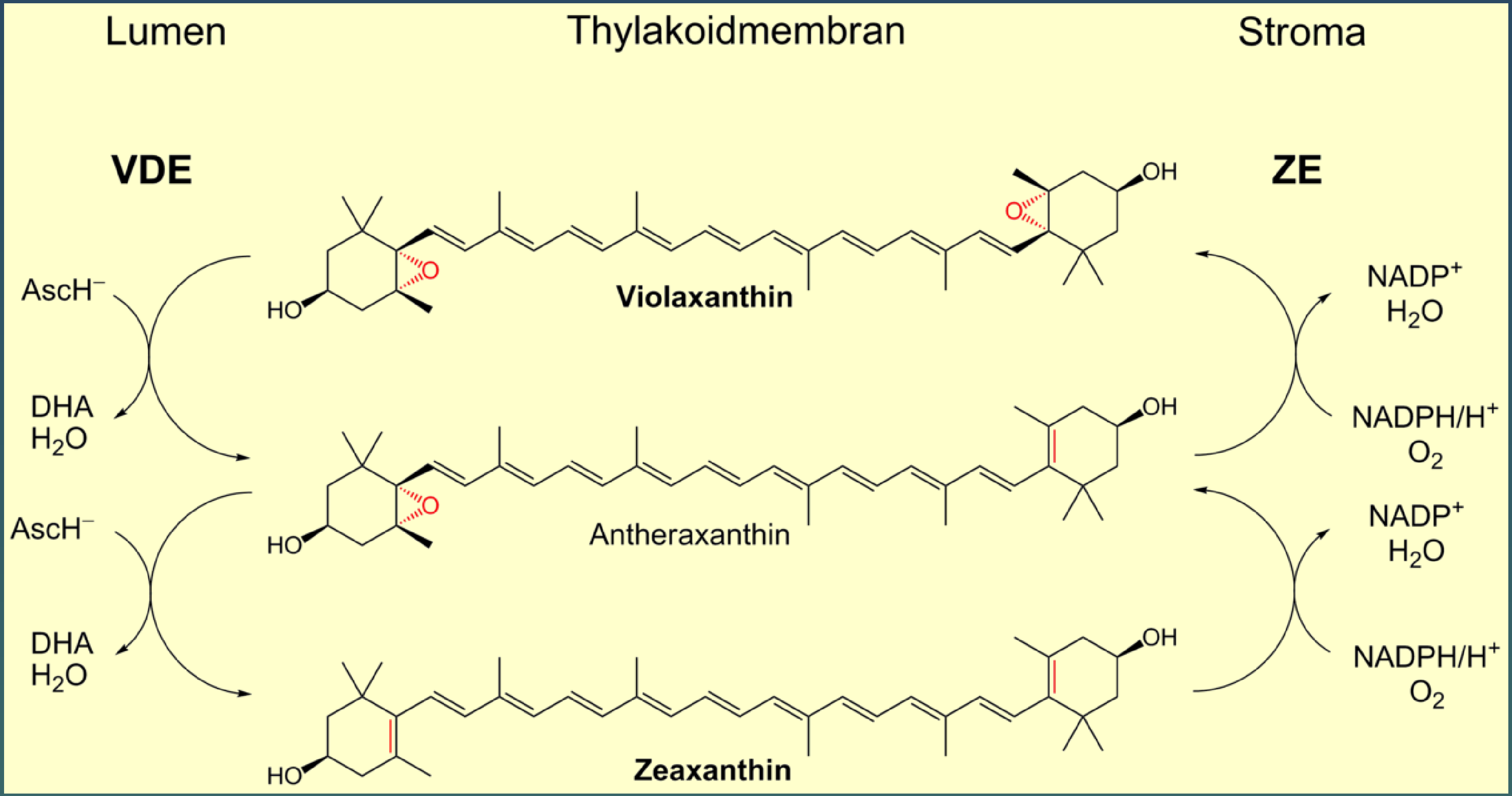


After Polle, 1998

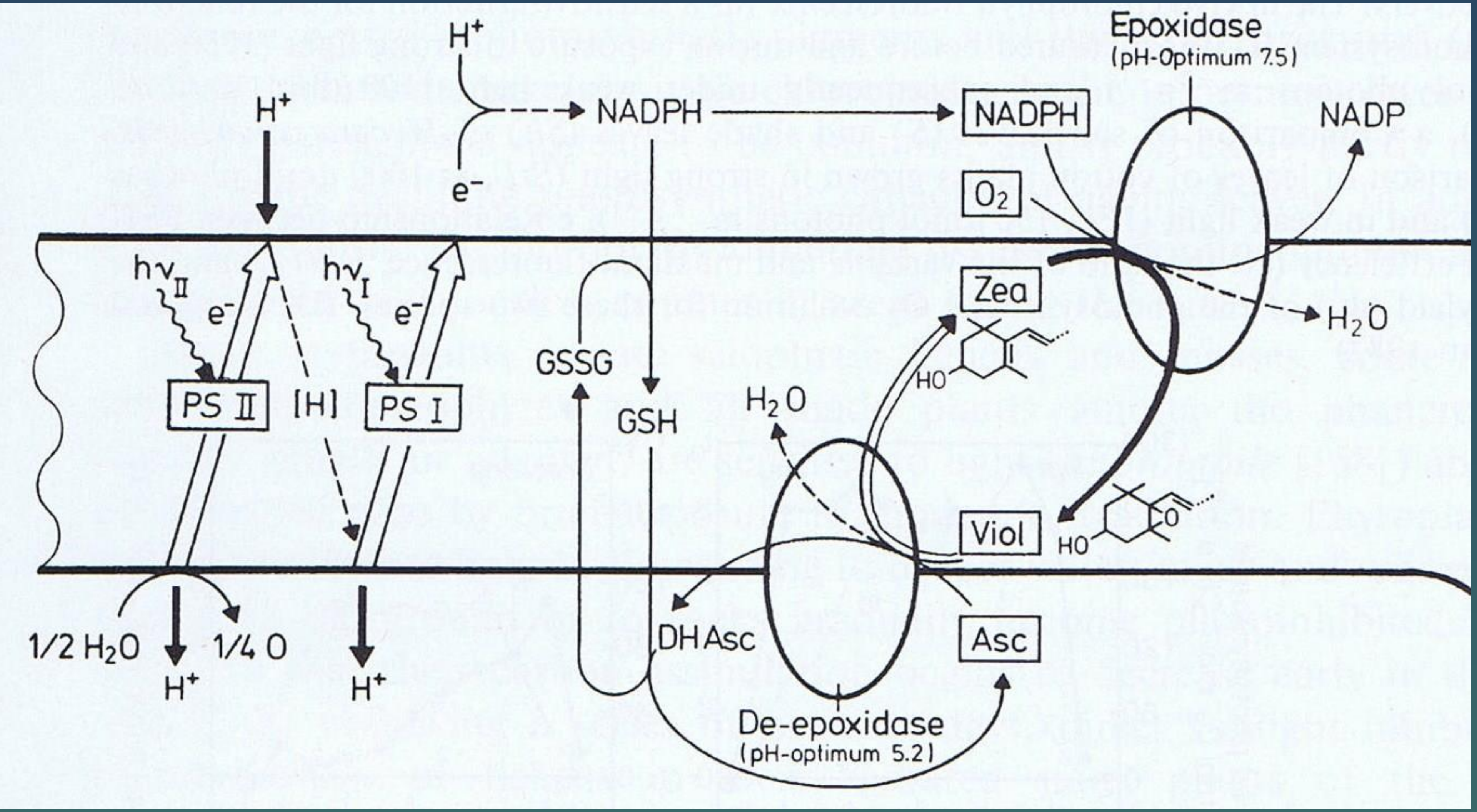
Xanthophylls important Carotenoids

- Xanthophyll cycle: enzymatic removal of epoxy groups from violaxanthin and antheraxanthin to create the de-epoxidised xanthophyll zeaxanthin.
- They stimulate energy dissipation by non-photochemical quenching (chlorophyll fluorescence) to protect against photo inhibition.
- During light stress **violaxanthin** is converted into **zeaxanthin** via the intermediate **antheraxanthin**.
- Acting as lipid protective anti-oxidant

Xanthophylls important Carotenoids

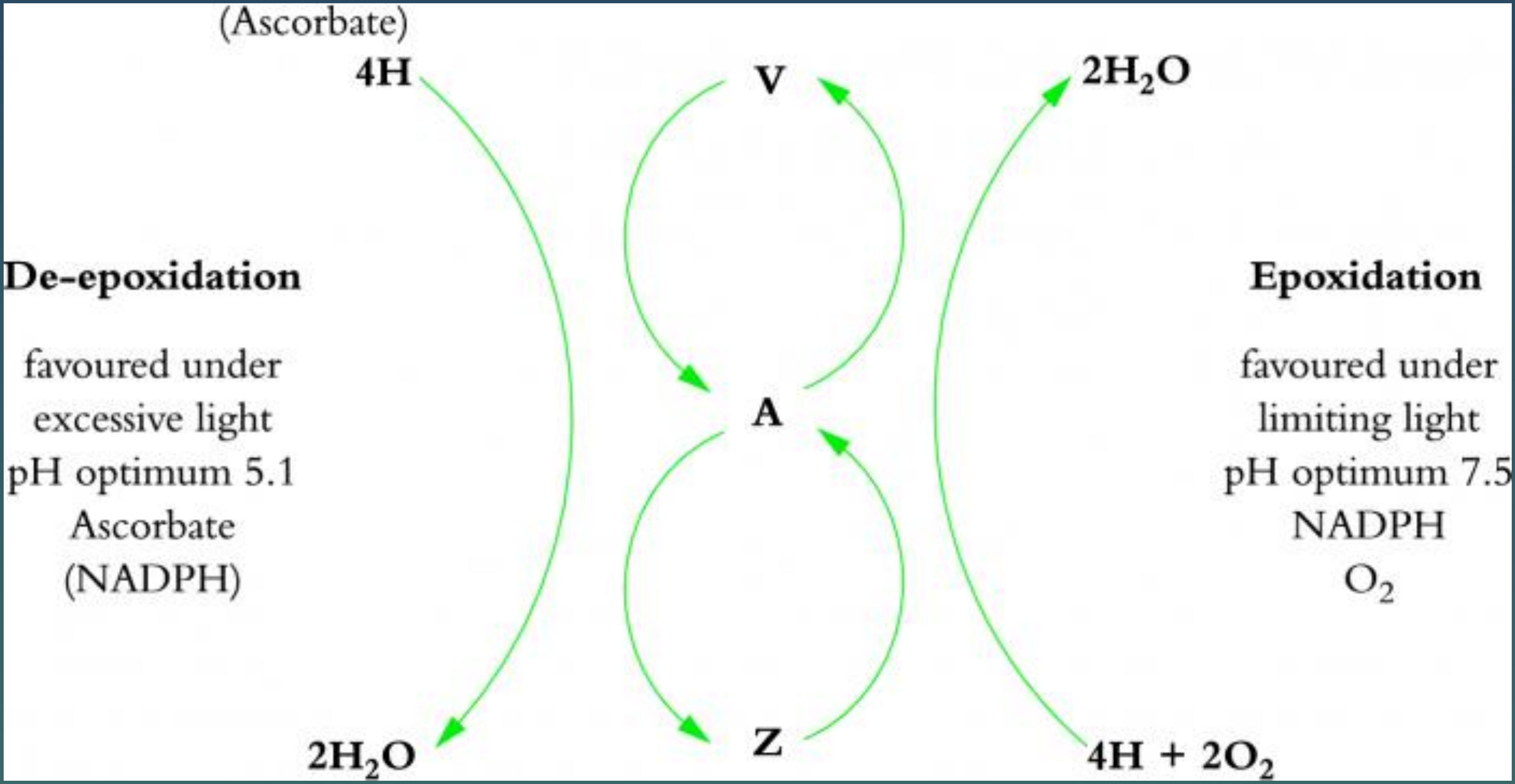


Xanthophyll cycle



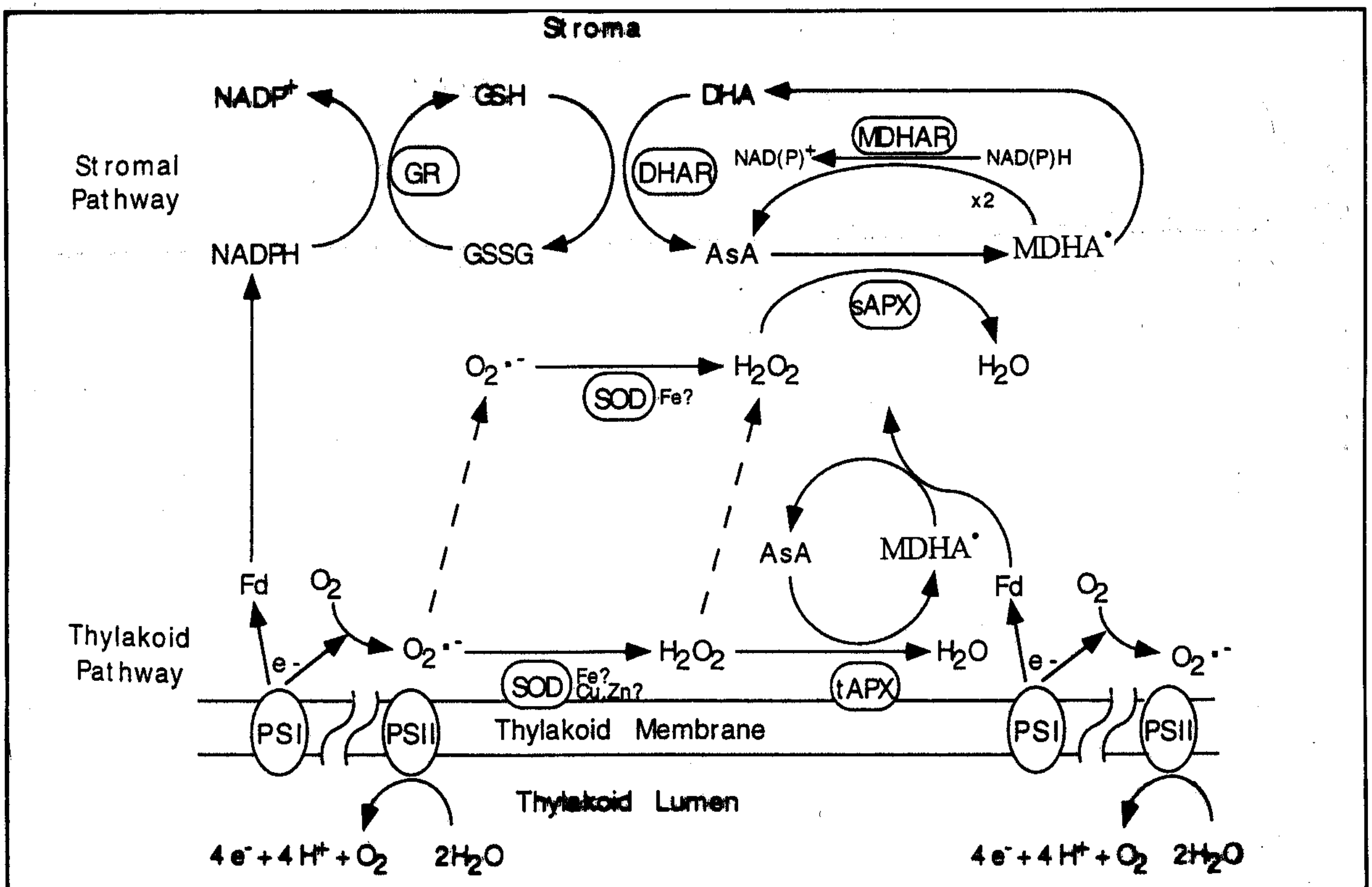
Lärcher, 1995

Xanthophyll cycle



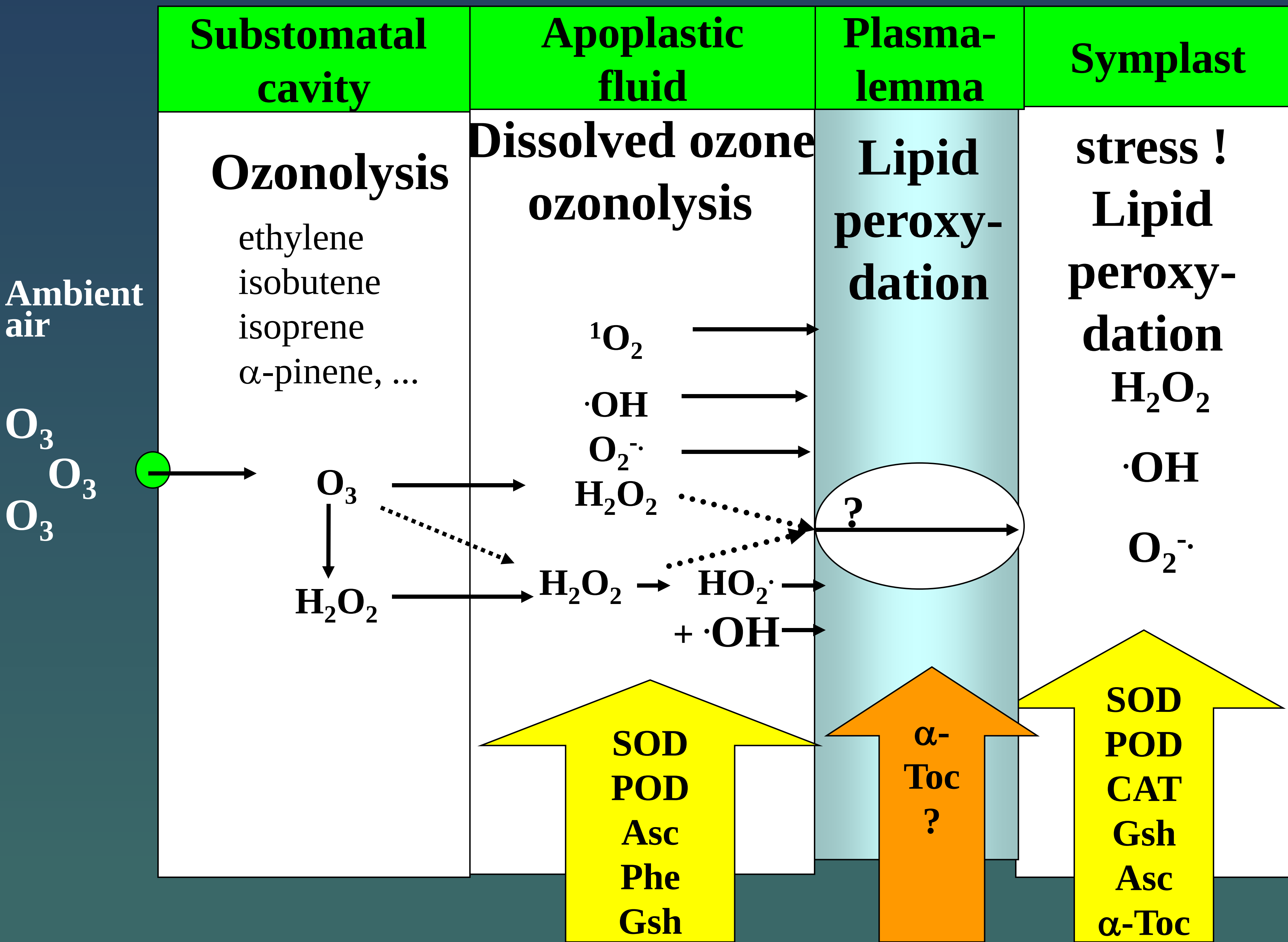
Robinson & Osmond 1994

Antioxidative metabolism in the chloroplasts

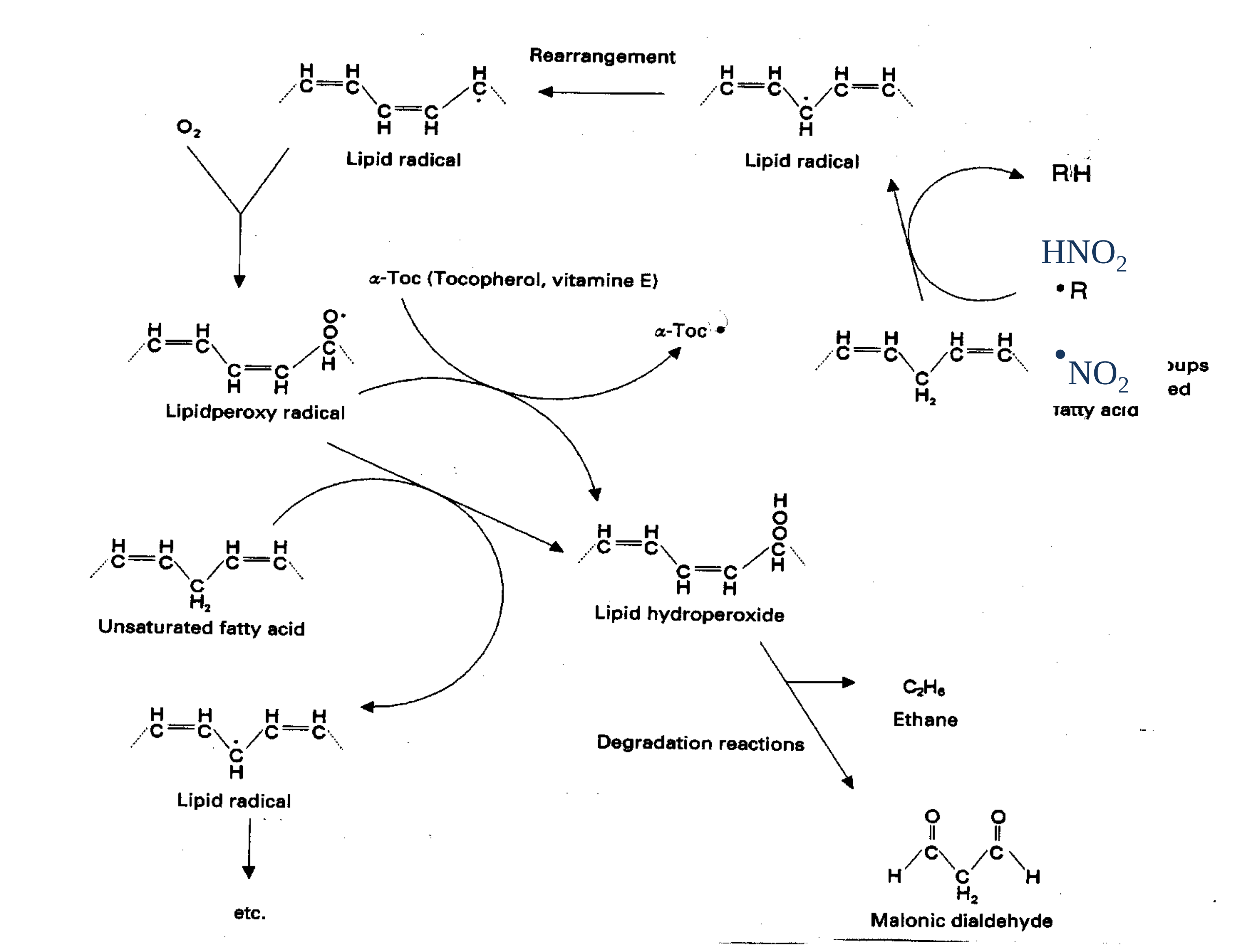


Alsher et al., 1998

Uptake and detoxification



Lipid peroxidation by NO_x

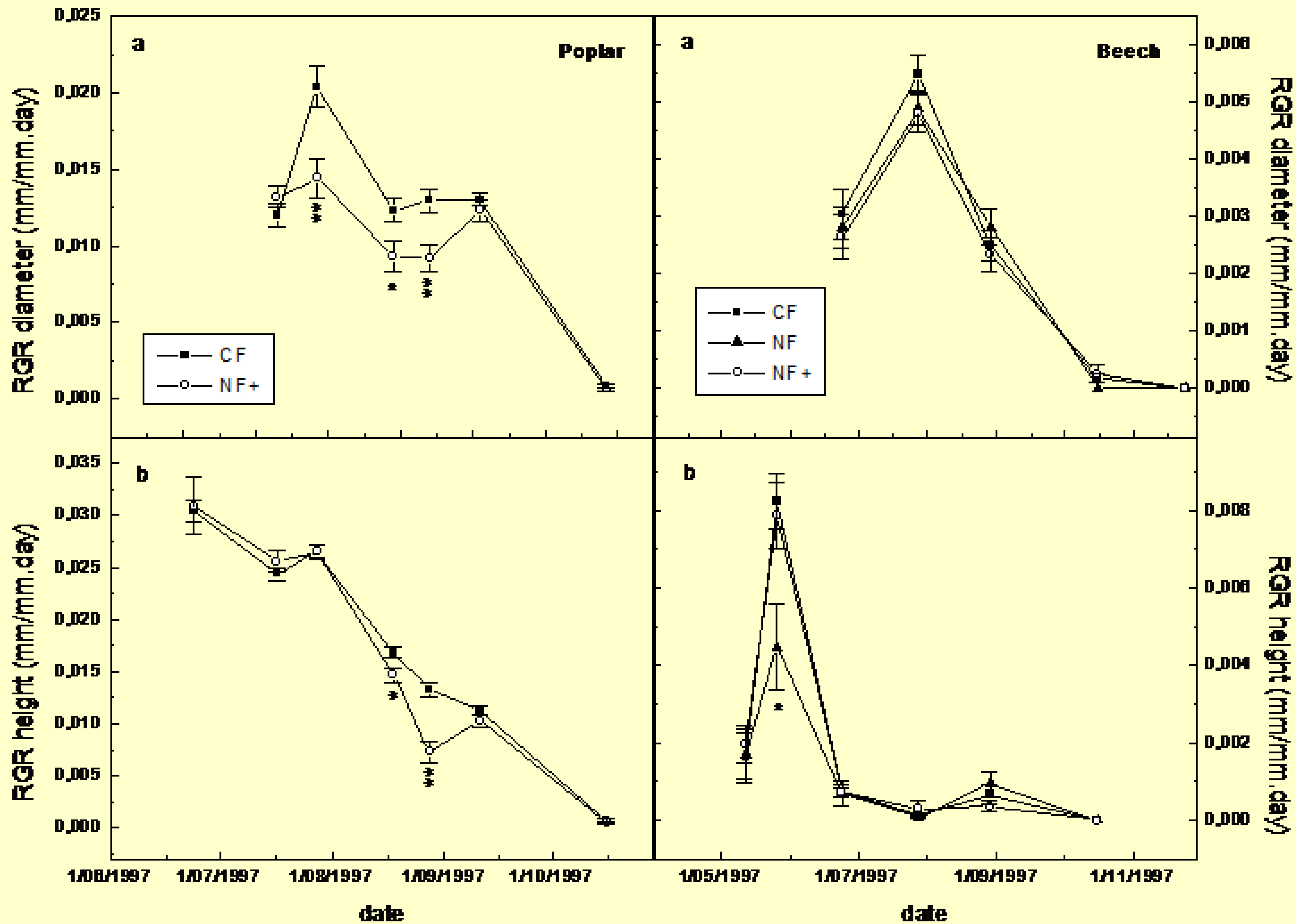


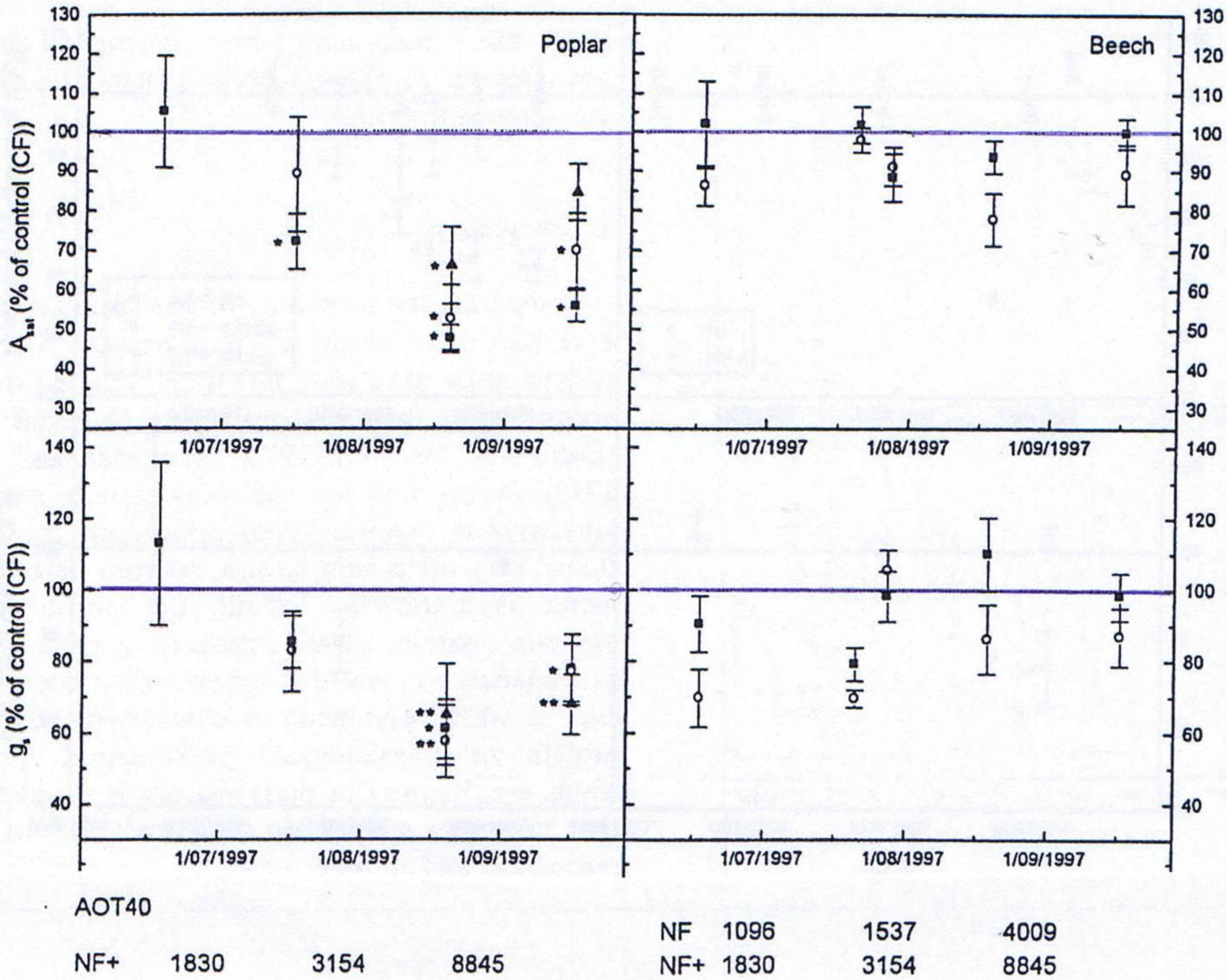
Ramge et al., 1993

Ozone sensitivity of trees

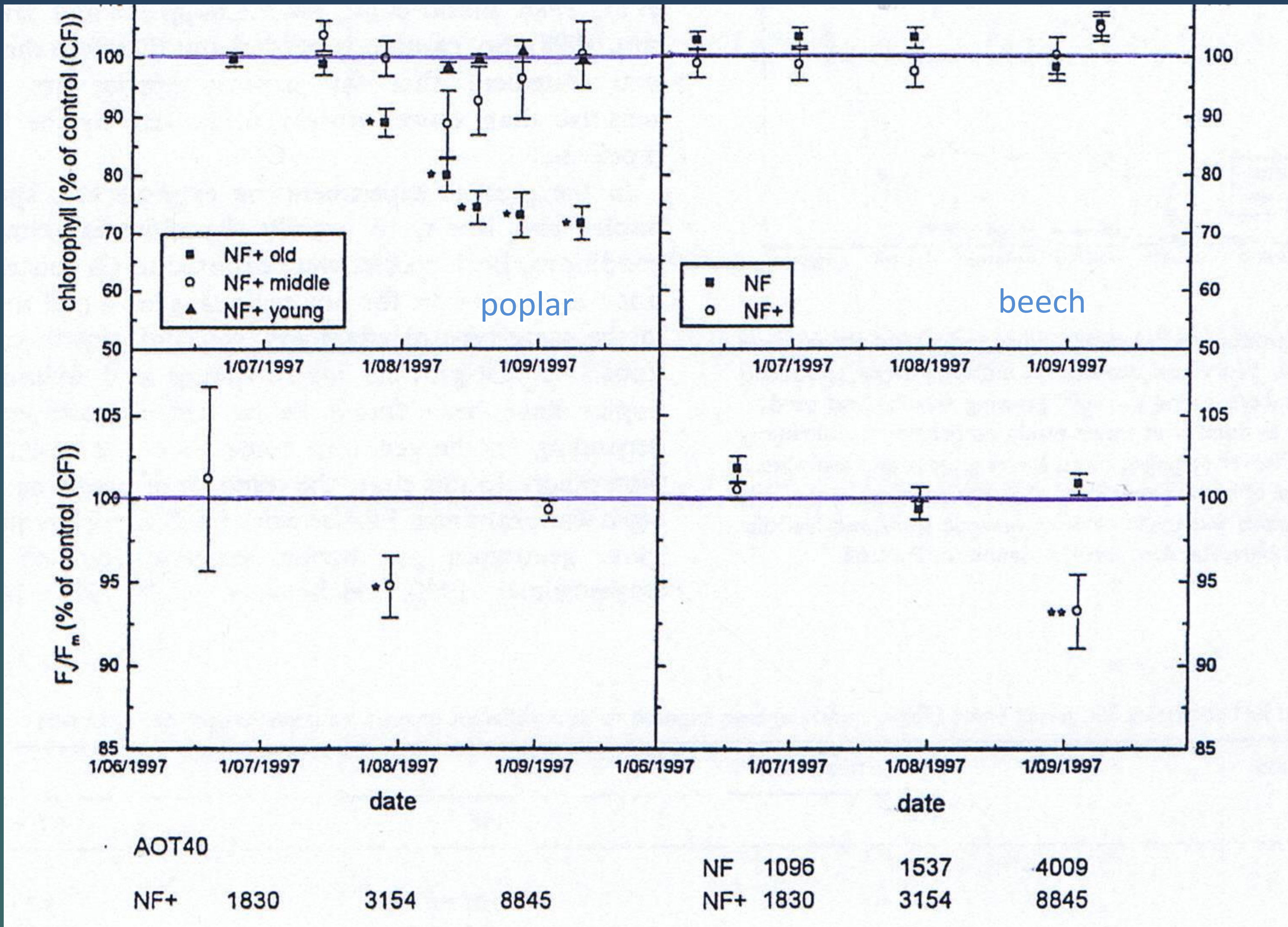
- Comparison of visible effects
- RGR of stem diameter and height growth
- Stomatal conductance (g_s)
- Light saturated photosynthesis (A_{sat})
- Chlorophyll content
- Chlorophyll fluorescence such as

  F_v/F_m: potential quantum yield of photosystem II


a
Poplar
RGR diameter (mm/mm.day)
0,025
0,020
0,015
0,010
0,005
0,000
CF
NF+
b
RGR height (mm/mm.day)
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
1/06/1997
1/07/1997
1/08/1997
1/09/1997
1/10/1997
date
a
Beech
RGR diameter (mm/mm.day)
0,006
0,005
0,004
0,003
0,002
0,001
0,000
CF
NF
NF+
b
RGR height (mm/mm.day)
0,008
0,006
0,004
0,002
0,000
1/05/1997
1/07/1997
1/09/1997
1/11/1997
date



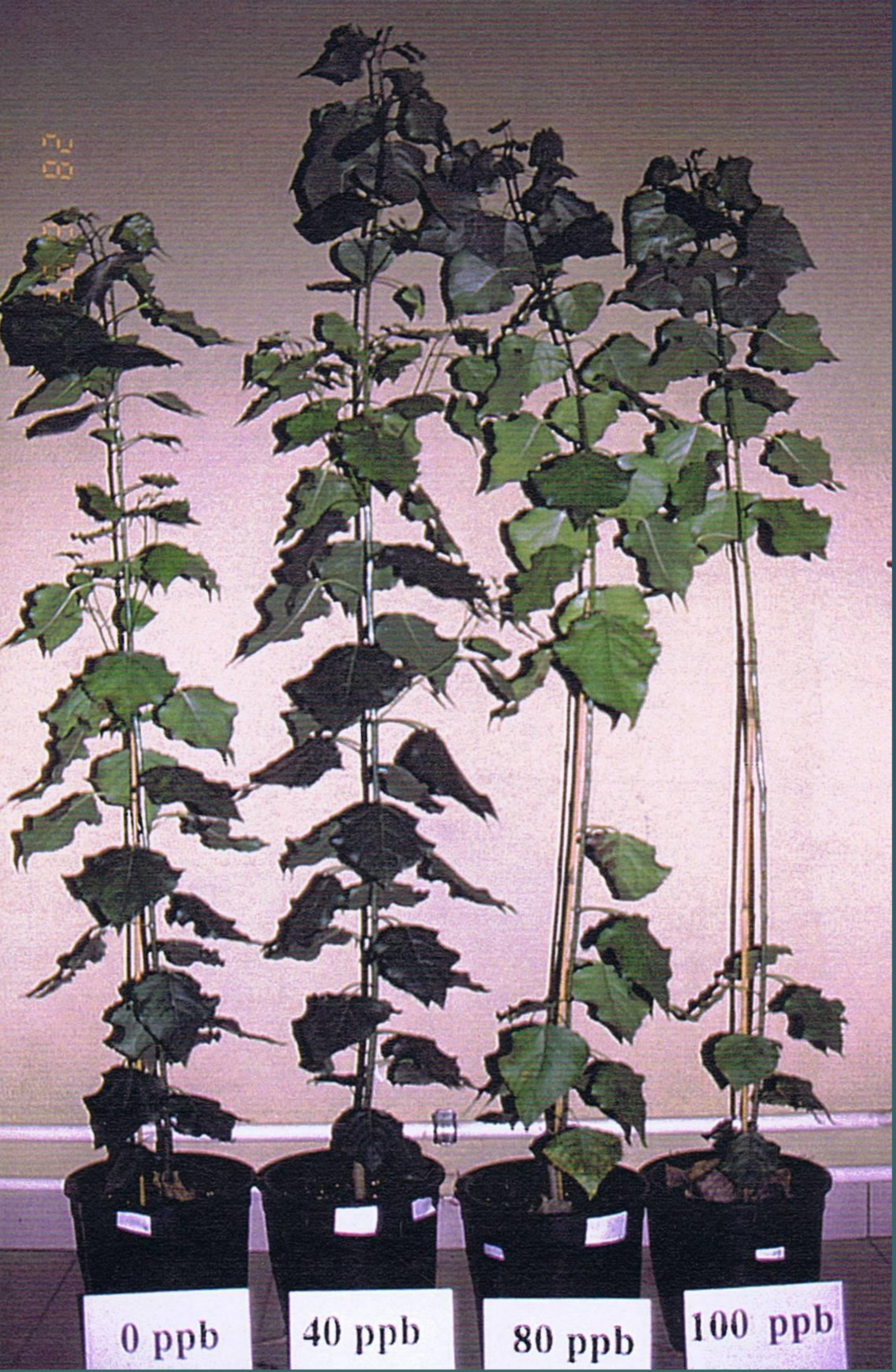
Poplar
Beech
A_{sat} (% of control (CF))
g_s (% of control (CF))
130
120
110
100
90
80
70
60
50
40
30
140
1/07/1997
1/08/1997
1/09/1997
AOT40
NF 1096 1537 4009
NF+ 1830 3154 8845

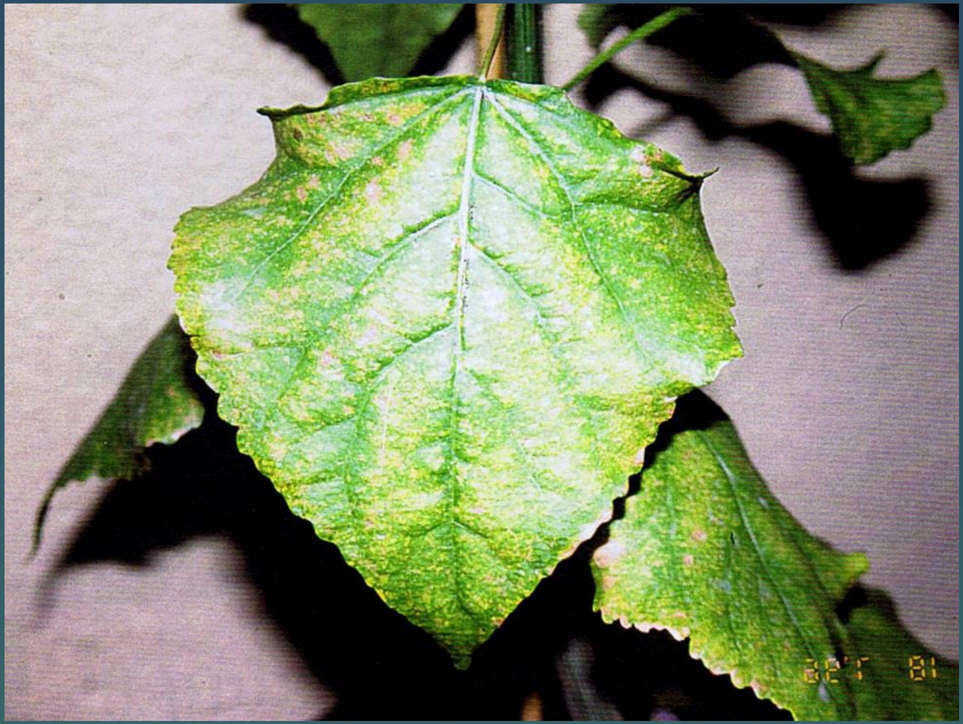


chlorophyll (% of control (CF))
NF+ old
NF+ middle
NF+ young
poplar
NF
NF+
beech
1/07/1997
1/08/1997
1/09/1997
F_v/F_m (% of control (CF))
1/06/1997
date
AOT40
NF+ 1830 3154 8845
NF 1096 1537 4009
NF+ 1830 3154 8845

Membrane injury

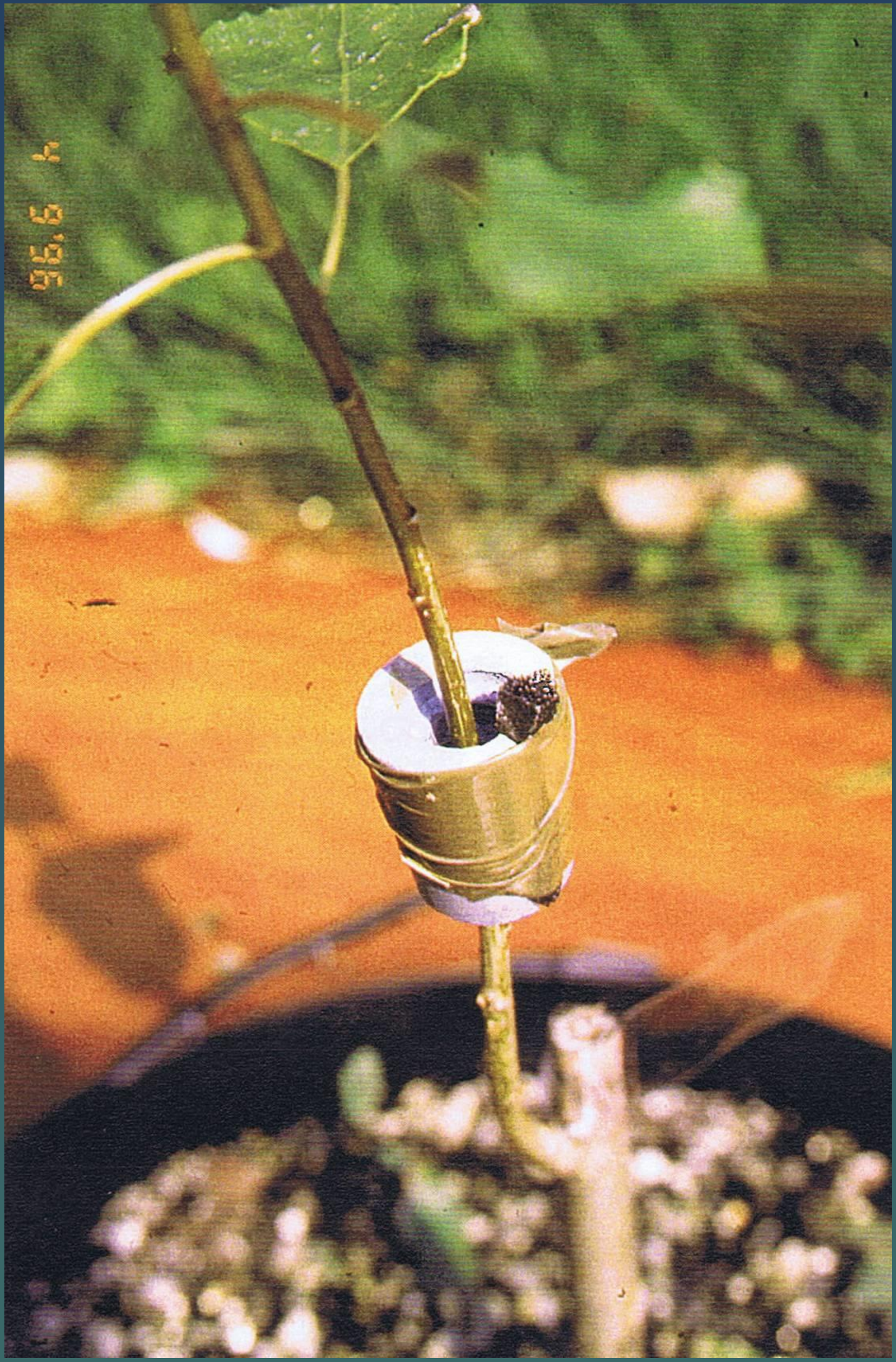
- Cell death is to some extend less harmful than slight injury. Other cells receive more resources and take over the function.
- Dark respiration is needed for the repair process.
- Plants are more sensitive to ozone damage in Nordic countries because of short nights.



0 ppb
40 ppb
80 ppb
100 ppb

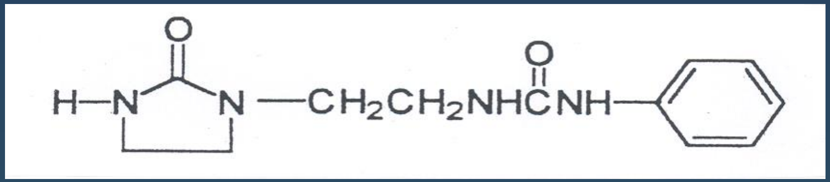



8 8'96

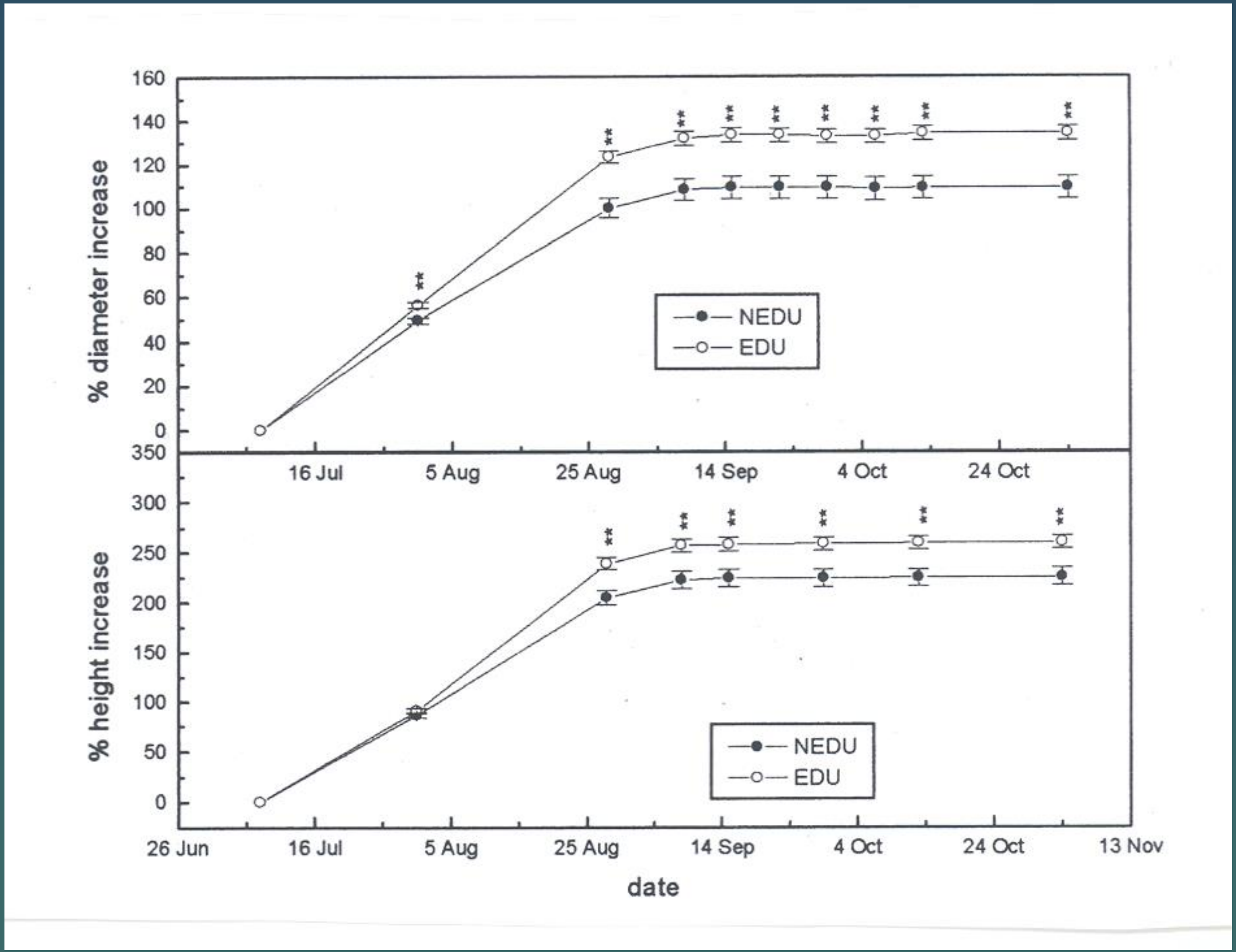
Using EDU as a research tool



Ozone effects on poplar



Ethylenediurea or
N-[2-(2-oxo-1-imidazolinidyl)ethyl]N’fenylurea



Flux modeling

- **Modeling of the ozone flux**

$$GO_3 = g_{max} \times g_{pot} \times \max\{g_{min}, (g_{light} \times g_{temp} \times g_{vpd})\}$$

  - GO_3: stomatal conductivity for ozone: nmol O_3 m^{-2} s^{-1}
  - g_{max}: average max. GO_3 on the total leaf surface
  - g_{min}: minimal stomatal conductivity during the day
  - Changes in g_{max} (realtively between 0-1) due to:
    - Phenological changes : g_{pot}
    - PFD: g_{light}
    - Temperature: g_{temp}
    - Vapor pressure deficit: g_{vpd}

- **Effective ozone flux (EF)**

$$EF = F(t) – D(t)$$

F(t): absorbed ozone dose on time t

D(t): defence capacity on time t

Flux based Critical level

terms	Abb.	unit	Description
Projected leaf area	PLA	m^2	Leaf surface (one side)
Stomatal O_3 flux	F_{st}	nmol m^{-2} PLA s^{-1}	
Stomatal O_3 flux above threshold Y	F_{st} Y	nmol m^{-2} PLA s^{-1}	Stomatal O_3 flux above a threshold of Y nmol m^{-2} PLA s^{-1}
Phytotoxic Ozone Dose (above threshold Y)	POD_Y	mmol m^{-2} PLA	Accumulated stomatal O_3 flux above threshold of Y (nmol m^{-2} PLA s^{-1})
Flux based Critical Level for ozone	CLe_f	mmol m^{-2} PLA	Accumulated flux above a threshold flux Y over over a period for day light hours.

Flux based critical levels for ozone

	Critical level – O_3 flux CLe_f	
	Accumulated flux	**Effect (% reduction)**
Spruce	**POD_1 = 8 mmol m^{-2}**	**Biomass (2%)**
Birch and beech	**POD_1 = 4 mmol m^{-2}**	**Biomass (4%)**

Effective ozone flux

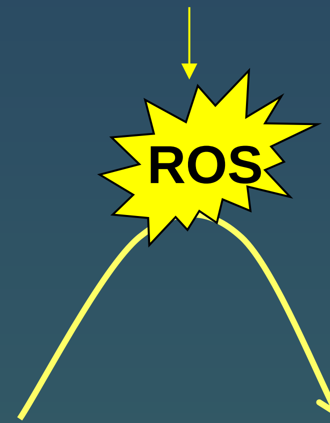
$$EF = F(t) - D(t)$$

- EF= effective ozone flux
- F(t) = absorbed dose or uptake into the leaf at a given point in time
- D(t) = defensive response at that time
  - D(t) is a function of photosynthesis since it provides the plant with photosynthate needed for defensive processes

Musselman & Massman, 1999

Antioxidative Capacity: Function

Ozone (O_3)



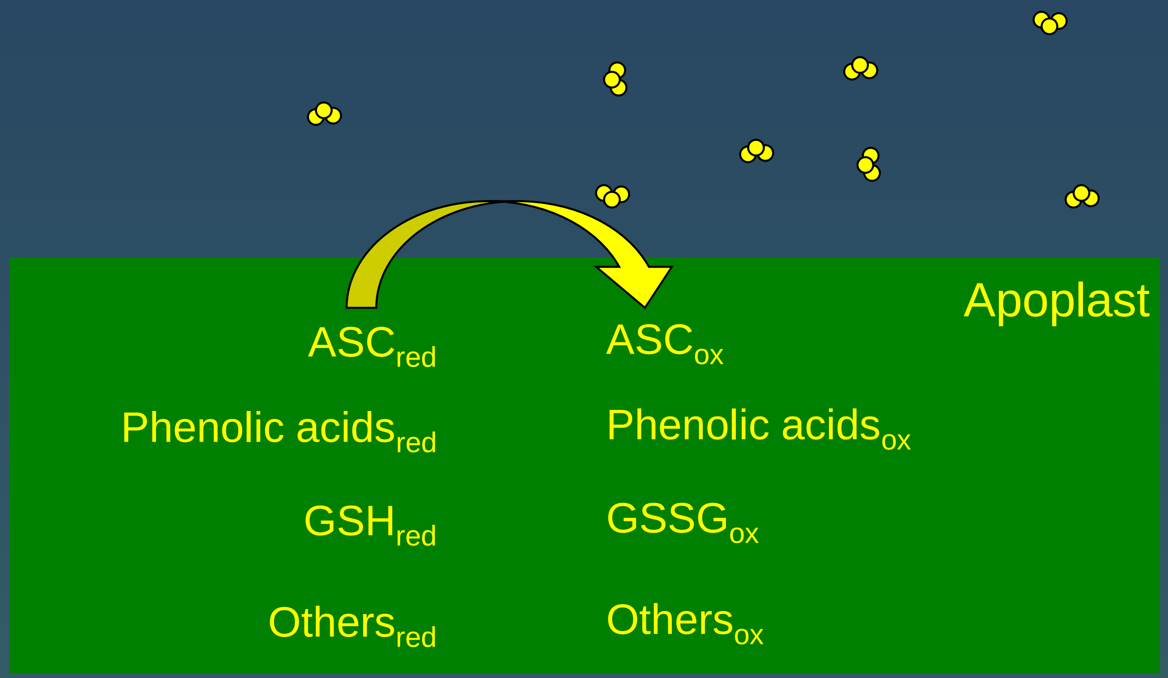
Antioxidant (Reduced) — Antioxidant (Oxidised)

Ascorbate (Red.) → **Dehydroascorbate (Ox.)**

Glutathione (Red.) → **Glutathione disulfide (Ox.)**

Other Antioxidant (Red.) → **Other Antioxidant (Ox.)**

Apoplastic Antioxidants



Problems:

- Relative importance of antioxidants unkown
- Interdepedence unkown
- Total Antioxidative power unkown
- Turnover rate of antioxidants unkown

Conclusions

The effective ozone flux is a very promising issue to determine the risk for ozone exposure in forests

Models to determine stomatal ozone uptake give already very good results but need to be further developed and tested

Much more research is needed to determine the defensive response because of its complexity

- **The ant oxidative power of phenolic acids is not well known**
- **Turnover rate of antioxidants is unknown**
- **Constitutive levels of antioxidants do not determine resistance**