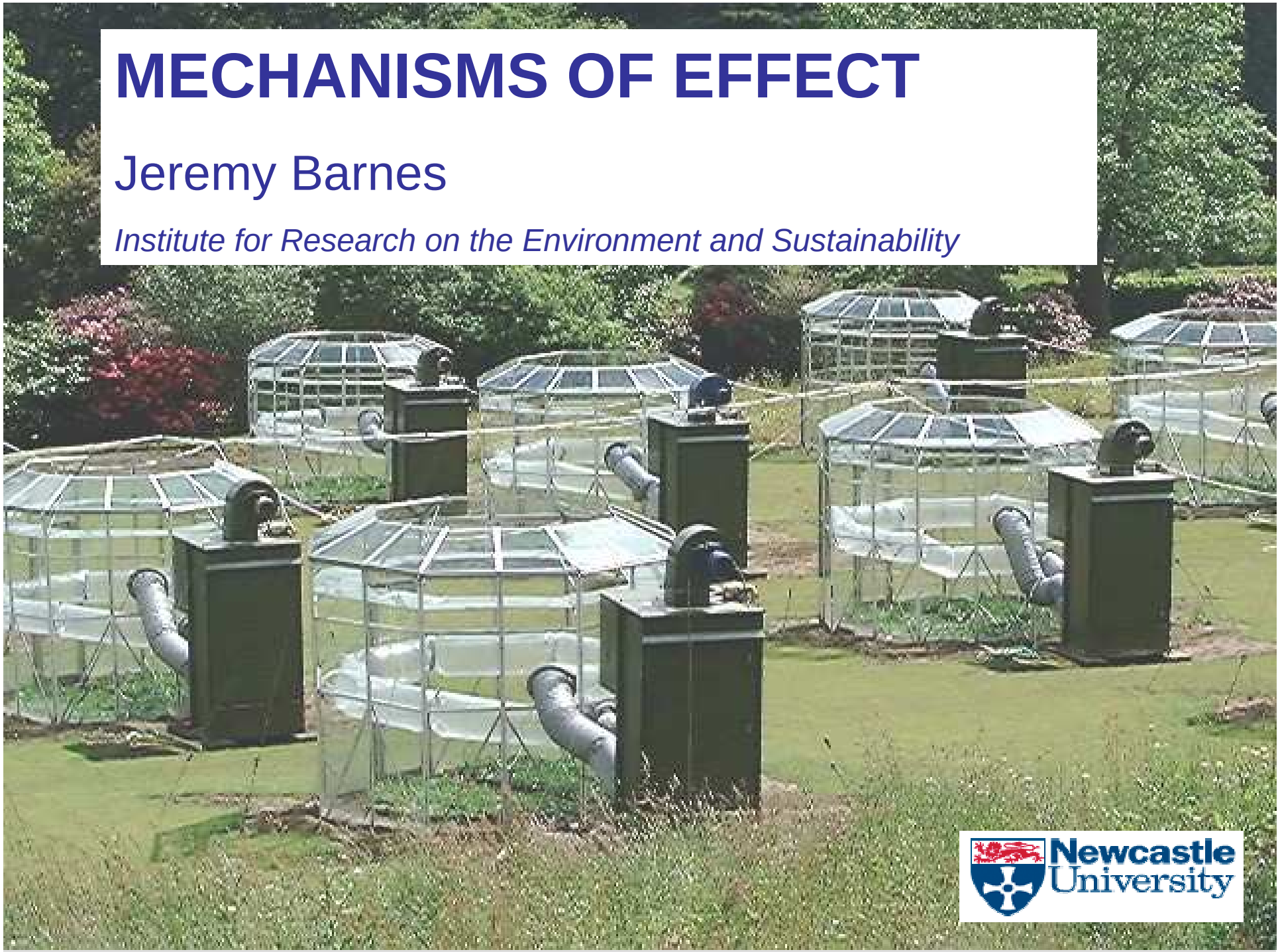
MECHANISMS OF EFFECT

Jeremy Barnes

Institute for Research on the Environment and Sustainability

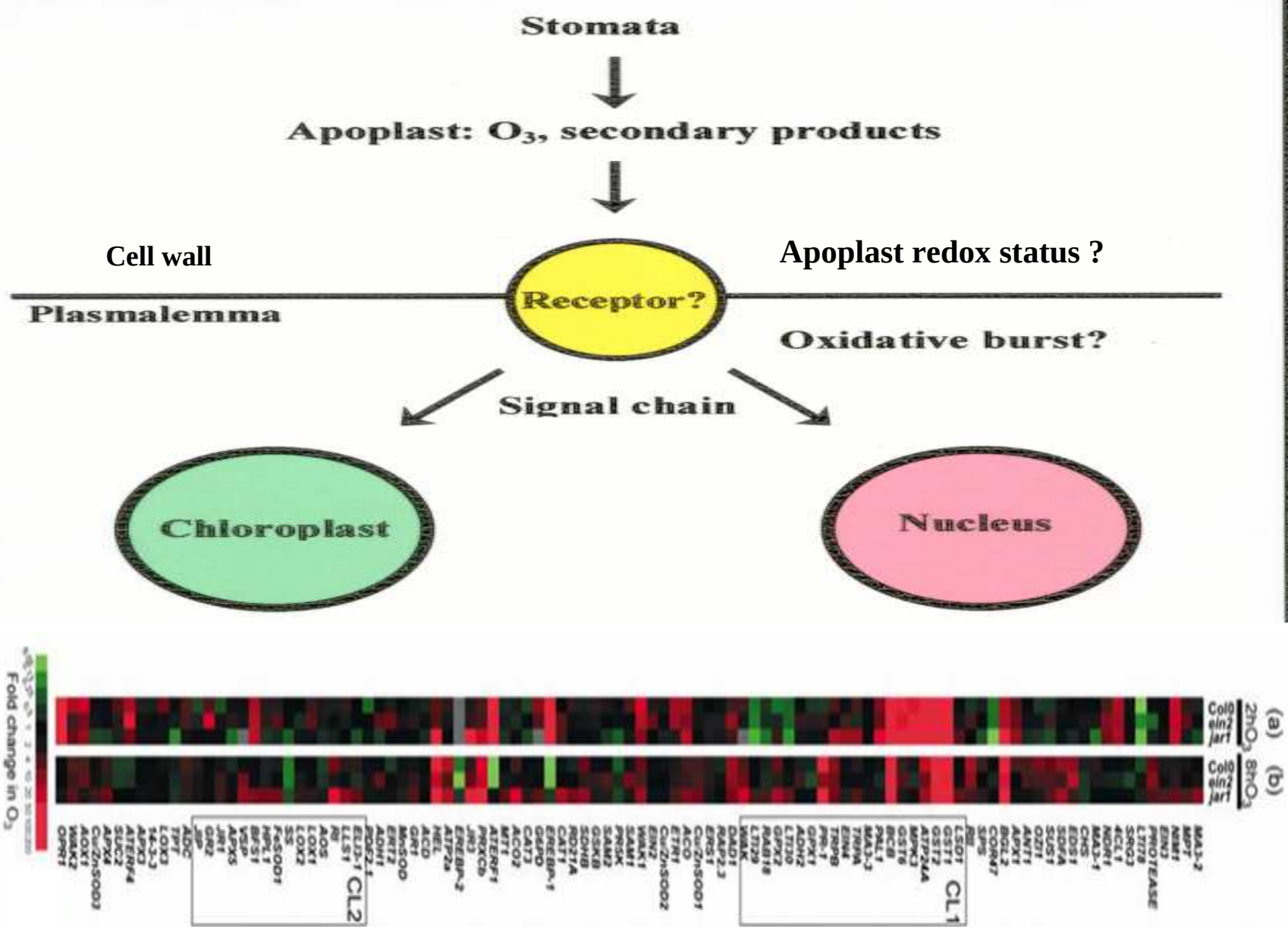


Newcastle University

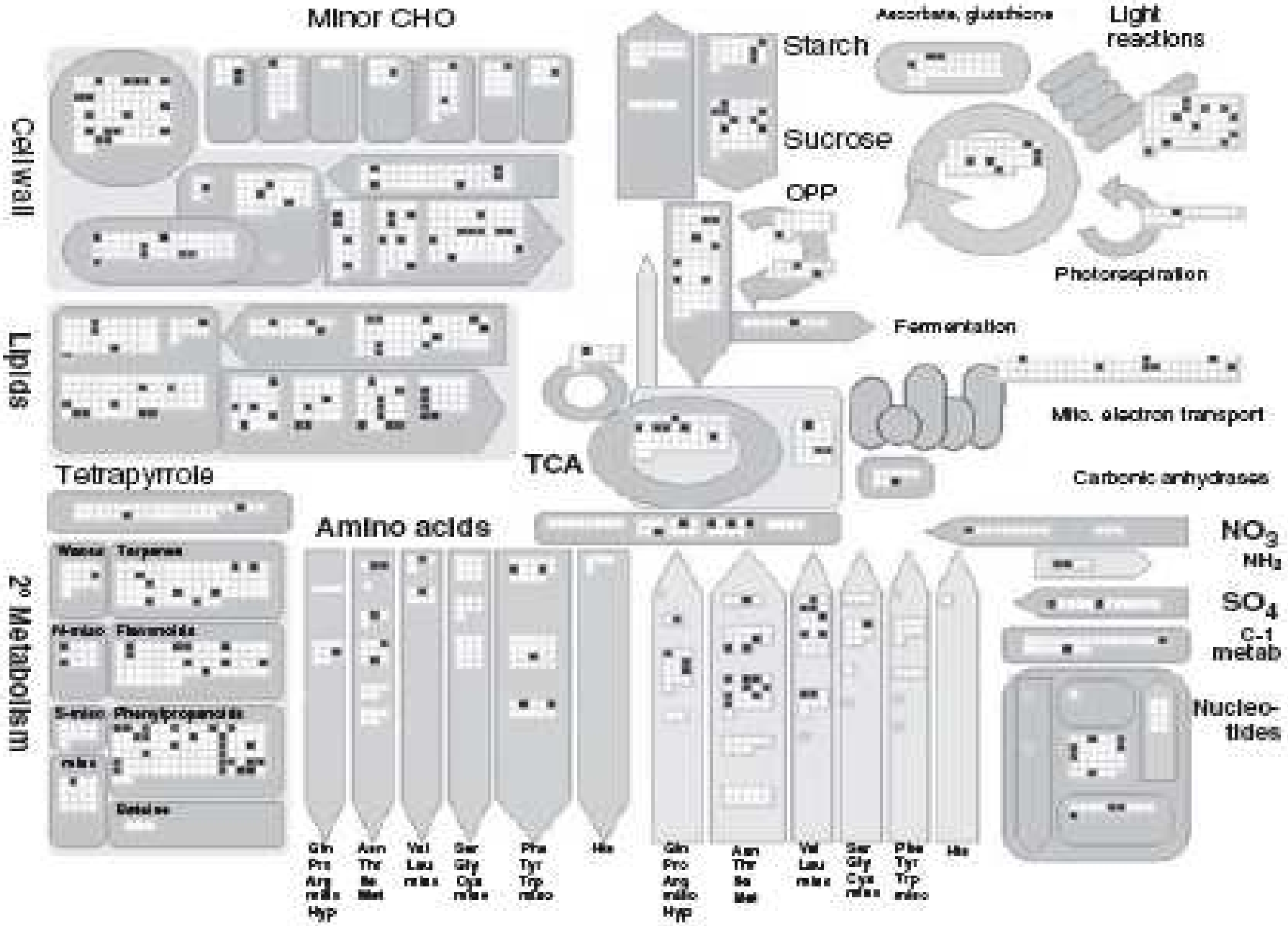
Ozone effects on vegetation

- Orchestration of plant responses
- Ozone detoxification *in planta*
- Selection for ozone resistance

Newcastle University



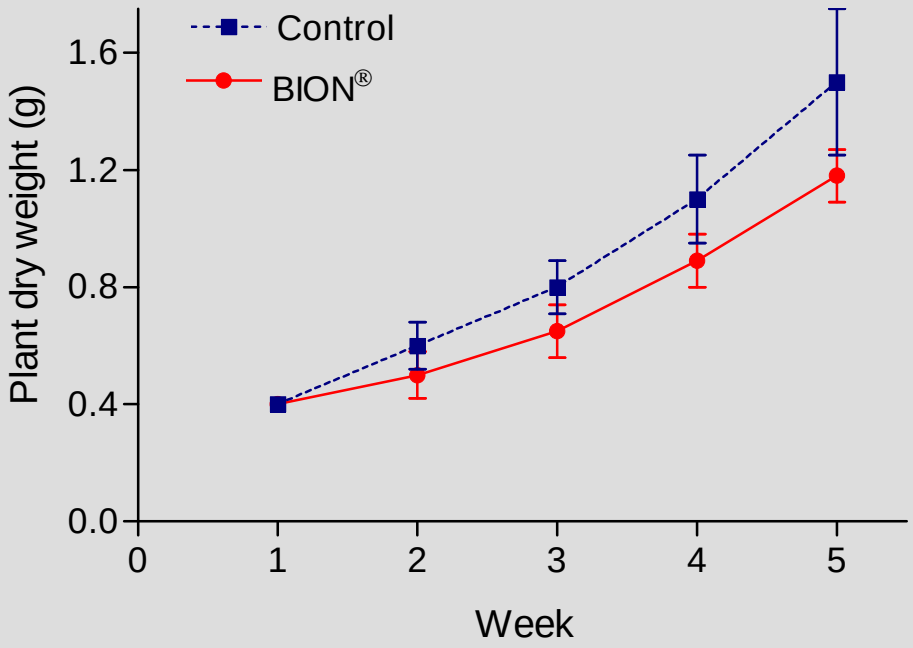
Microarray data abstracted from Tuominen *et al*. 2004 *Plant Journal* **39**: 59-69



Arabidopsis thaliana WS ecotype exposed to 25% extra ozone using soyFACE system [www.soyFACE.uiuc.edu]
gene expression changes depicted in MapMan format Li *et al.* 2006 *Plant Cell and Environment* **29**: 854-868

‘Costs’ associated with defence gene induction related to SAR

BION® = benzothiadiazole; a synthetic mimic of SA-action

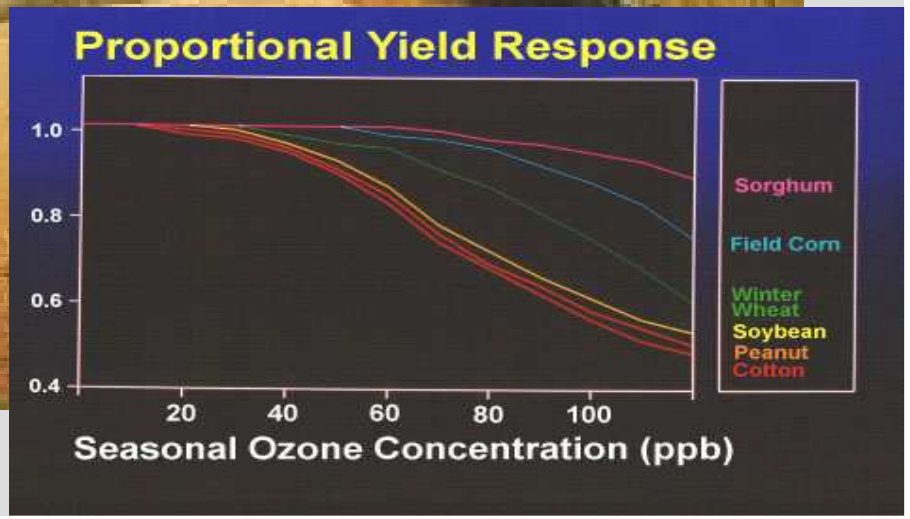


Heil *et al.* (2000) *Journal of Ecology* **88**: 645-654

Carbon utilization (i.e. metabolic costs) spiral

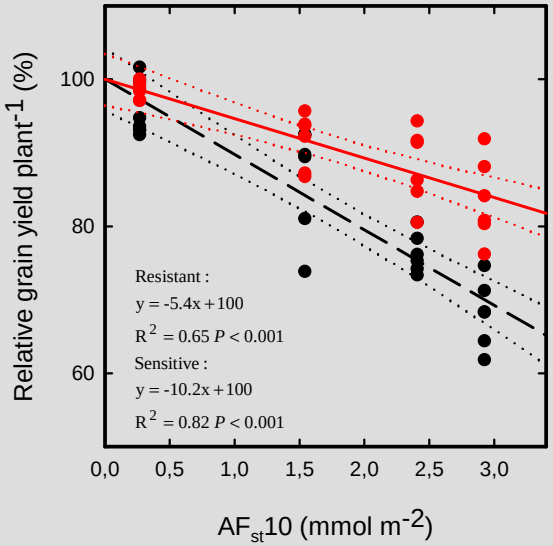
Carbon fixation down-regulated

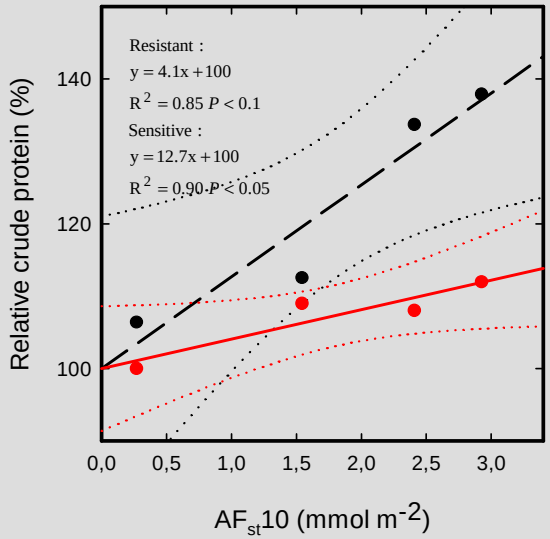
Carbon allocation patterns altered



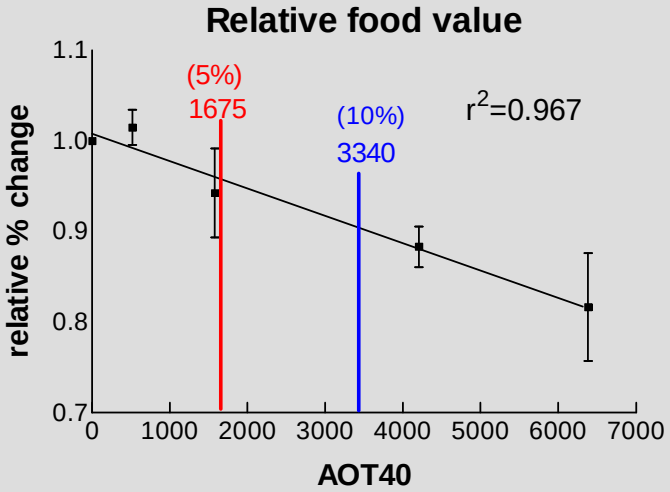
Knowledge gaps: Quality shifts

Produce





Vegetation



Knowledge gaps: ‘direct’ effects

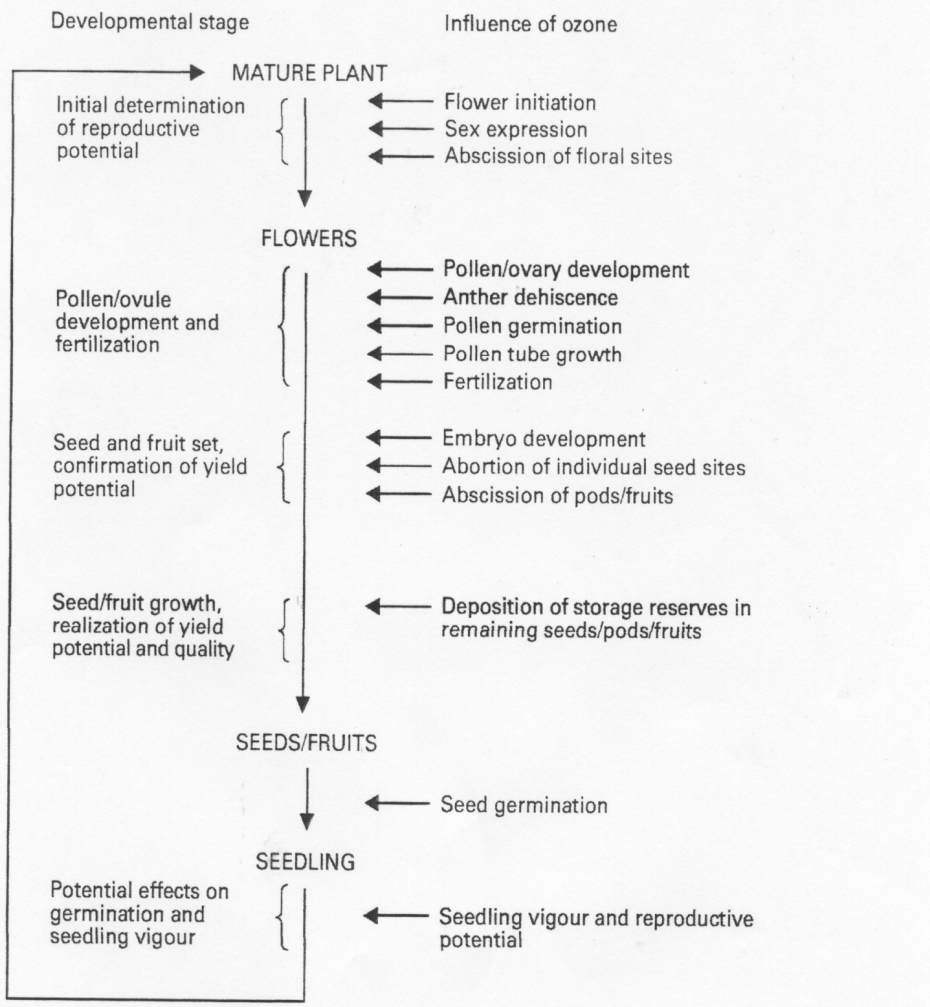
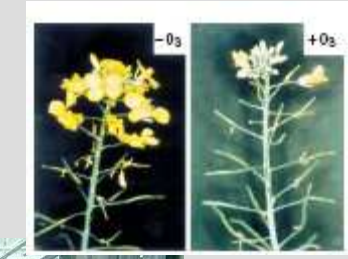
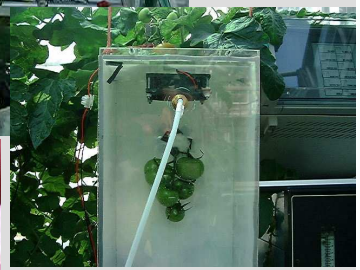
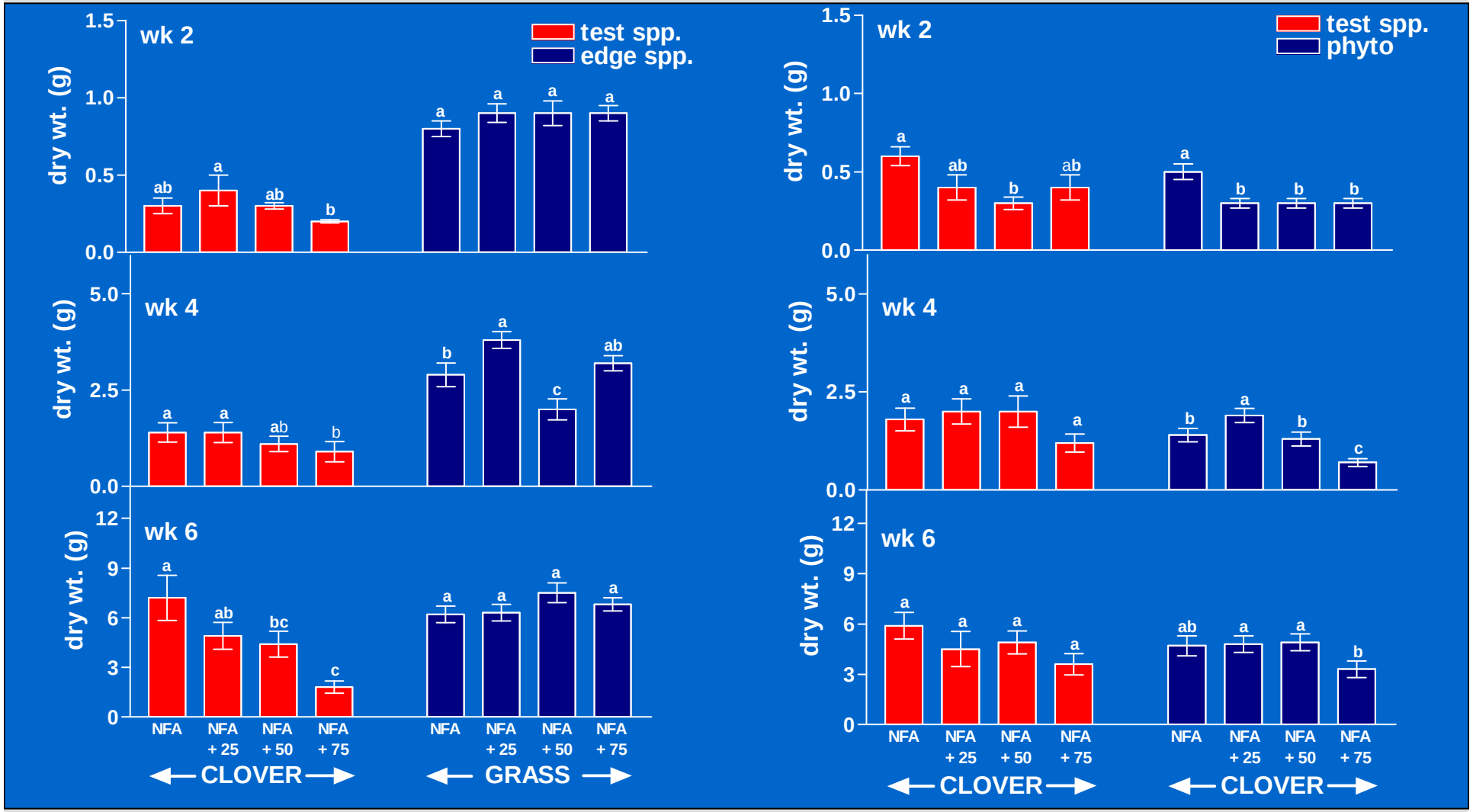


Fig. 1. Potential points of interaction between ozone and processes affecting sexual reproduction in higher plants (*after Wolters & Martens, 1987*).





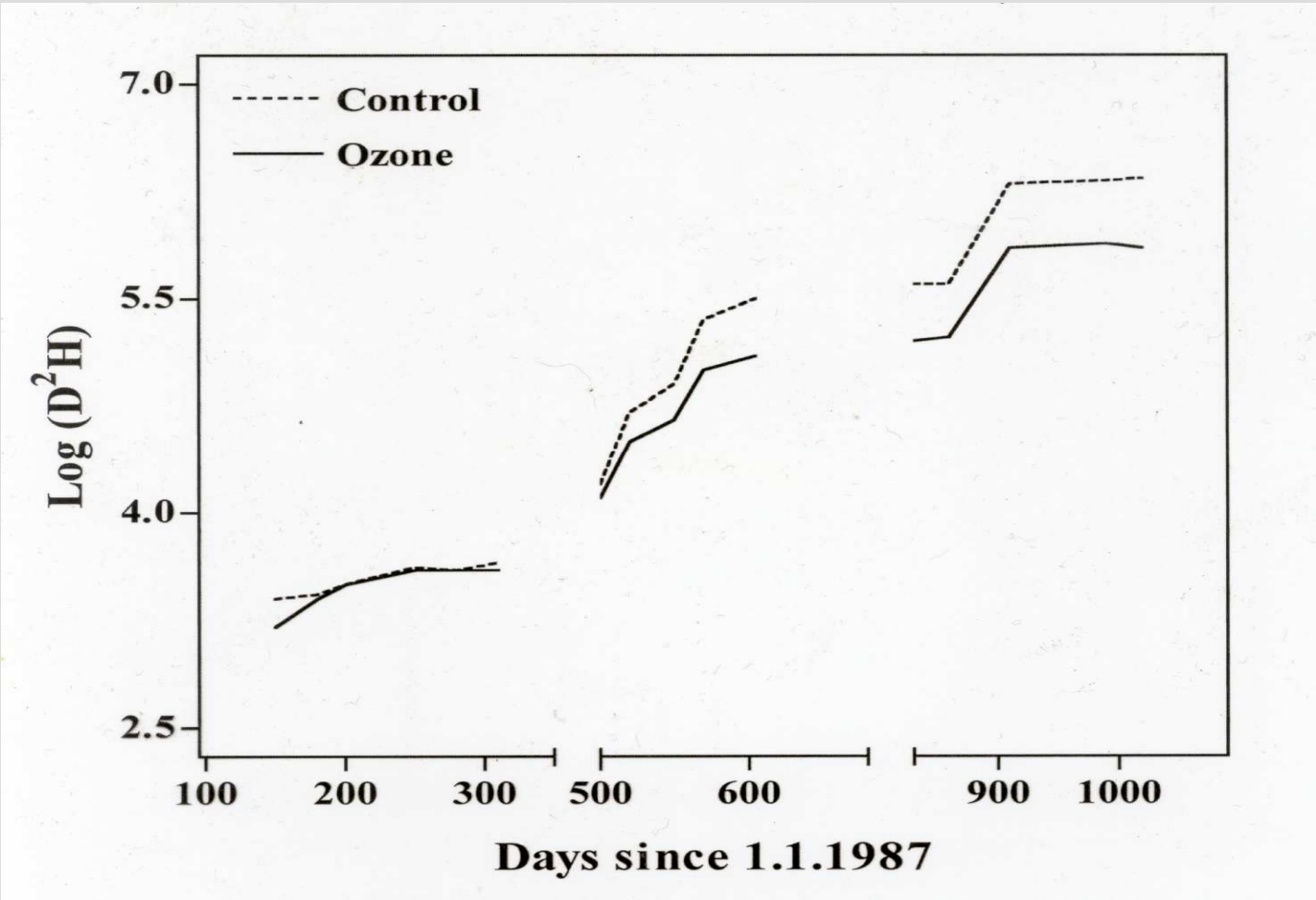
Knowledge gaps: competition



Mixed Mesocosms

Mono Mesocosms

Knowledge gaps: long-term effects on perennial species

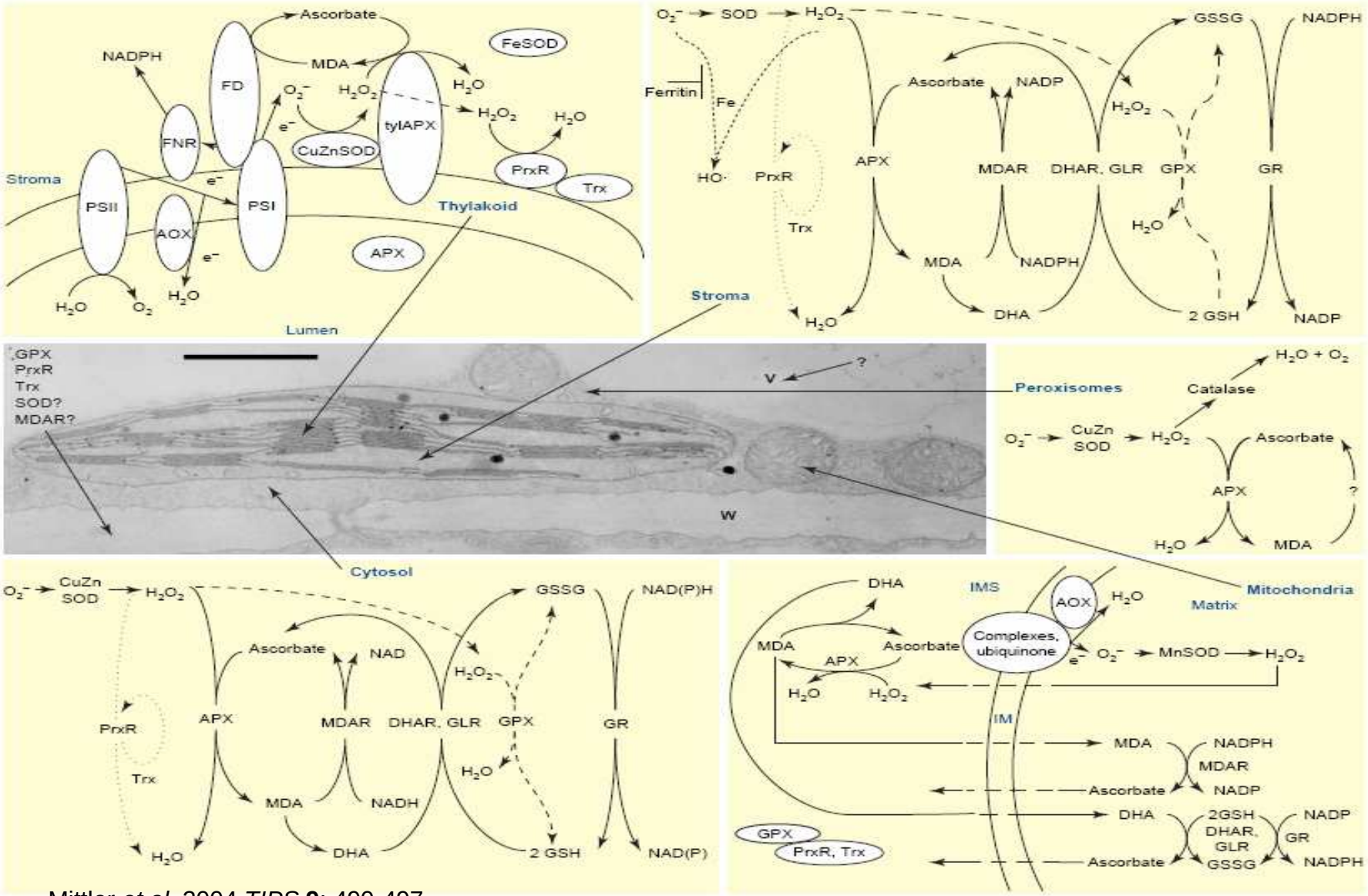


Wellburn, Barnes *et al* (1996)

Knowledge gaps: management practices

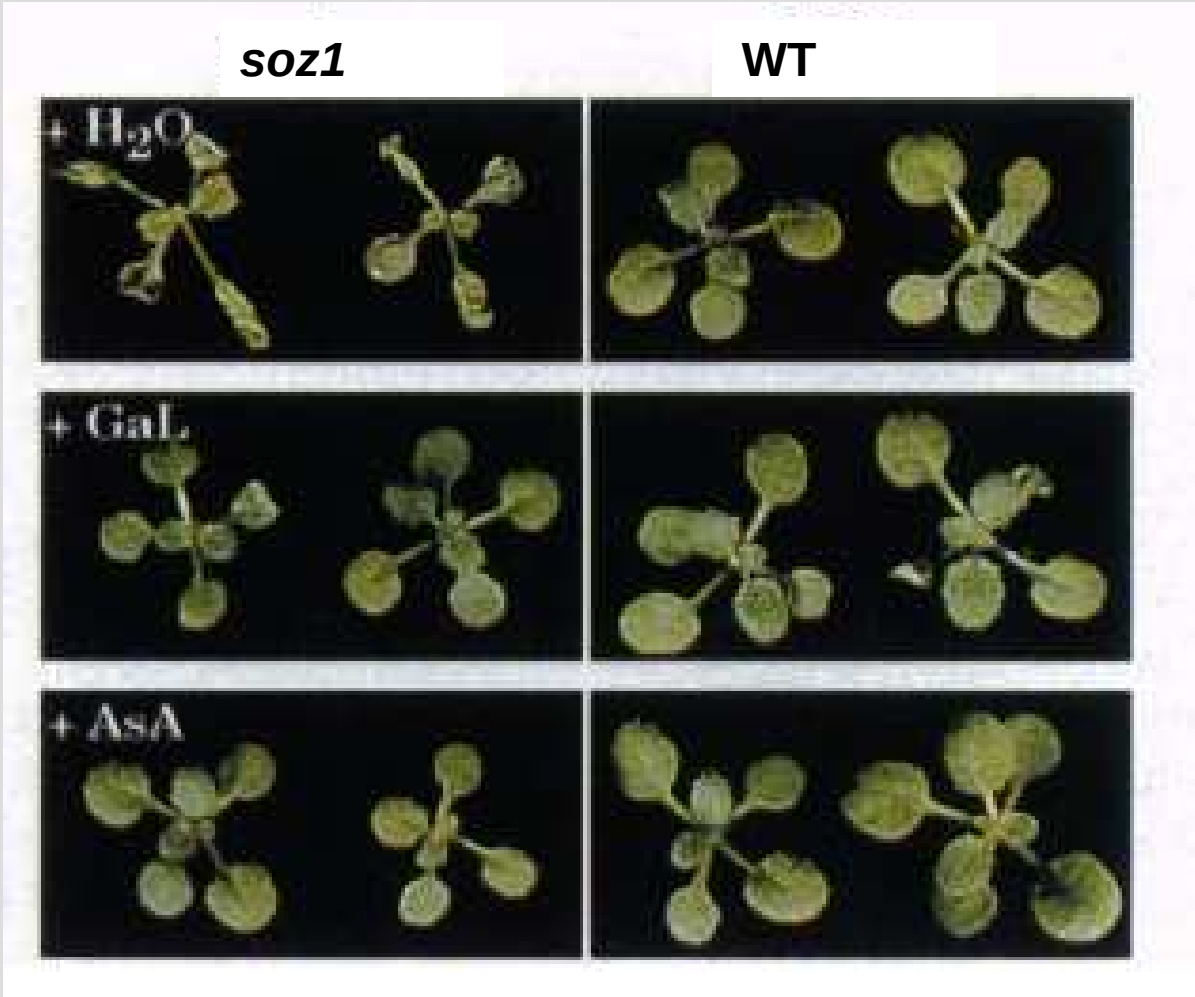
investment in field (realworld) fumigation facilities

Ozone detoxification *in planta*



Mittler *et al*. 2004 *TIPS* **9**: 490-497

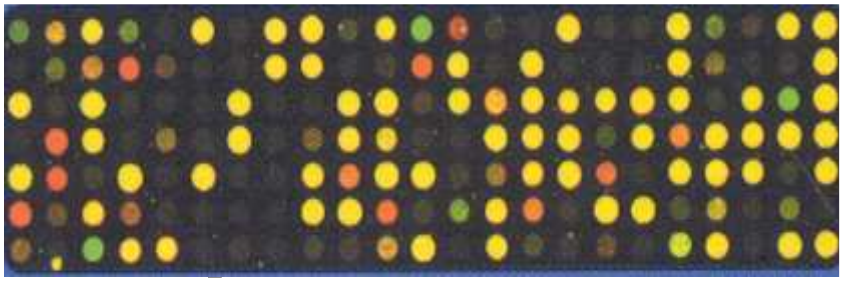
soz1 [later renamed *vtc1*]: semi-dominant single gene mutation resulting in 30% of WT L-ascorbic acid (Vitamin C) content

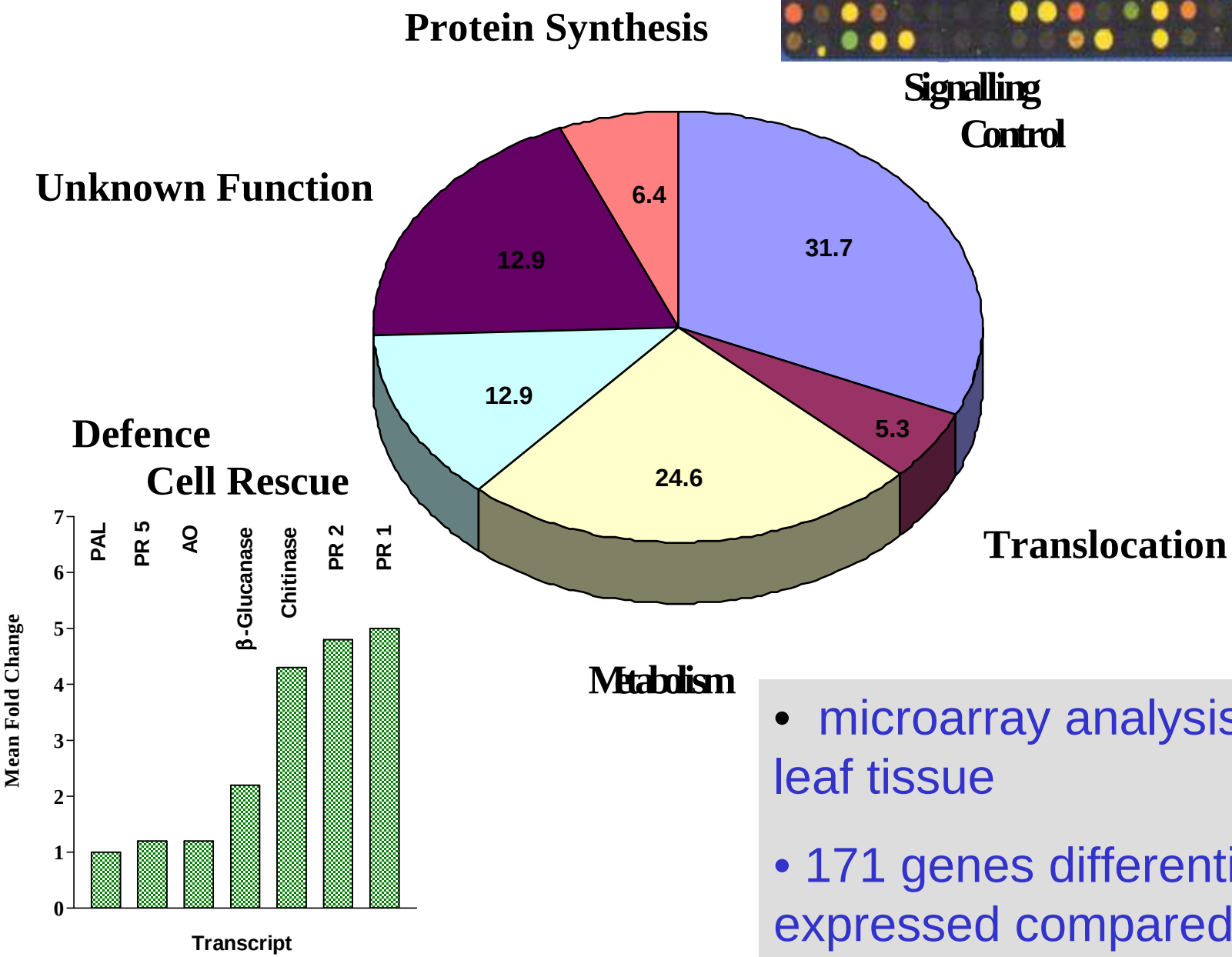


400 ppb ozone 8 h

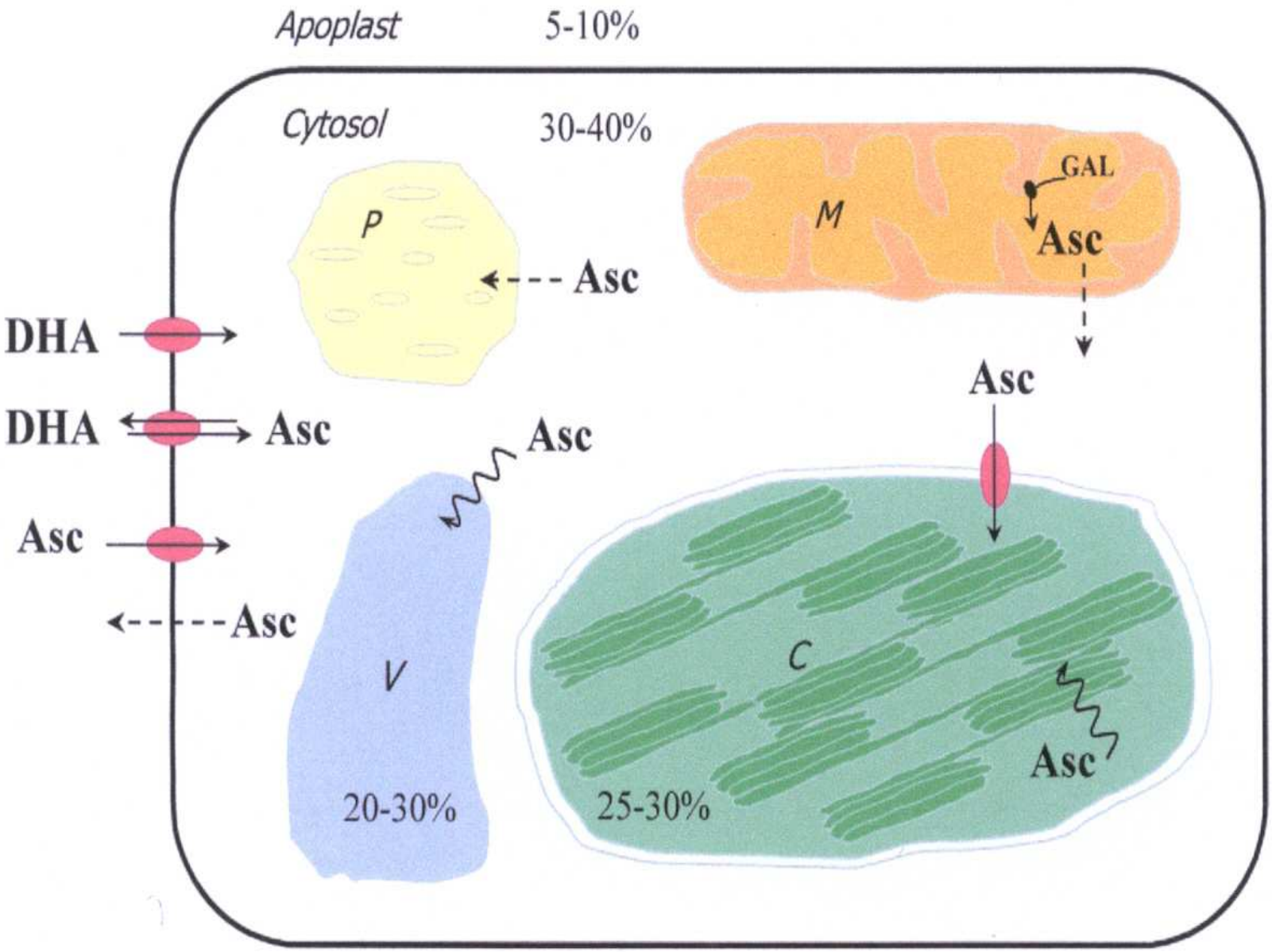
Conklin *et al.* 1996 *PNAS* **93**: 9970-9974

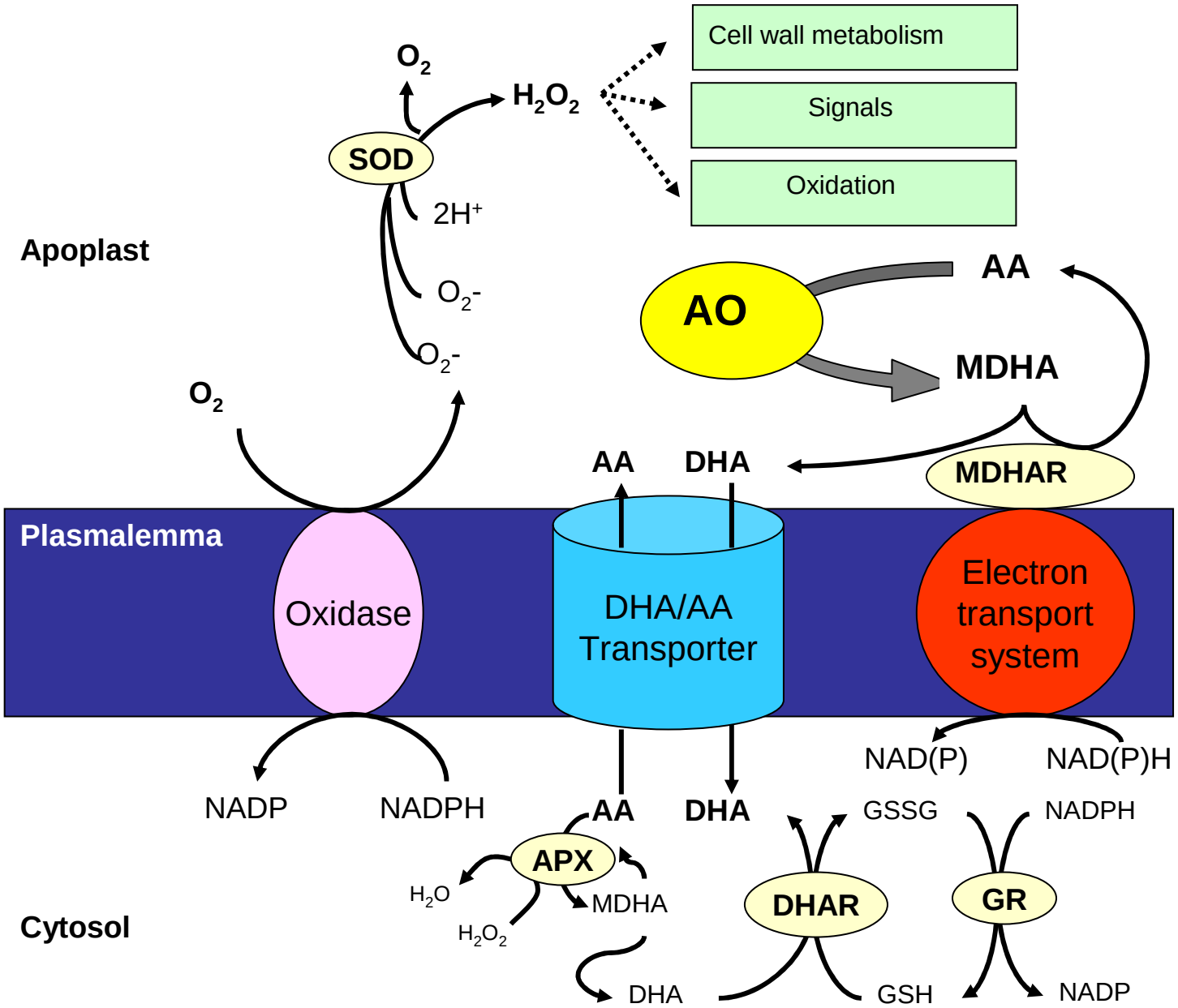
Pastori *et al*. (2003) *Plant Cell* **15**: 939-951



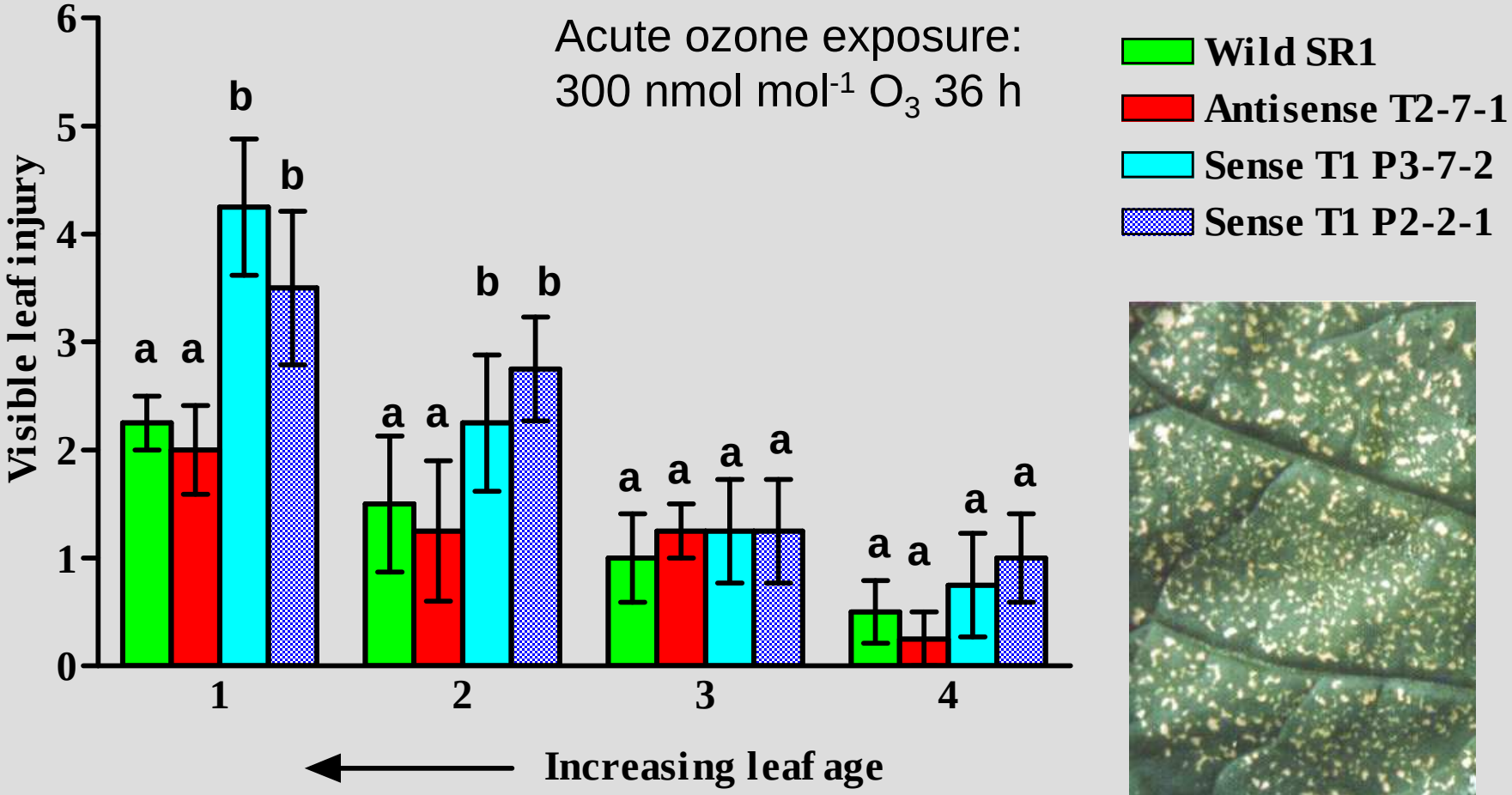


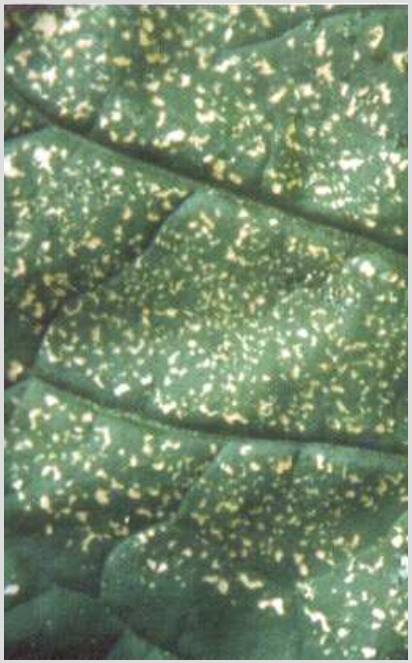
- microarray analysis *vtc1* leaf tissue
- 171 genes differentially expressed compared WT


Apoplast
5-10%
Cytosol
30-40%
P
Asc
M
GAL
Asc
DHA
DHA
Asc
Asc
Asc
Asc
Asc
V
20-30%
C
25-30%
Asc


Cell wall metabolism
Signals
Oxidation
O_2
H_2O_2
SOD
$2H^+$
O_2^-
O_2^-
Apoplast
AO
AA
MDHA
O_2
AA
DHA
MDHAR
Plasmalemma
Oxidase
DHA/AA Transporter
Electron transport system
NADP
NADPH
NAD(P)
NAD(P)H
AA
DHA
GSSG
NADPH
APX
H_2O
H_2O_2
MDHA
DHAR
GR
Cytosol
DHA
GSH
NADP

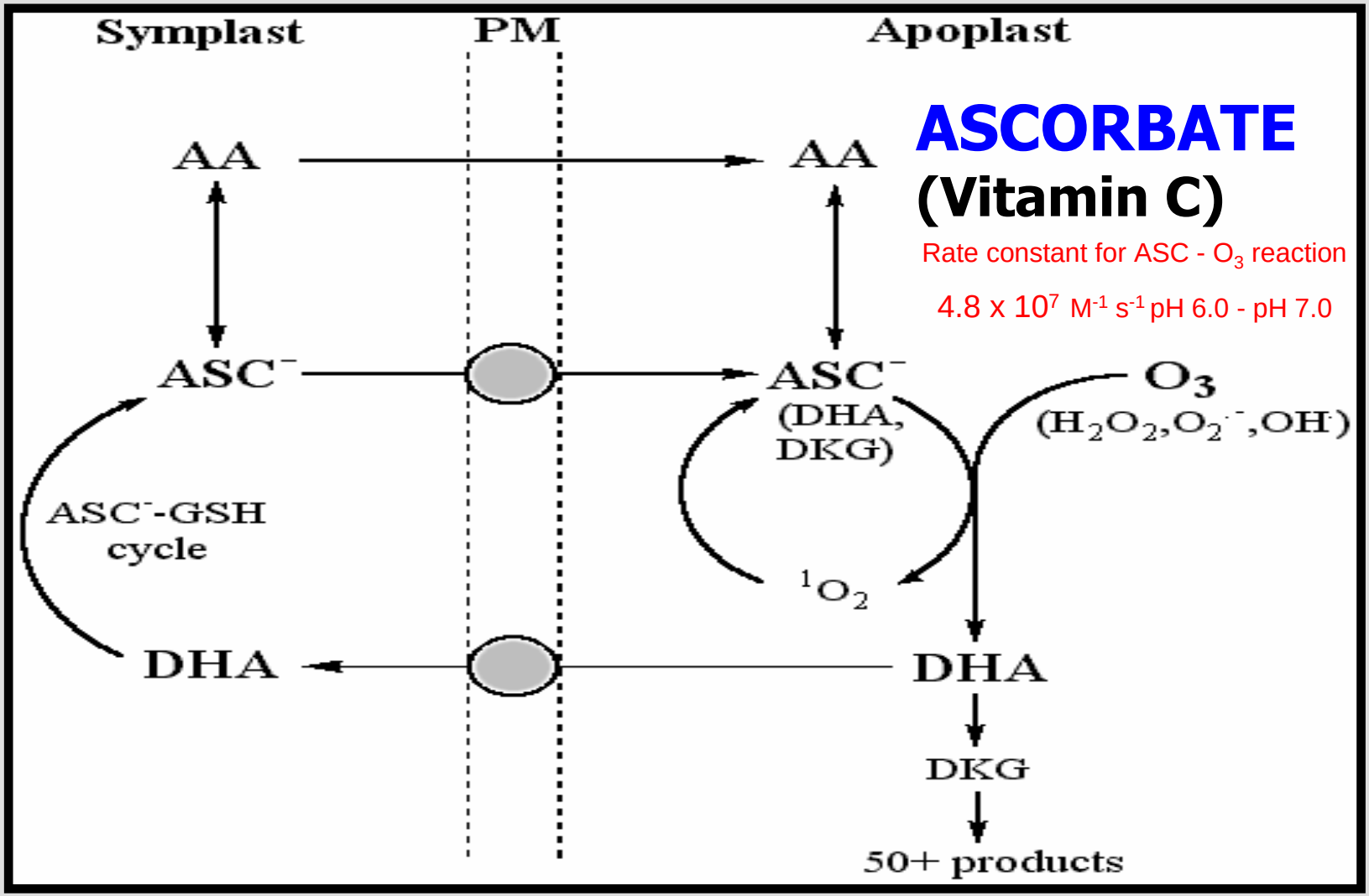
Ozone sensitivity consistent with $[ASC]_{apo}$





Smith H, Foyer CH, Barnes JD, unpublished

Cell wall-localised ASC constitutes a first line of defence against ozone



Barnes et al. (1996) *Environmental Pollution and Plant Responses*

Cell wall-localised ASC content/redox status controls aspects of defence-related gene expression

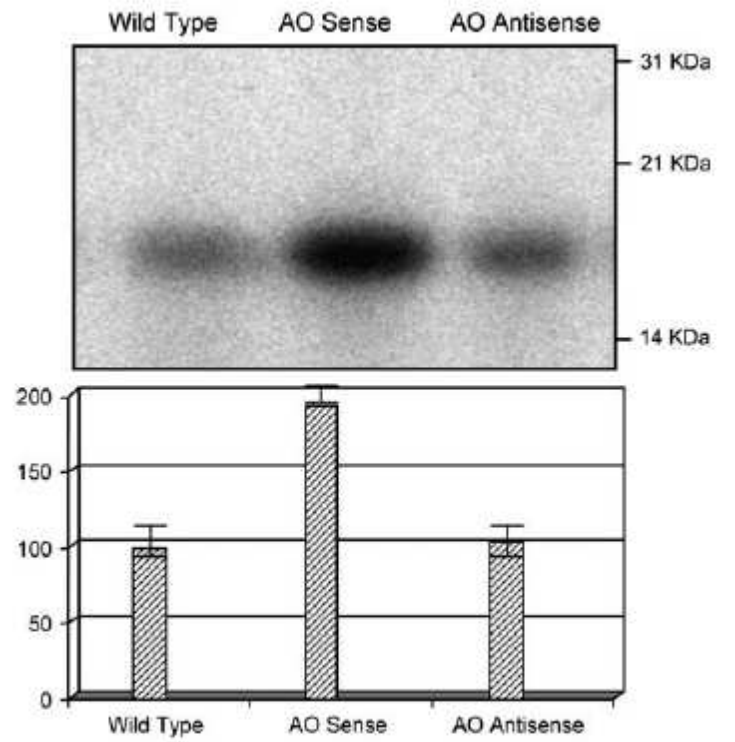


Figure 3. Comparison of MAPK activity of leaves from untransformed controls (wild type), *AO* sense, and *AO* antisense plant MAPK activity was determined by the relative phosphorylation (top) of MBP in vitro

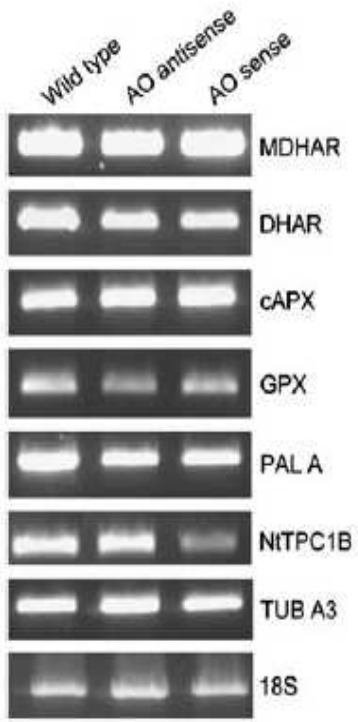
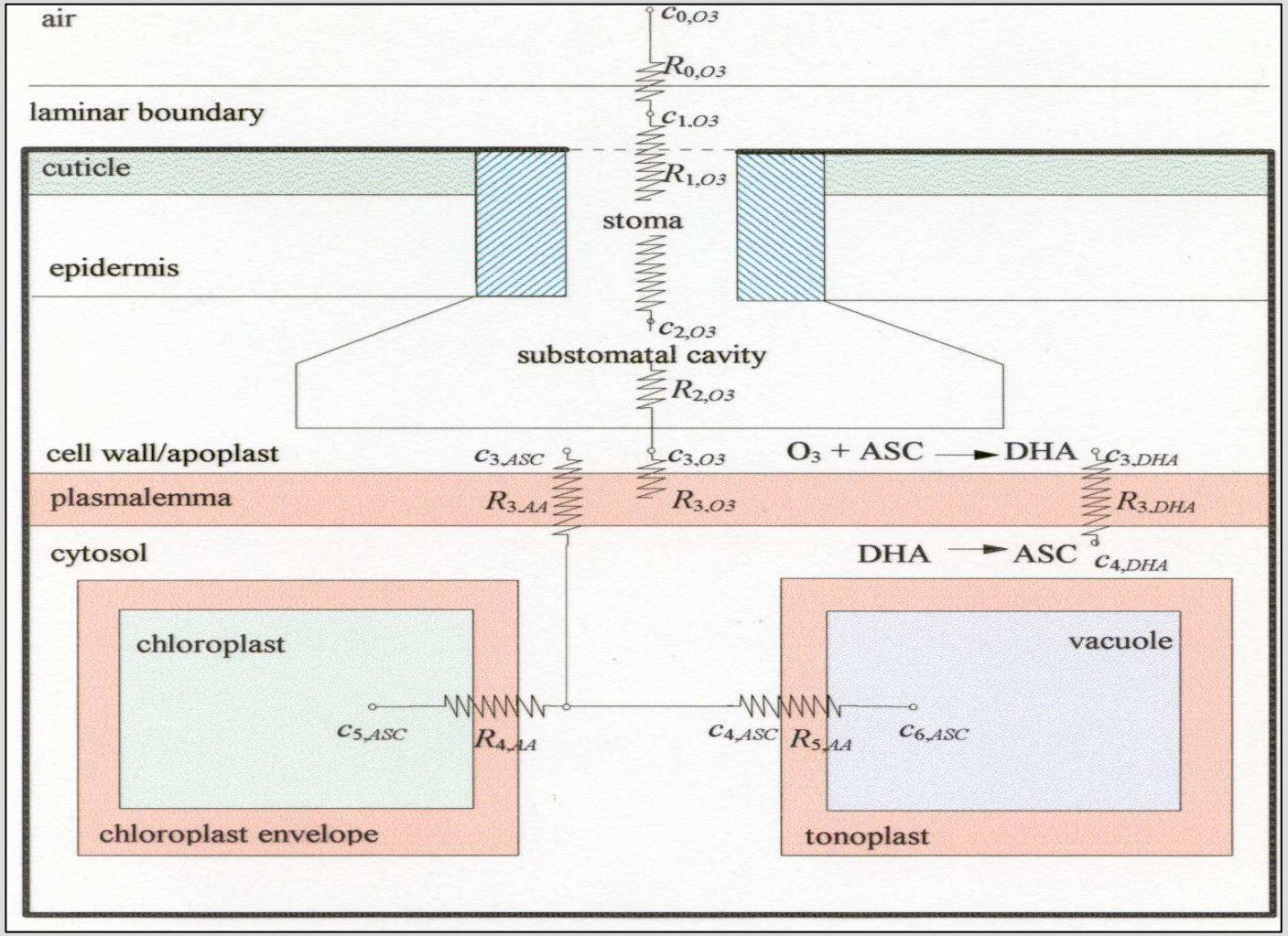


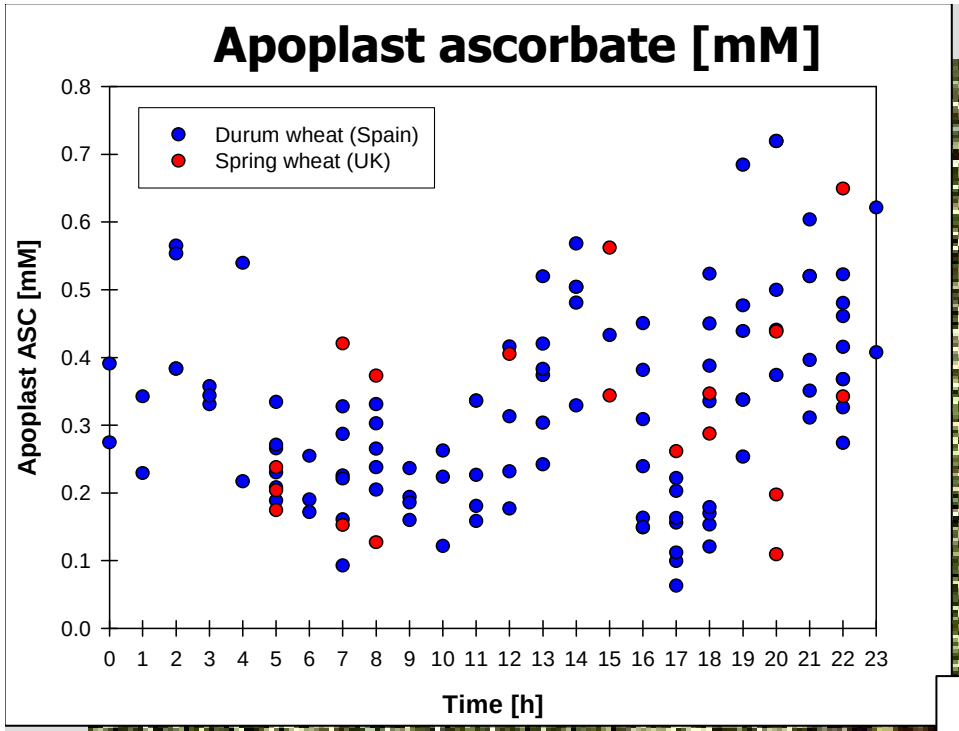
Figure 6. Relative abundance of transcripts encoding a selection of antioxidant enzymes and the ion channel NtTPC1B. Transcript abundance was measured by semiquantitative RT-PCR in leaves from untransformed controls (wild type), *AO* sense, and *AO* antisense lines:

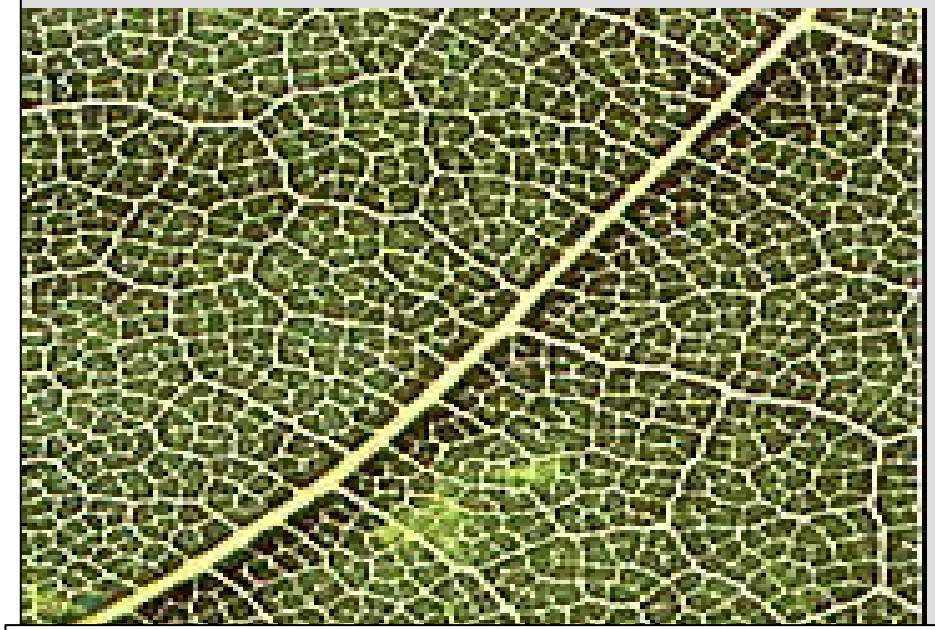
Pignocchi , Barnes *et al* (2006) Plant Physiol. 141: 423-449

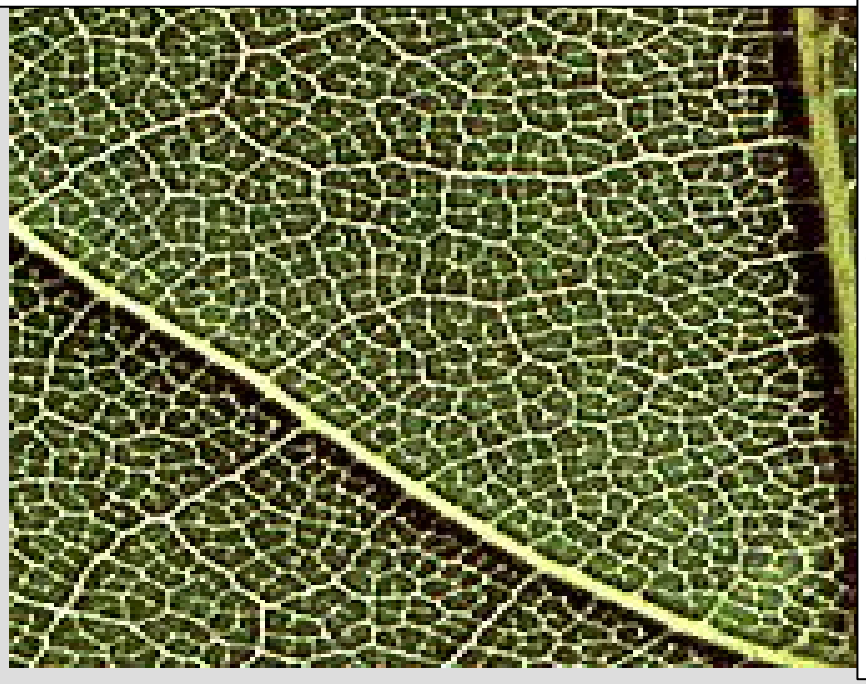
SODA: Modelled diffusion-reaction network

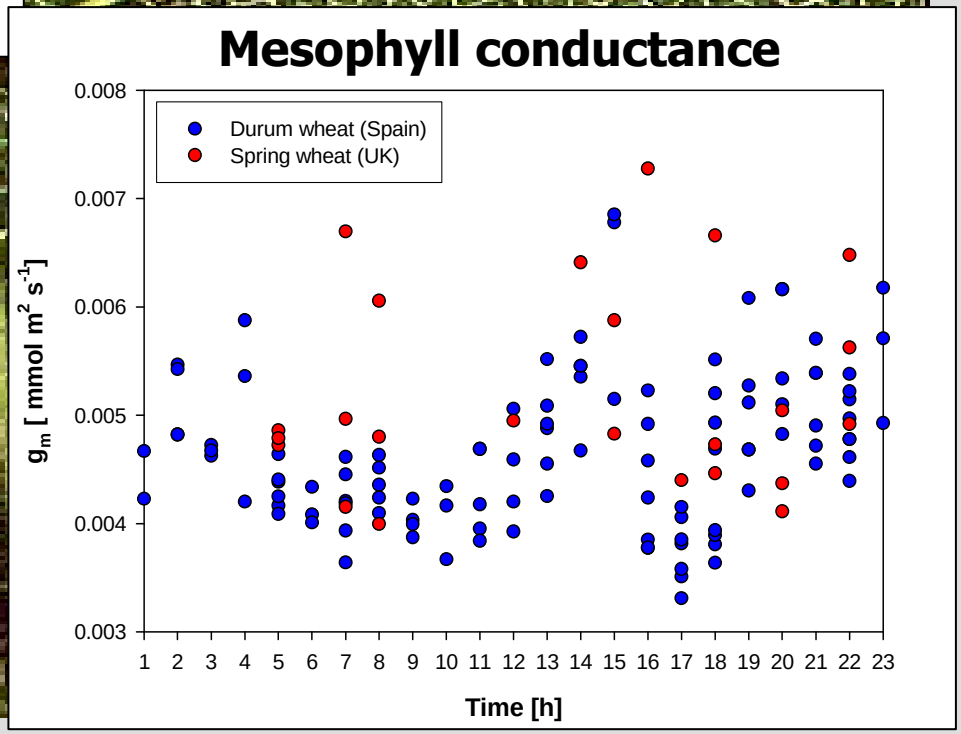


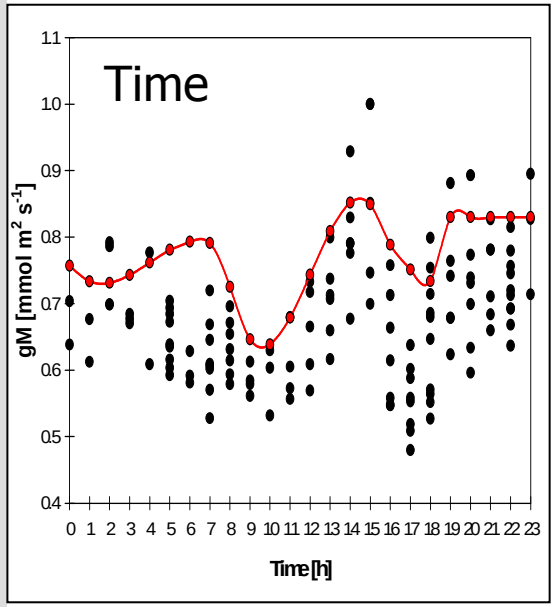
Plöchl, Barnes et al. (2000) Simulating ozone detoxification … Planta 210: 454-467

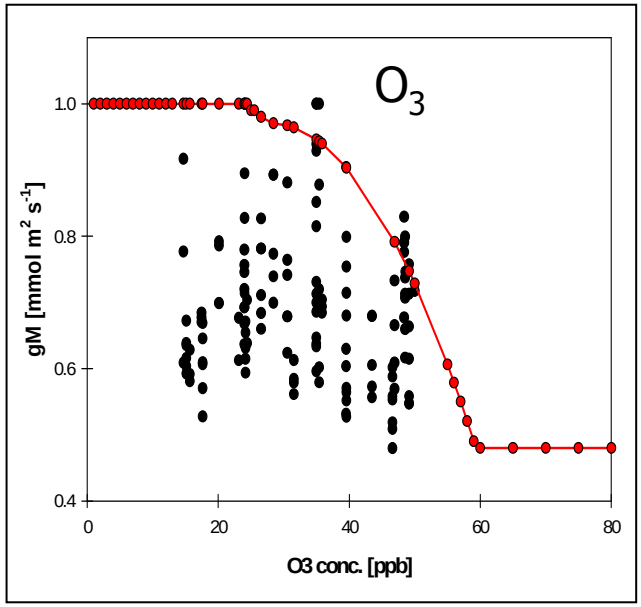

Apoplast ascorbate [mM]
Durum wheat (Spain)
Spring wheat (UK)
Apoplast ASC [mM]
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Time [h]

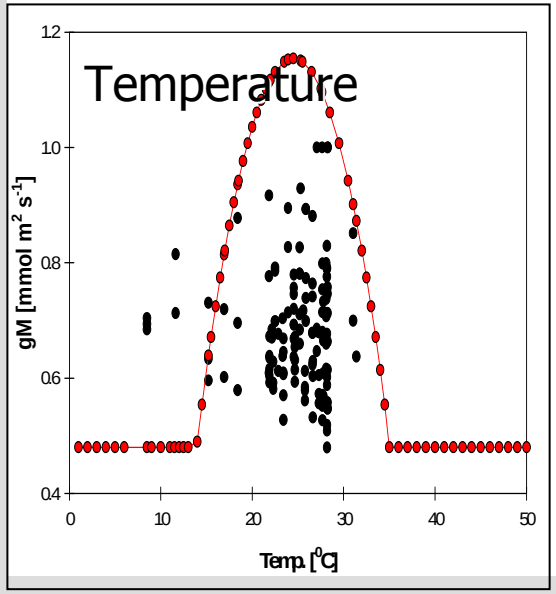


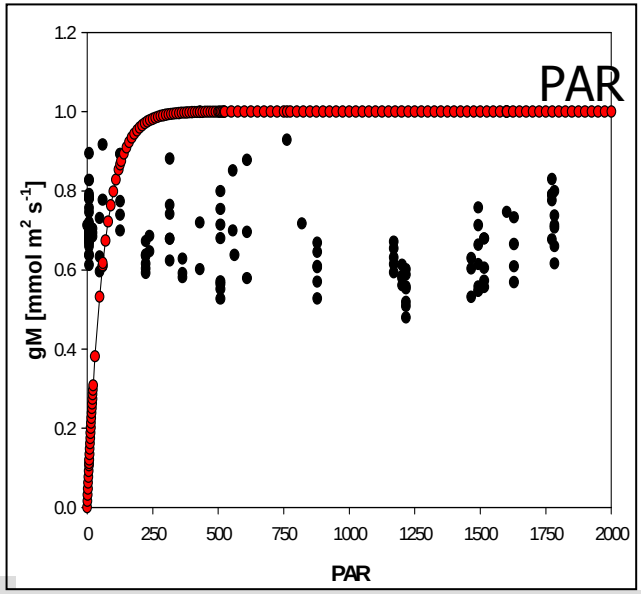


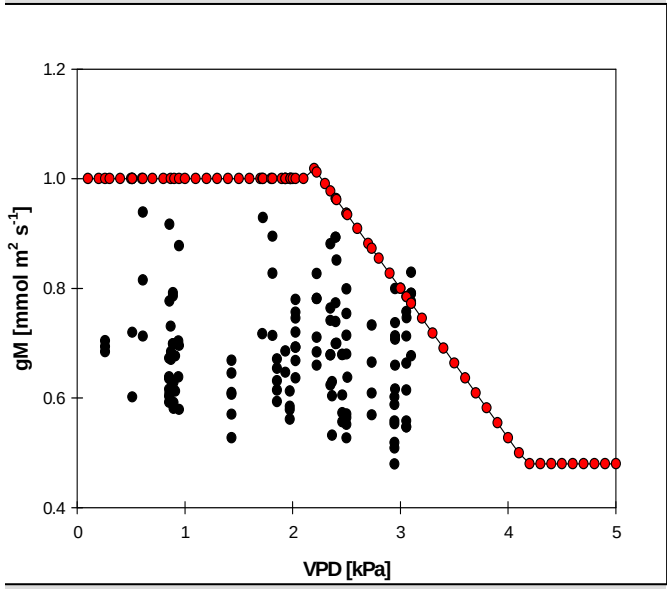

Mesophyll conductance
Durum wheat (Spain)
Spring wheat (UK)
g_m [mmol m^2 s^{-1}]
0.003
0.004
0.005
0.006
0.007
0.008
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Time [h]


Time
gM [mmol m2 s-1]
Time [h]


O3
gM [mmol m2 s-1]
O3 conc. [ppb]

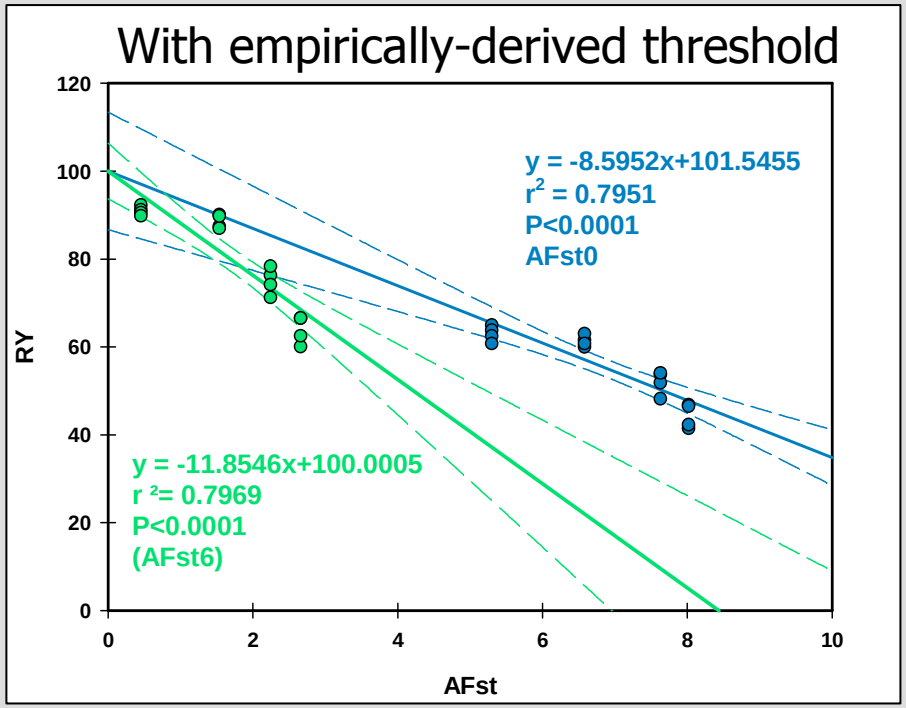

Temperature
gM [mmol m2 s-1]
Temp. [0C]

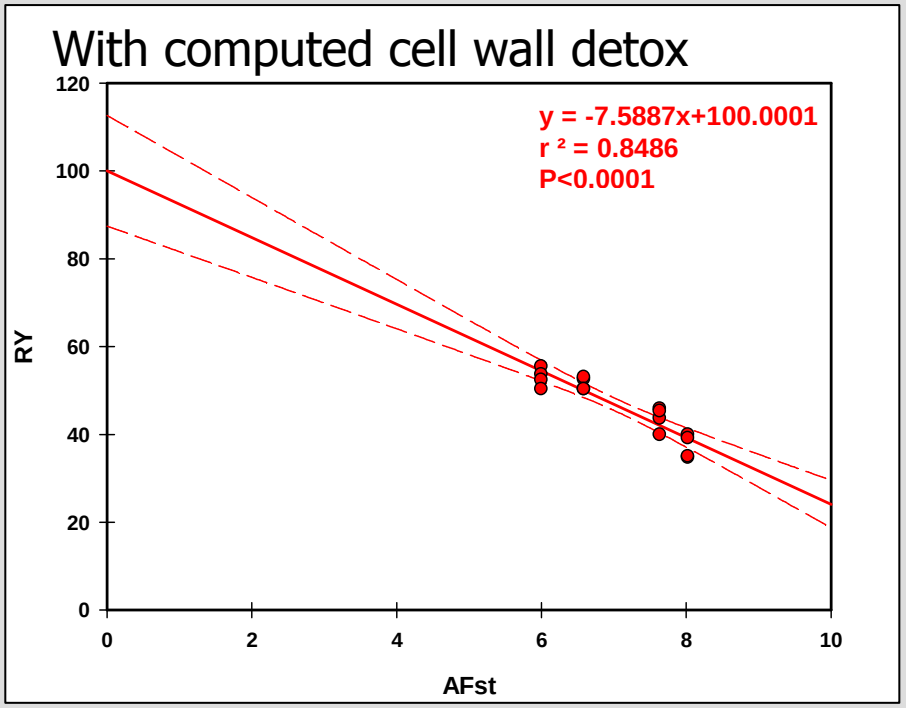

PAR
gM [mmol m2 s-1]
PAR


gM [mmol m2 s-1]
VPD [kPa]

Winter wheat flux-response

(for sensitive genotypes)

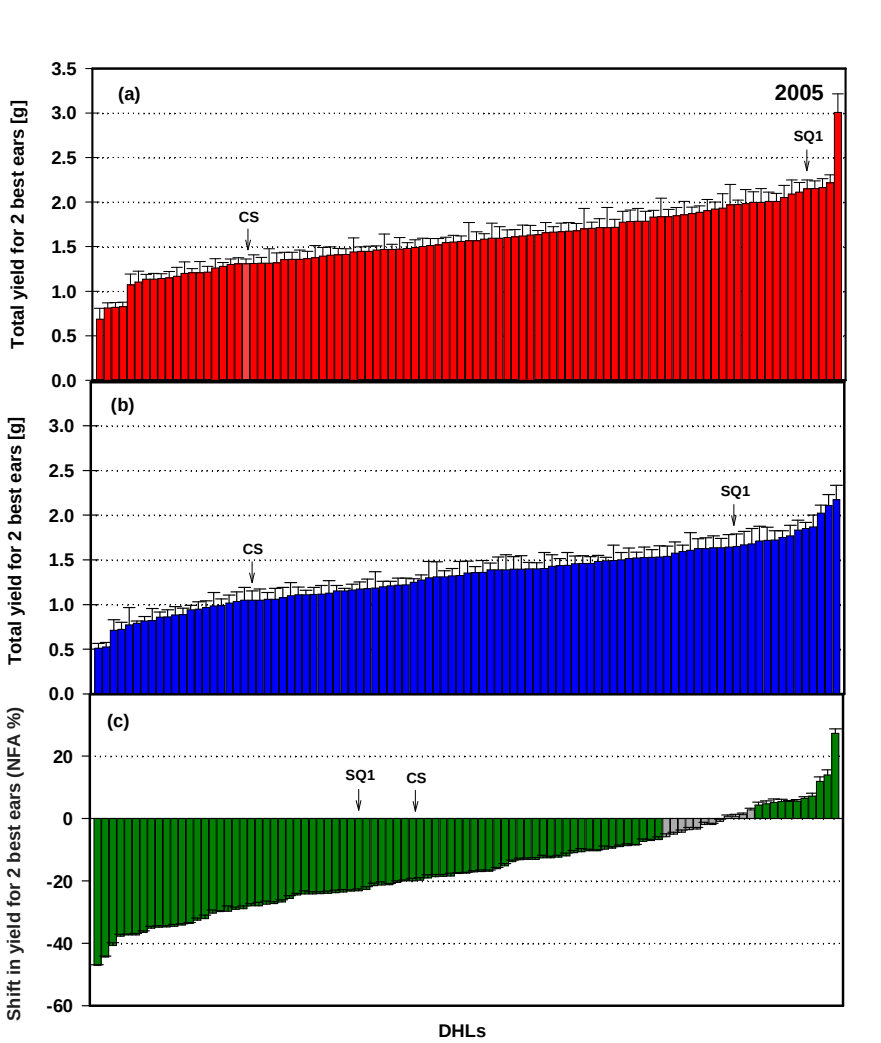




Knowledge gaps

- Greater understanding internal diffusion-reaction network/wall biochemistry
- Additional experimental data to test/improve/validate detox modelling approaches
- Quantification and dissection of carbon costs

Selection for ozone resistance



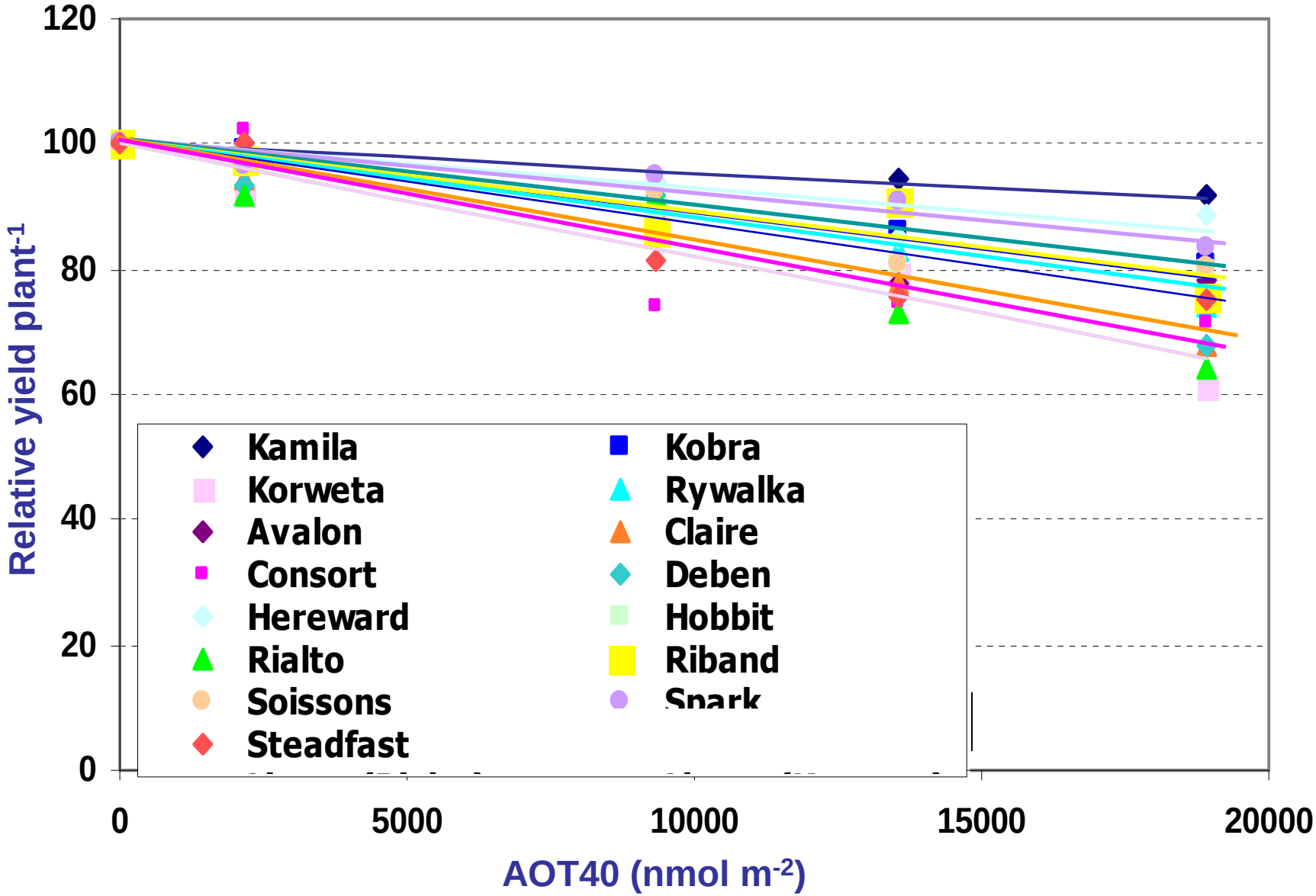
NFA

NFA+50 nmol mol $^{-1}$ O_3

O_3-induced shift in yield

CS X SQ1 yield (2005)

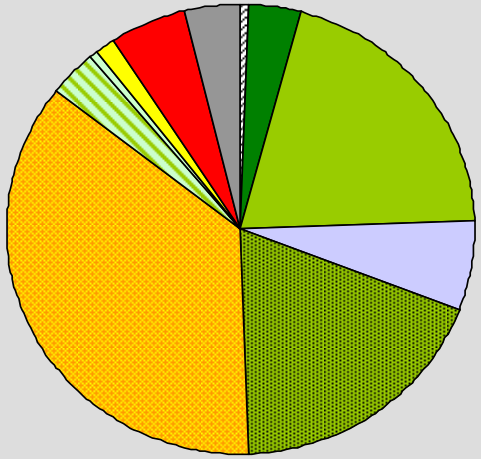
Kaminska, Barnes & Quarrie, unpublished


120
100
80
60
40
20
0
Relative yield plant-1
0
5000
10000
15000
20000
AOT40 (nmol m-2)
Kamila
Korweta
Avalon
Consort
Hereward
Rialto
Soissons
Steadfast
Kobra
Rywalka
Claire
Deben
Hobbit
Riband
Spark

Effect of 2050 upland ozone climate on a mesotrophic grassland community [MG3b] - mesocosm study (ongoing)

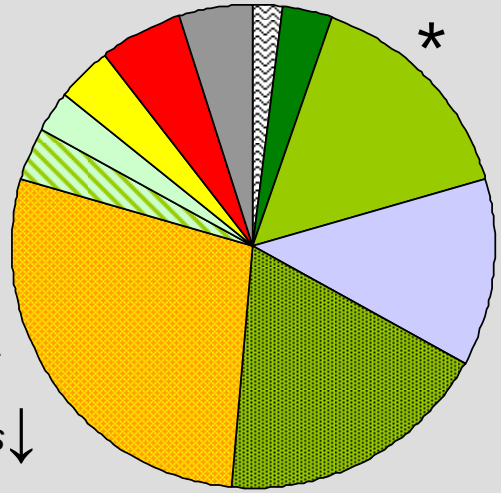
2006 Biomass

Present-day UK upland ozone climate



2050 UK Upland ozone climate

Briza media↓

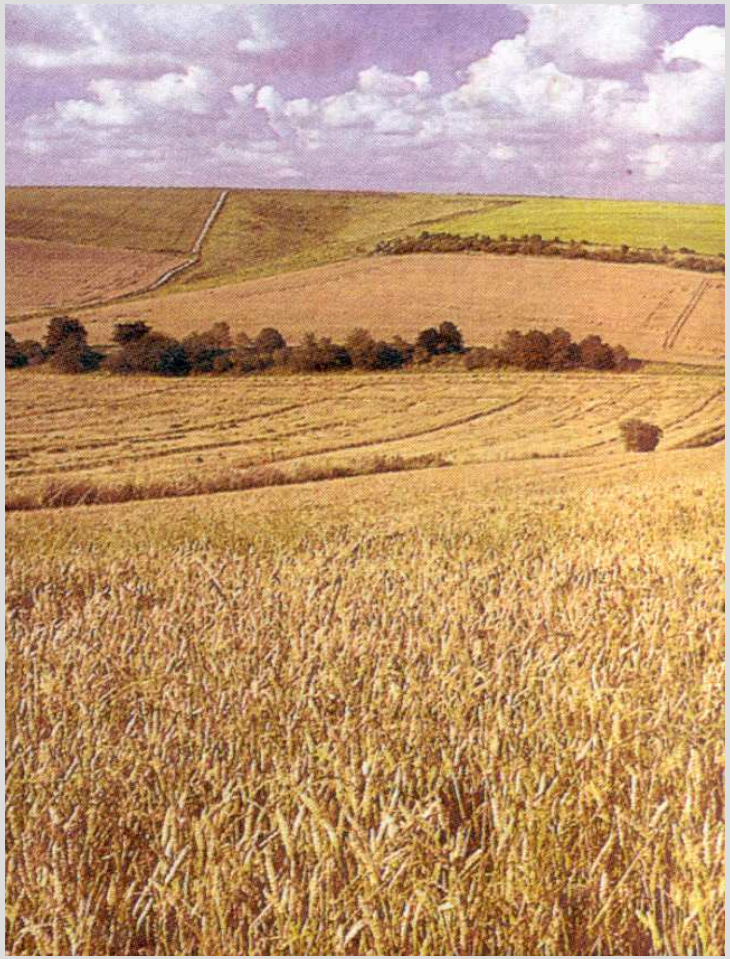


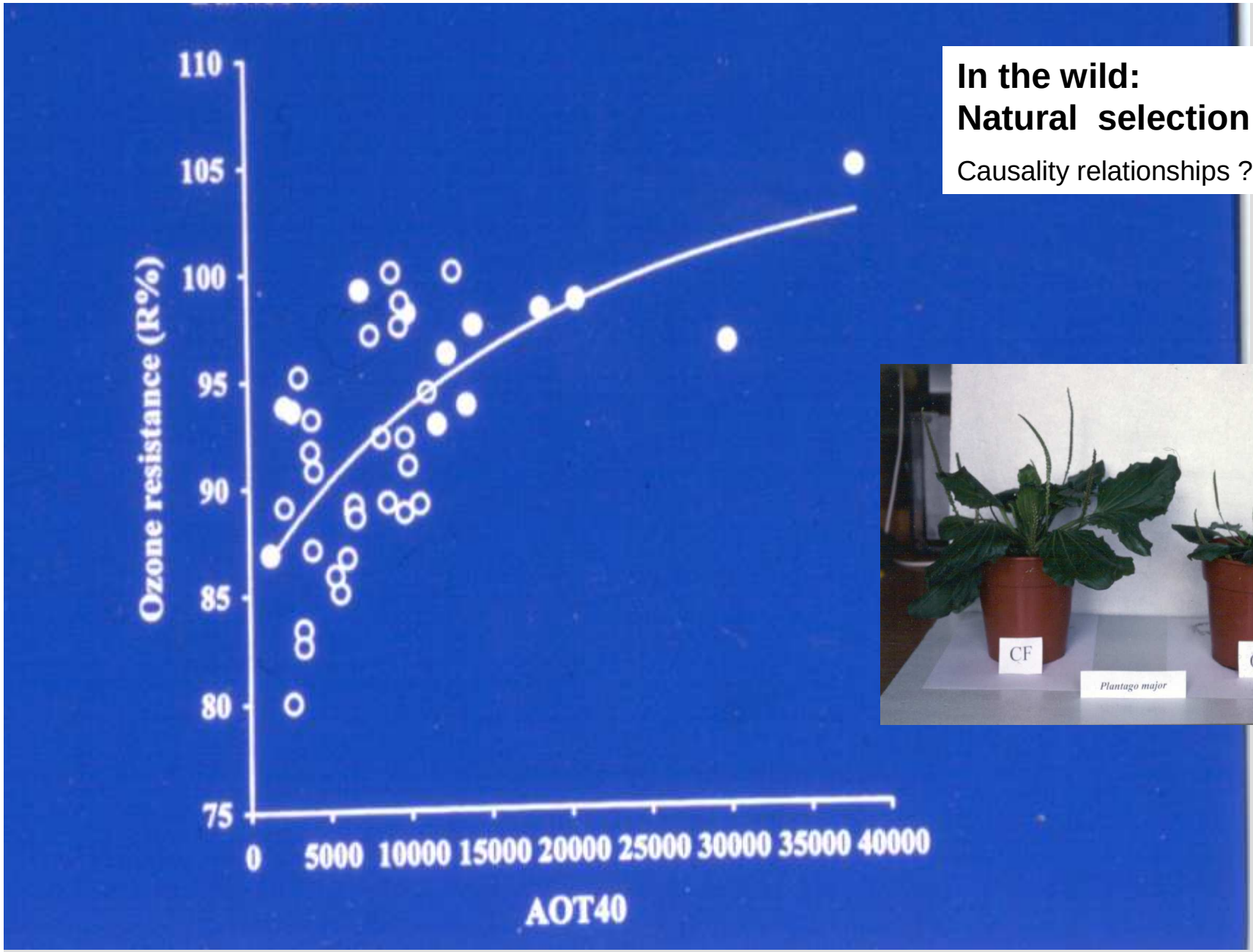
L. corniculatus↓

Selection for ozone resistance

Crops/ornamentals: inadvertent selection

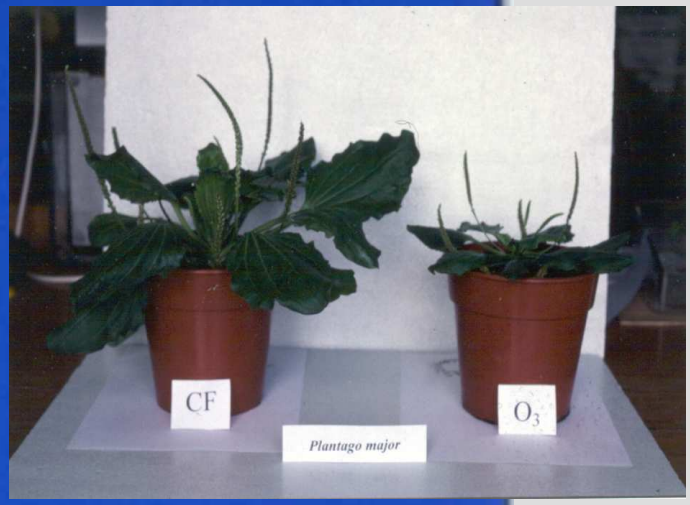
High yields/best appearance regardless of causality





In the wild:
Natural selection

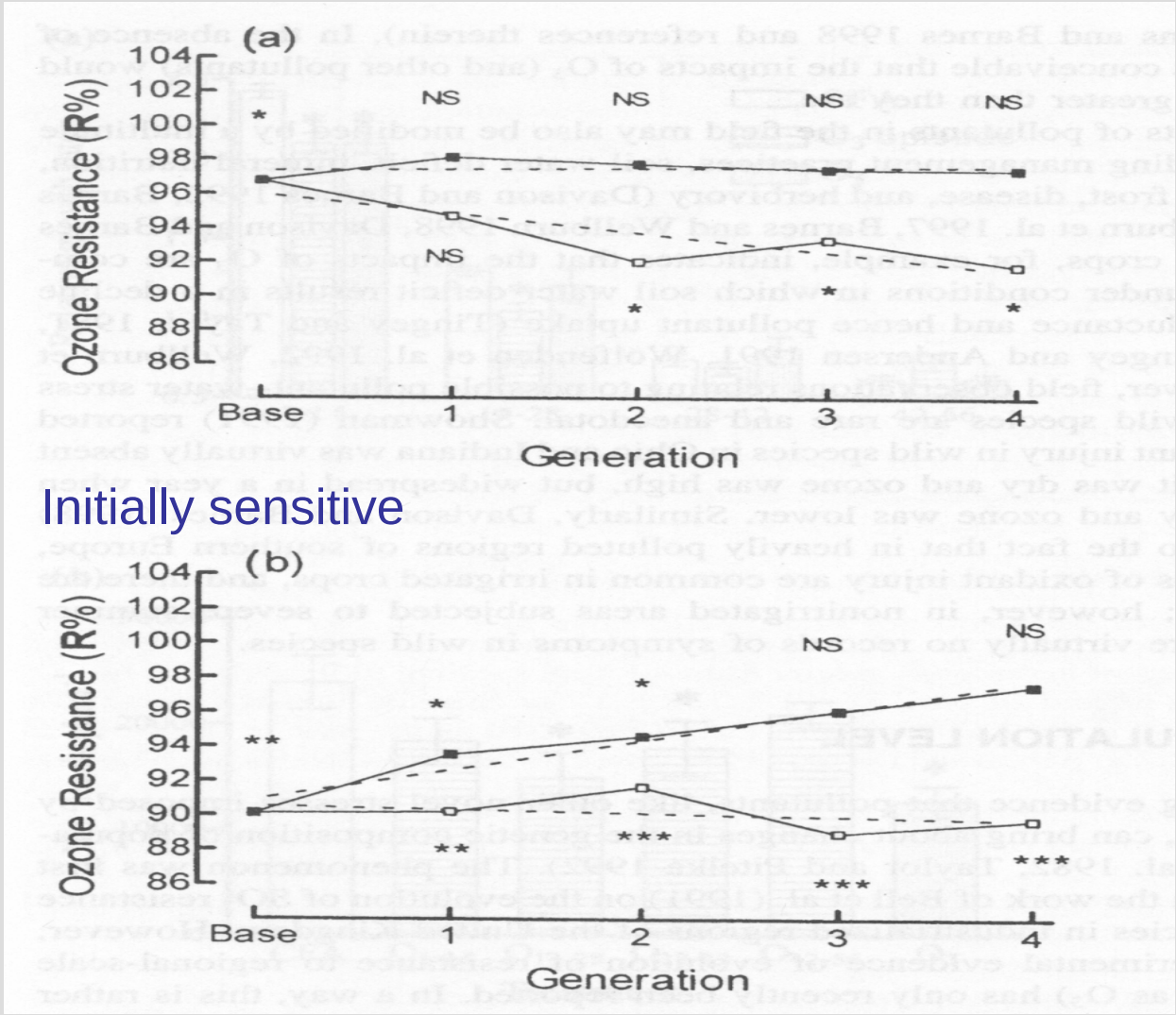
Causality relationships ?



Source: Davison & Barnes (1998) *New Phytol* 139: 135-151

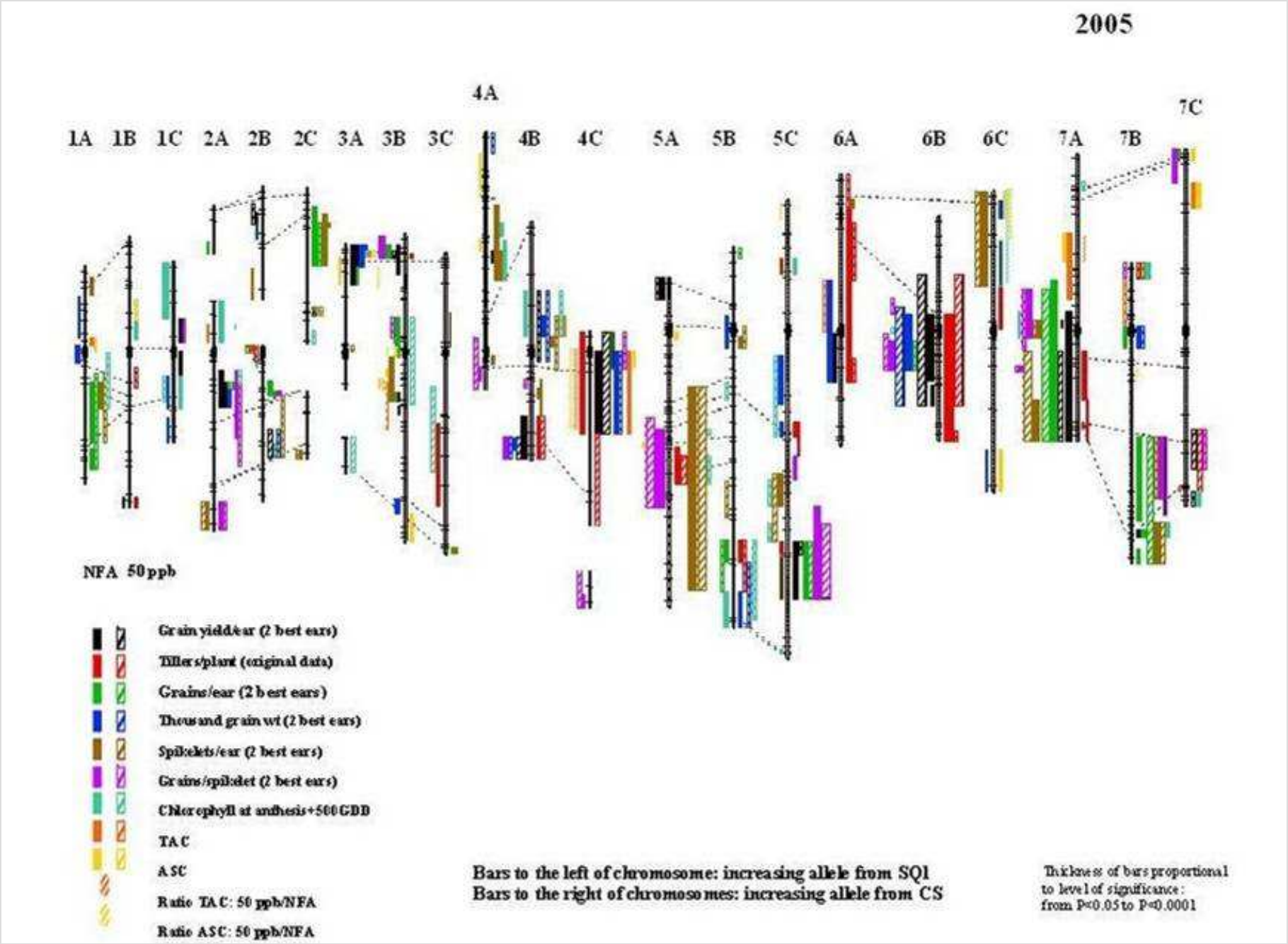
Directed selection

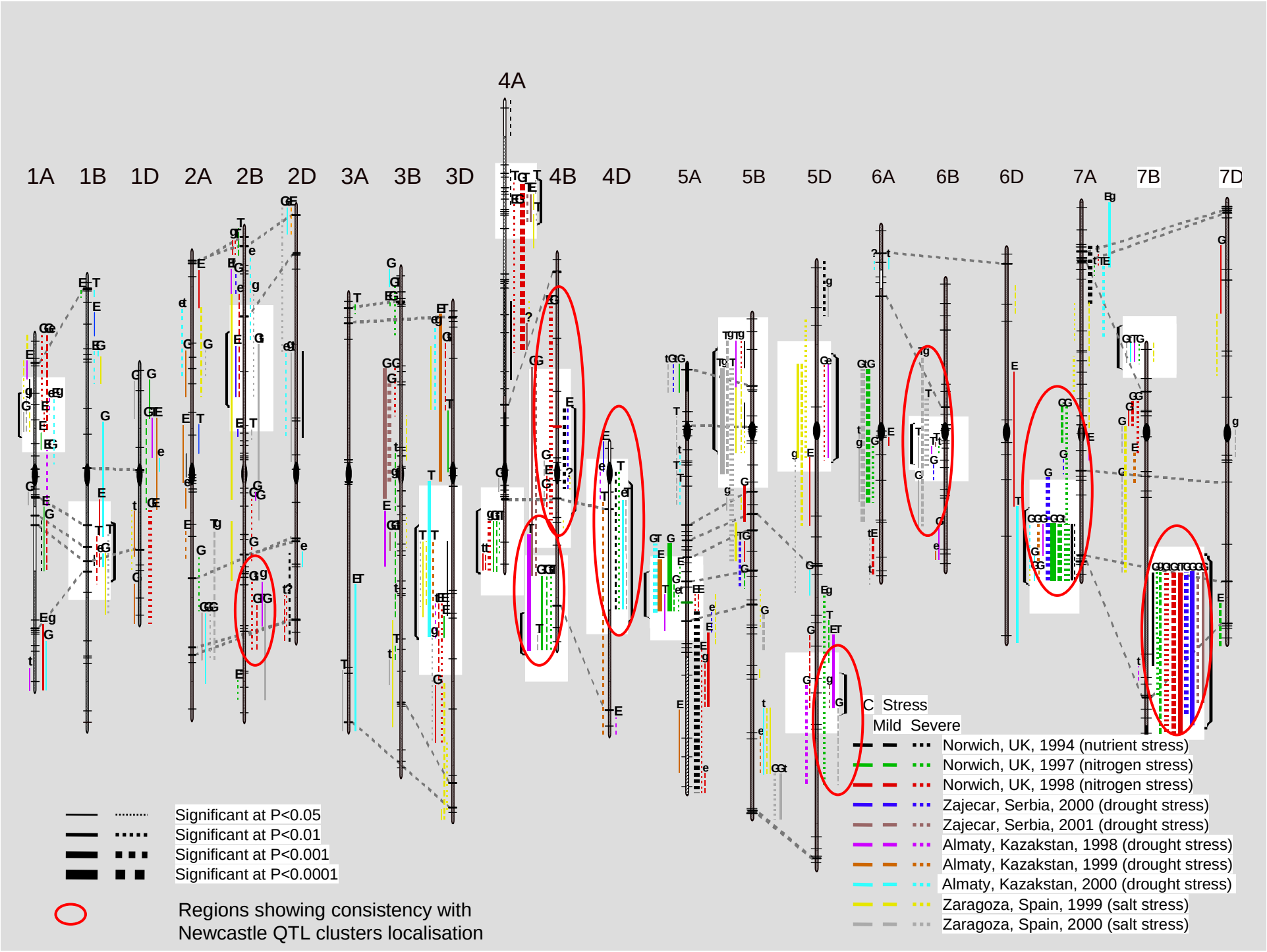
Initially resistant



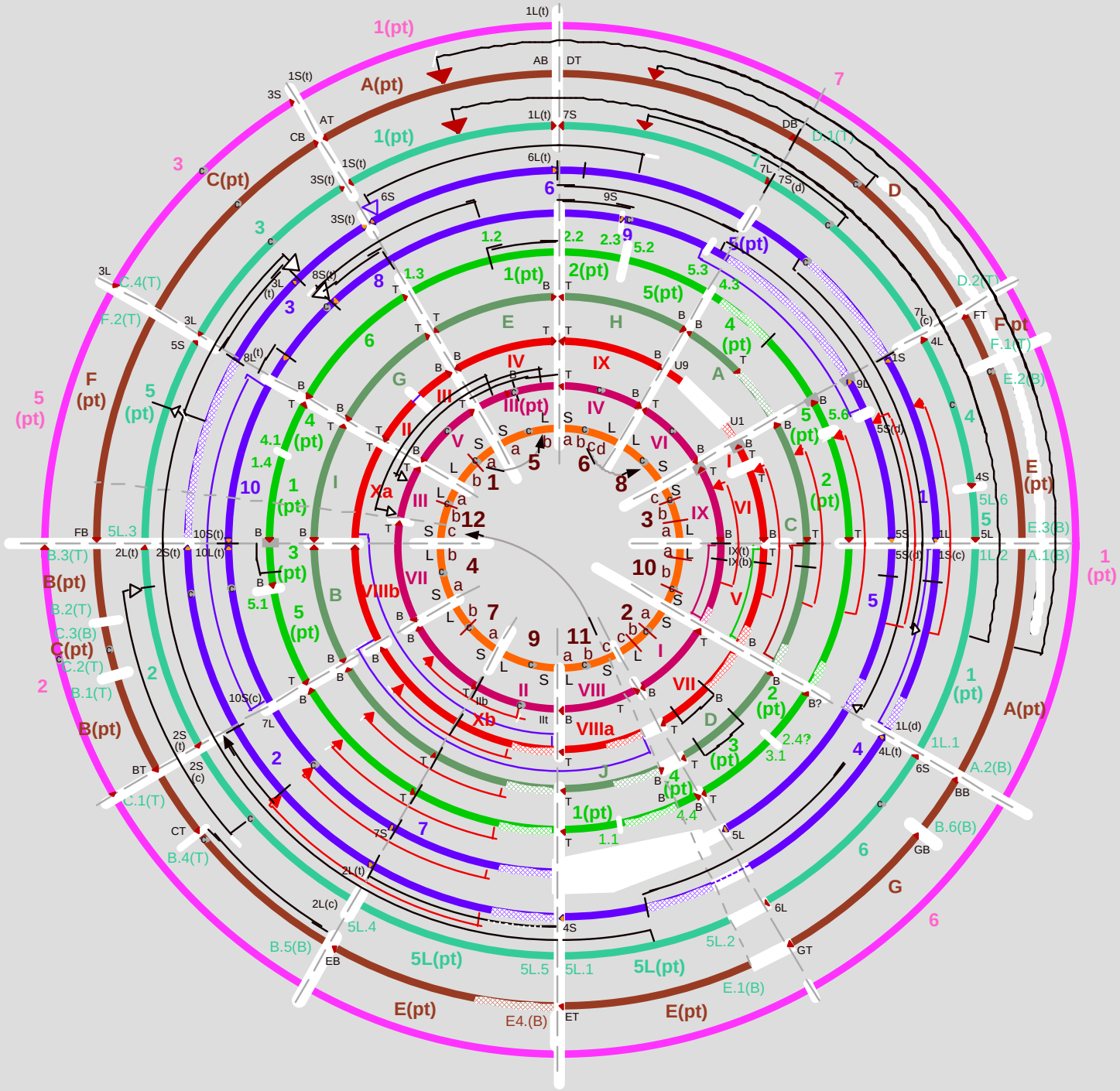
Initially sensitive

Source: Whitfield et al. (1997) *New Phytol*. 137: 645-655


2005
1A 1B 1C 2A 2B 2C 3A 3B 3C 4A 4B 4C 5A 5B 5C 6A 6B 6C 7A 7B 7C
NFA 50 ppb
Grain yield/ear (2 best ears)
Tillers/plant (original data)
Grains/ear (2 best ears)
Thousand grain wt (2 best ears)
Spikelets/ear (2 best ears)
Grains/spikelet (2 best ears)
Chlorophyll at anthesis+500 GDD
TAC
ASC
Ratio TAC: 50 ppb/NFA
Ratio ASC: 50 ppb/NFA
Bars to the left of chromosome: increasing allele from SQ1
Bars to the right of chromosomes: increasing allele from CS
Thickness of bars proportional to level of significance: from P<0.05 to P<0.0001


1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Significant at P<0.05
Significant at P<0.01
Significant at P<0.001
Significant at P<0.0001
Regions showing consistency with
Newcastle QTL clusters localisation
C Stress
Mild Severe
Norwich, UK, 1994 (nutrient stress)
Norwich, UK, 1997 (nitrogen stress)
Norwich, UK, 1998 (nitrogen stress)
Zajecar, Serbia, 2000 (drought stress)
Zajecar, Serbia, 2001 (drought stress)
Almaty, Kazakstan, 1998 (drought stress)
Almaty, Kazakstan, 1999 (drought stress)
Almaty, Kazakstan, 2000 (drought stress)
Zaragoza, Spain, 1999 (salt stress)
Zaragoza, Spain, 2000 (salt stress)

GRASS GENOMES



Lolium

Oats

Triticeae

Maize

Pearl millet

Sorghum

Sugar cane

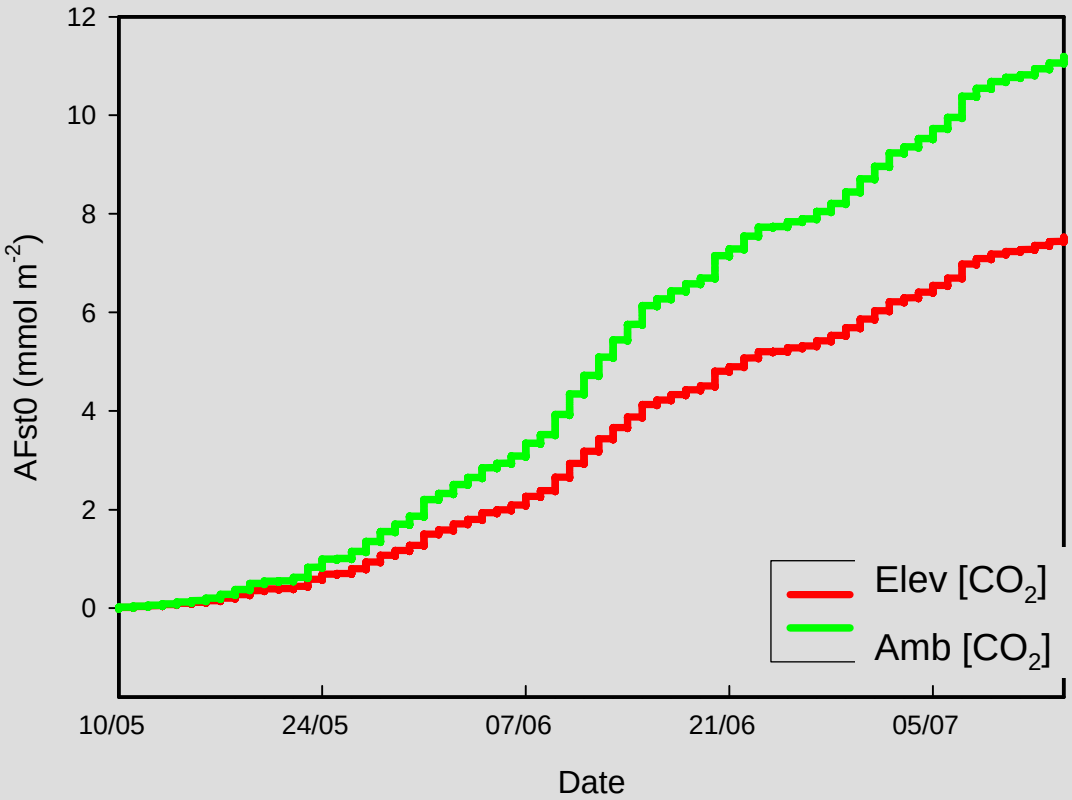
Foxtail millet

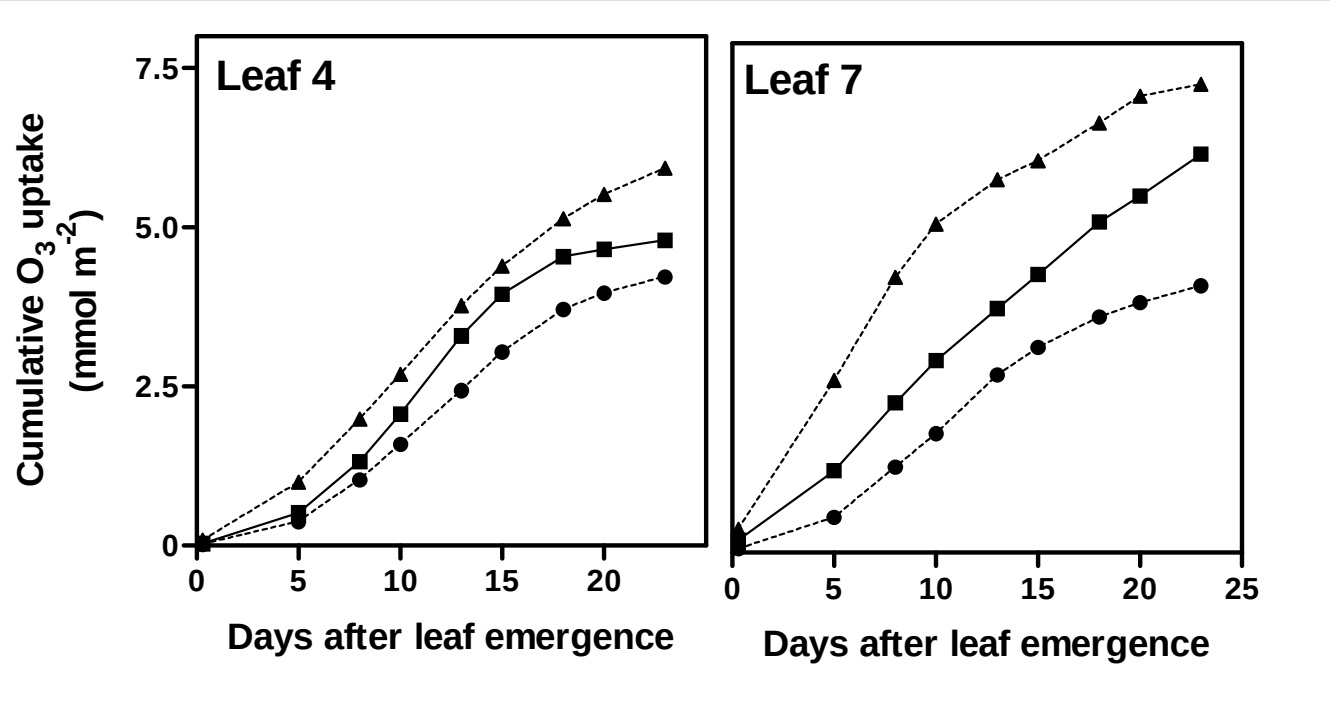
Rice

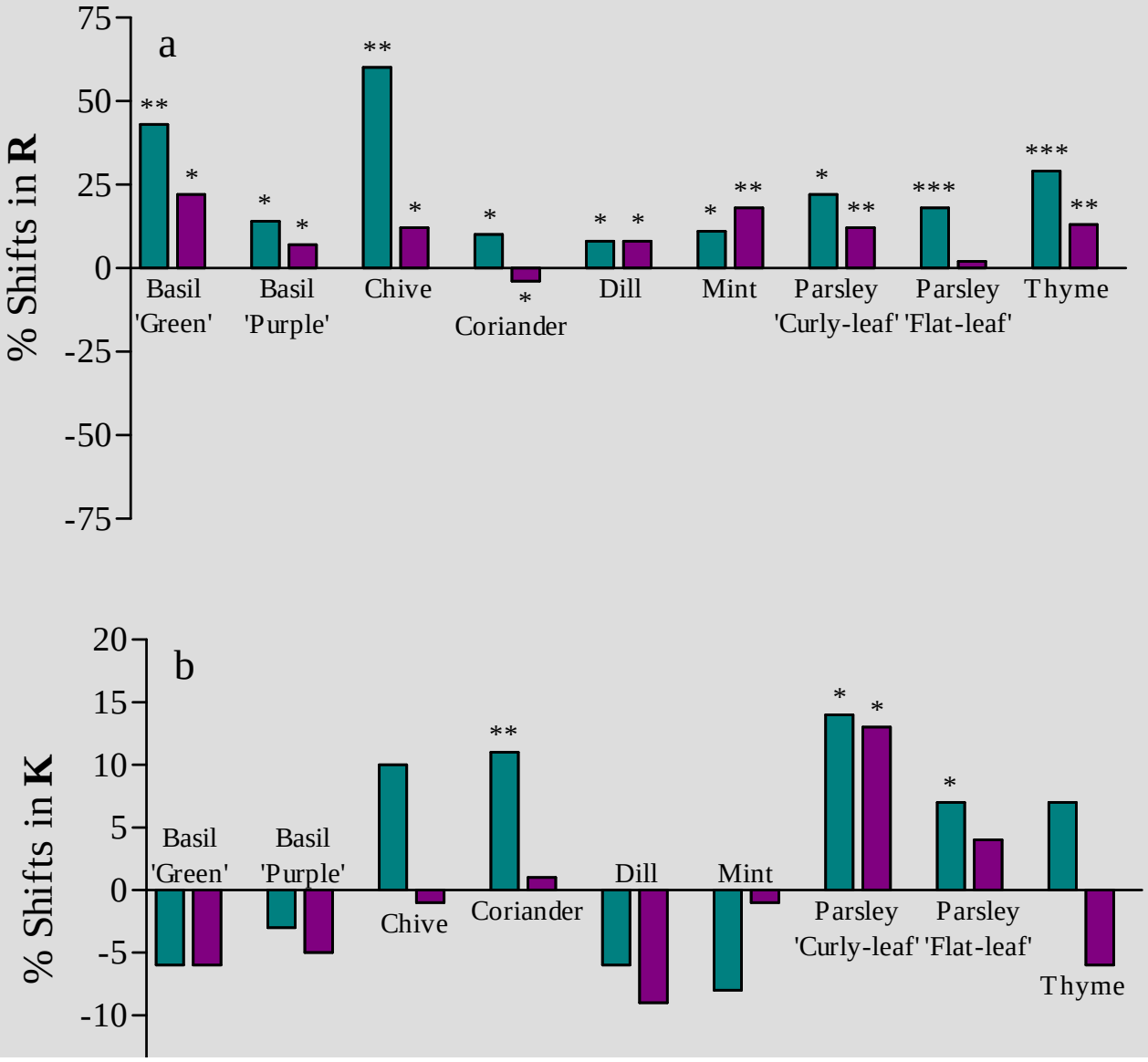
Knowledge gaps

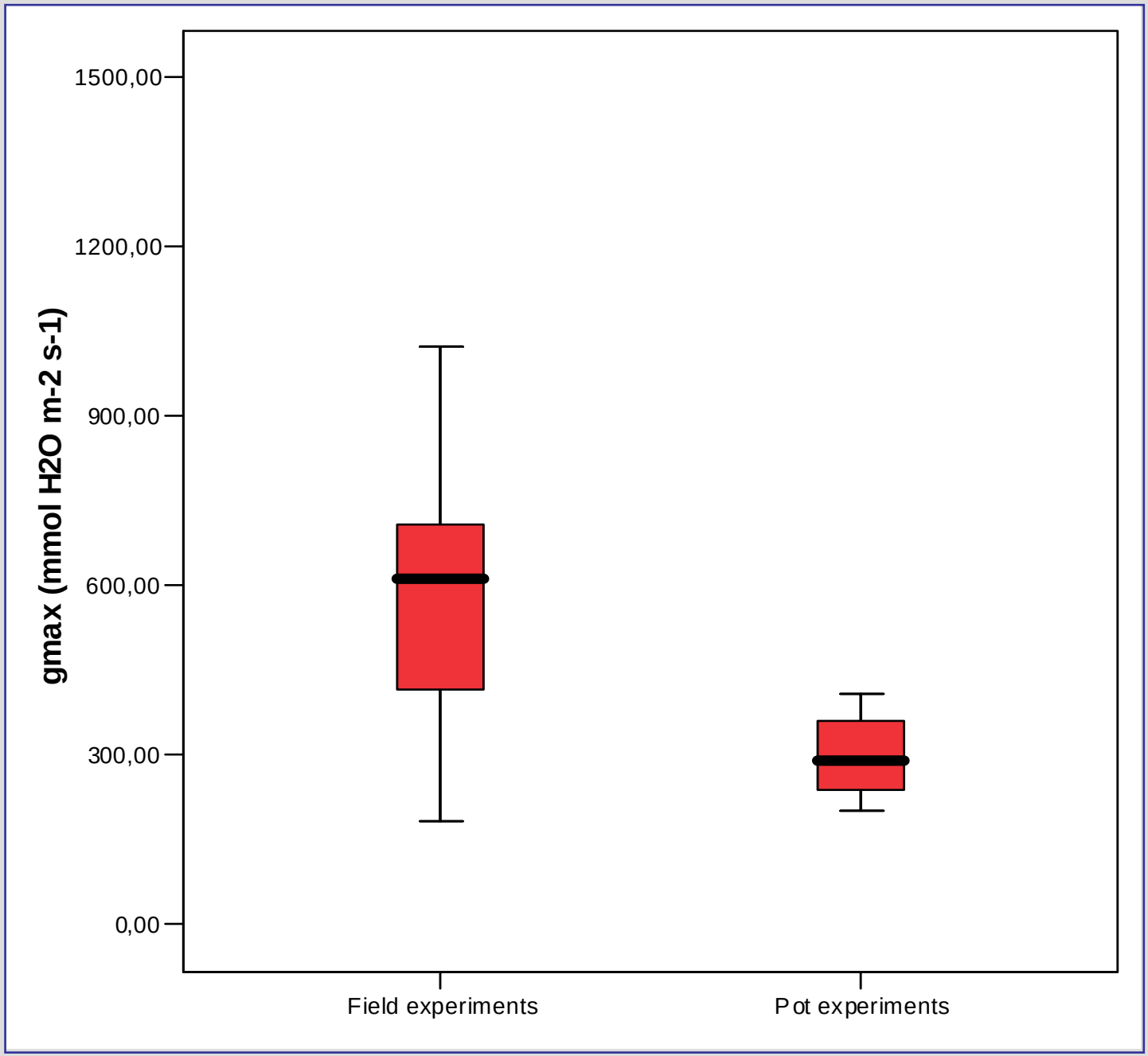
- Key QTLs/genes governing yield in O_3-enriched environments
- No studies ongoing using commercial germplasm
- Facilities limitations to undertake required screening on mapping populations and exposure of field-scale plots

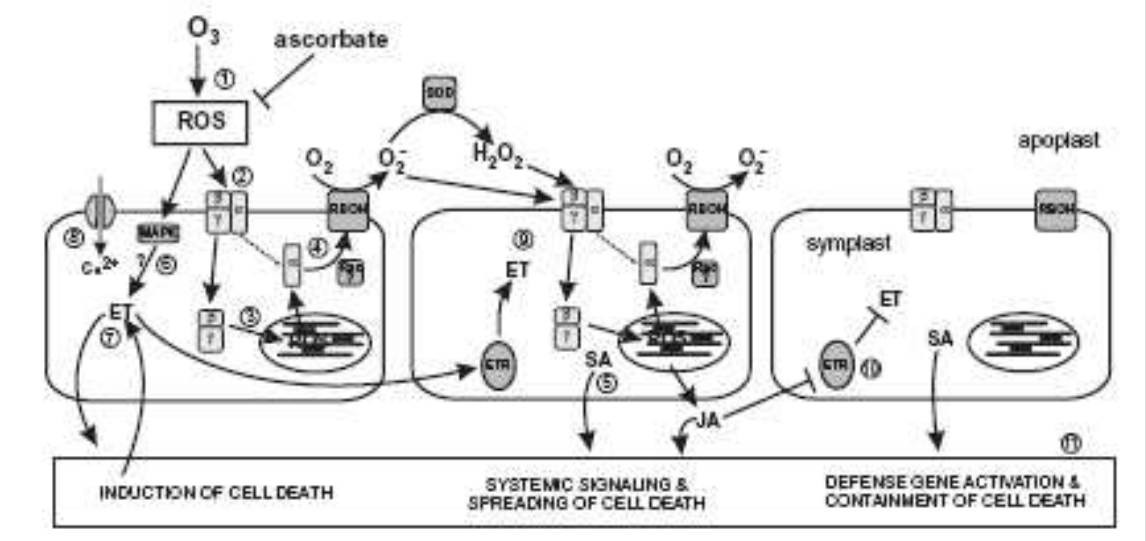
ozone flux – spring wheat



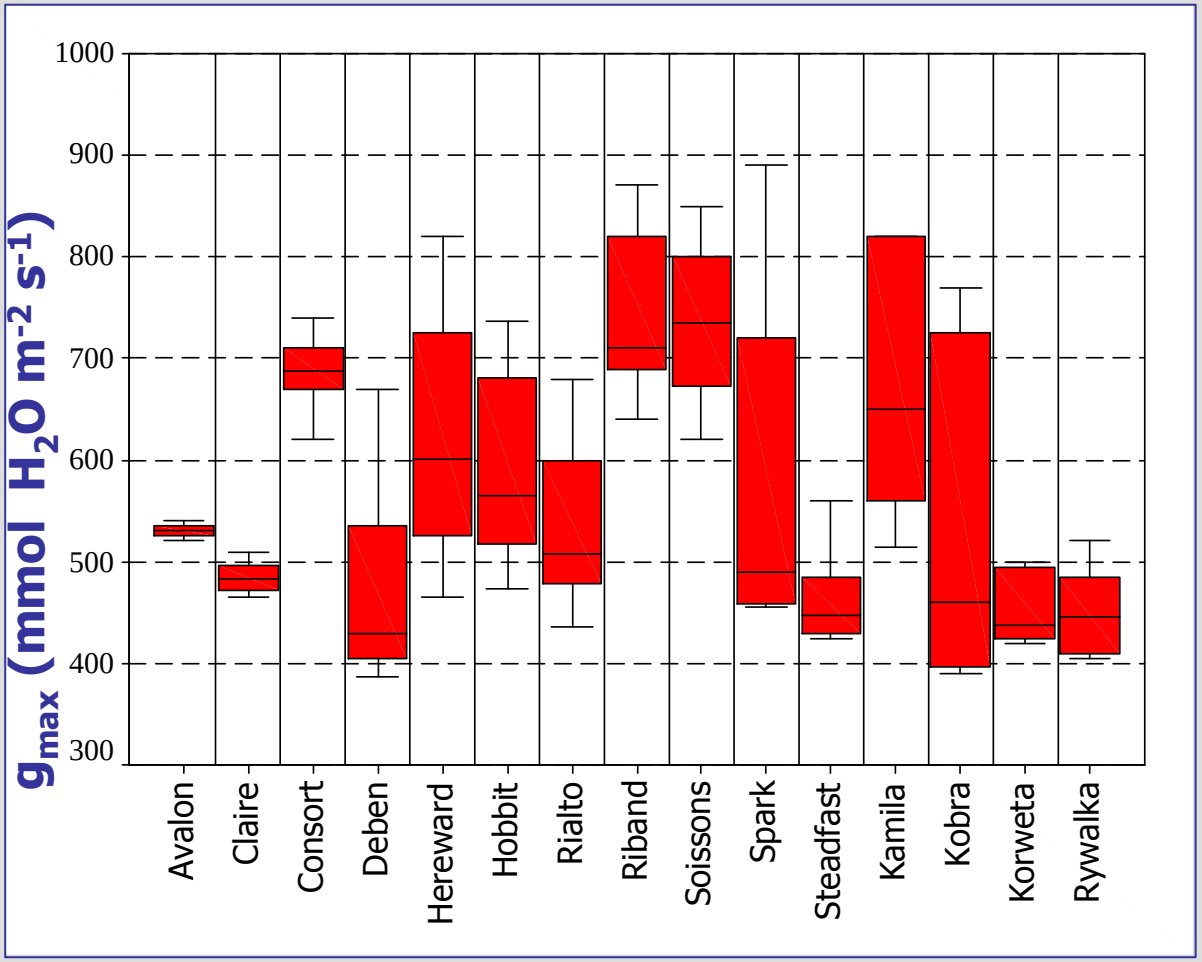

Leaf 4
Leaf 7
Cumulative O_3 uptake
(mmol m^{-2})
7.5
5.0
2.5
0
0
5
10
15
20
25
Days after leaf emergence
Days after leaf emergence


a
% Shifts in R
75
50
25
0
-25
-50
-75
Basil 'Green'
Basil 'Purple'
Chive
Coriander
Dill
Mint
Parsley 'Curly-leaf'
Parsley 'Flat-leaf'
Thyme
b
% Shifts in K
20
15
10
5
0
-5
-10
Basil 'Green'
Basil 'Purple'
Chive
Coriander
Dill
Mint
Parsley 'Curly-leaf'
Parsley 'Flat-leaf'
Thyme

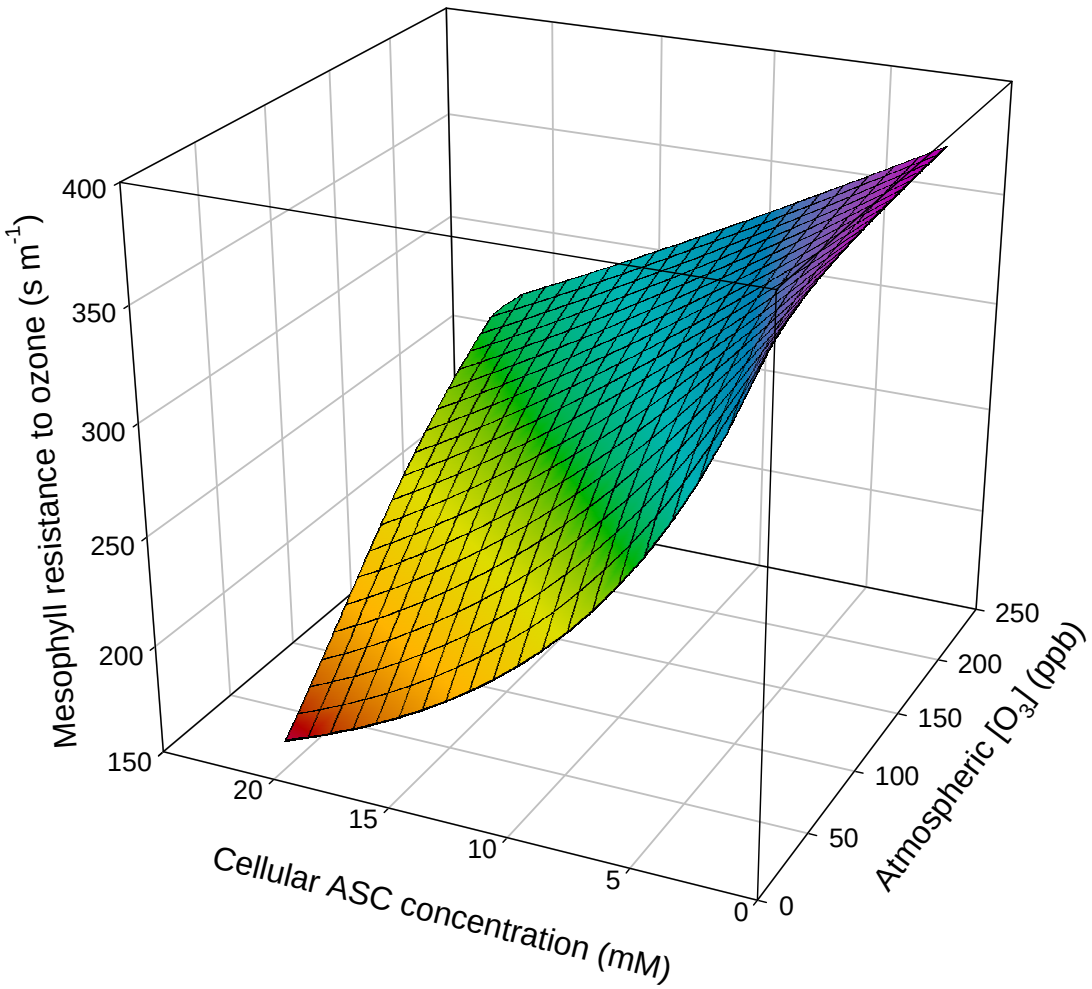

gmax (mmol H2O m-2 s-1)
1500,00
1200,00
900,00
600,00
300,00
0,00
Field experiments
Pot experiments


O3
ascorbate
ROS
SOD
H2O2
O2
O2-
RBOH
apoplast
symplast
MAPK
Ca2+
ET
ETR
SA
JA
INDUCTION OF CELL DEATH
SYSTEMIC SIGNALING &
SPREADING OF CELL DEATH
DEFENSE GENE ACTIVATION &
CONTAINMENT OF CELL DEATH

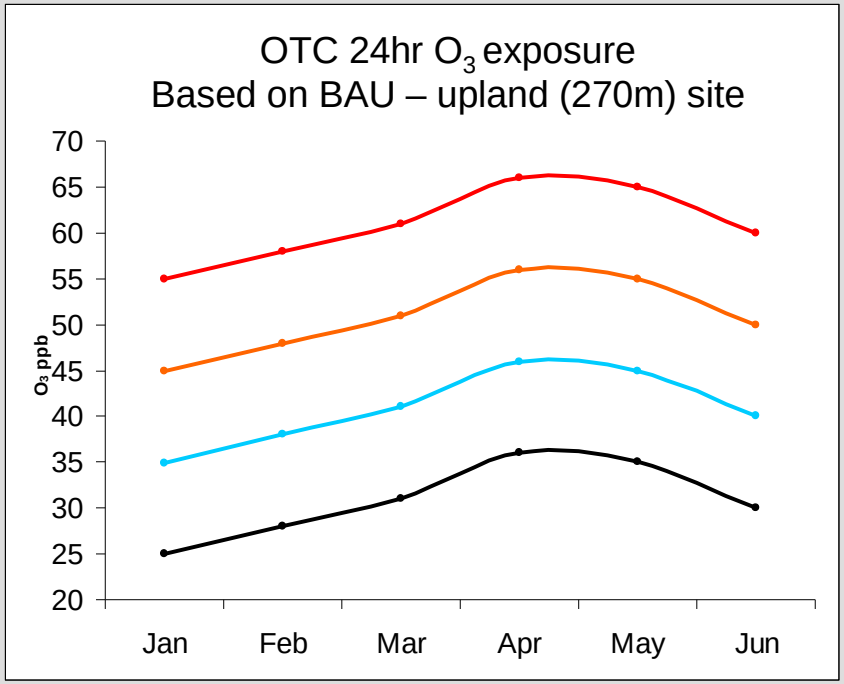
Knowledge gaps: variation in stomatal conductance



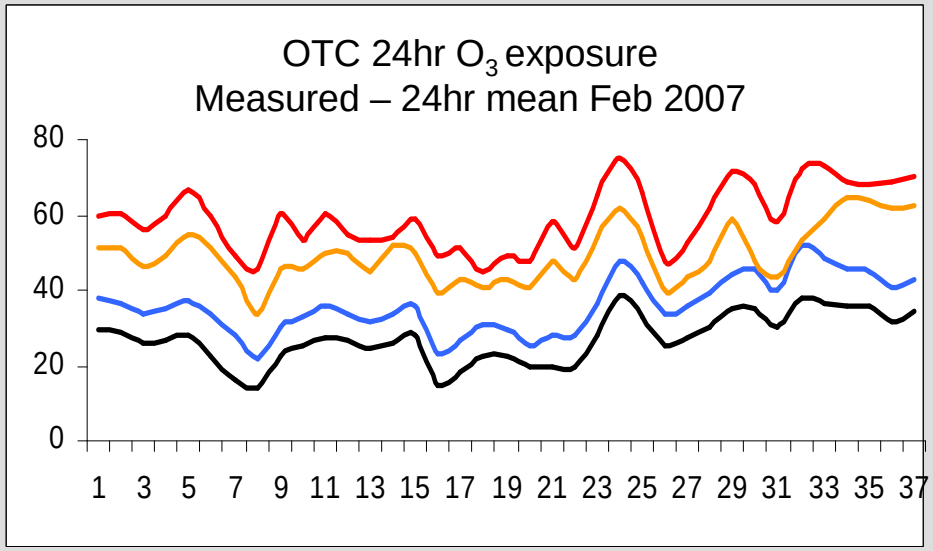
Winter wheat
Average g_{max} = 587 mmol H_2O m-2 s-1



- illustration of how reactive absorption with apoplast components (e.g. ASC) can reduce mesophyll resistance



NFA
NFA +10 ppb (2030)
NFA + 20 ppb (2100)
NFA + 30 ppb (2100+)



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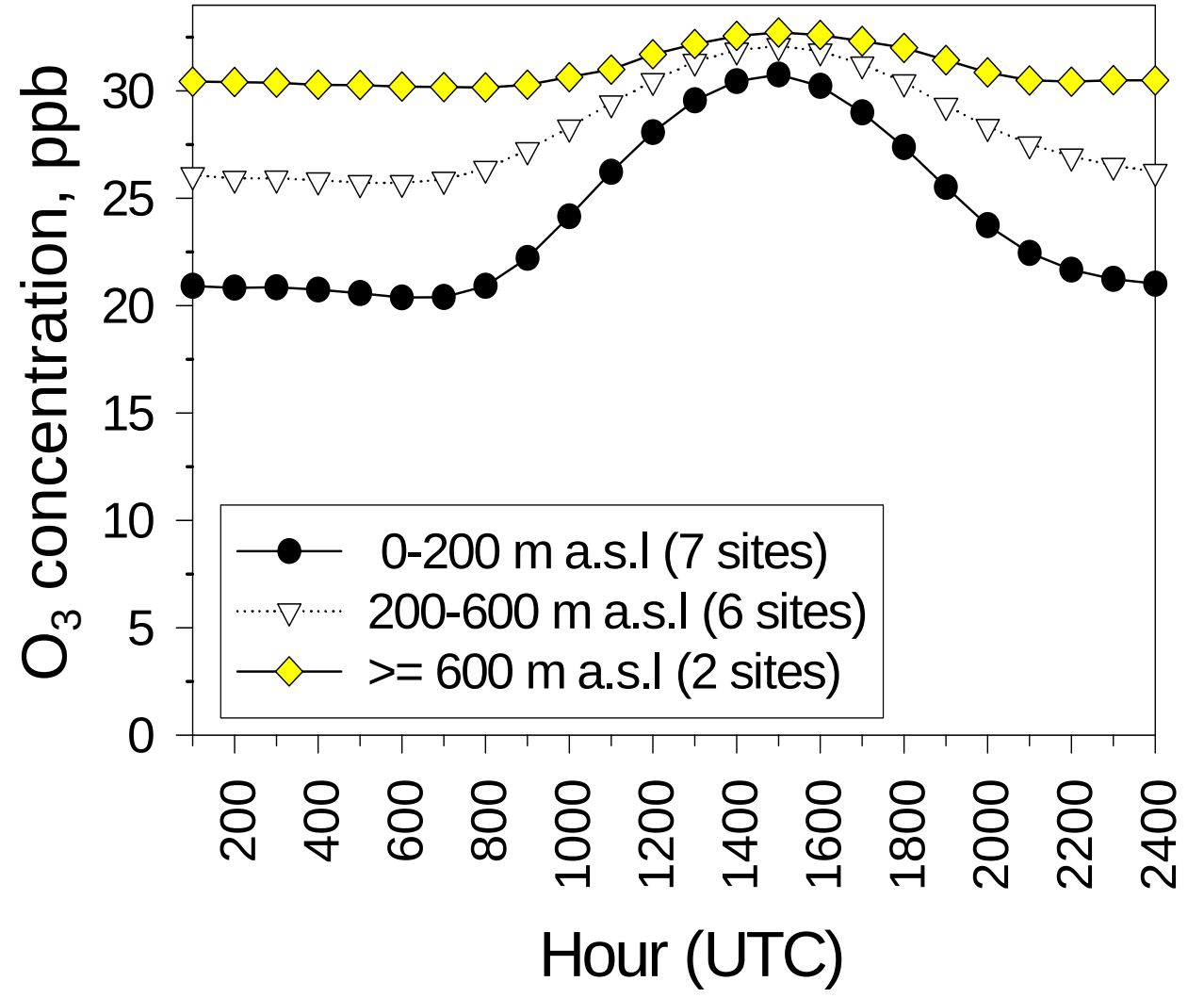
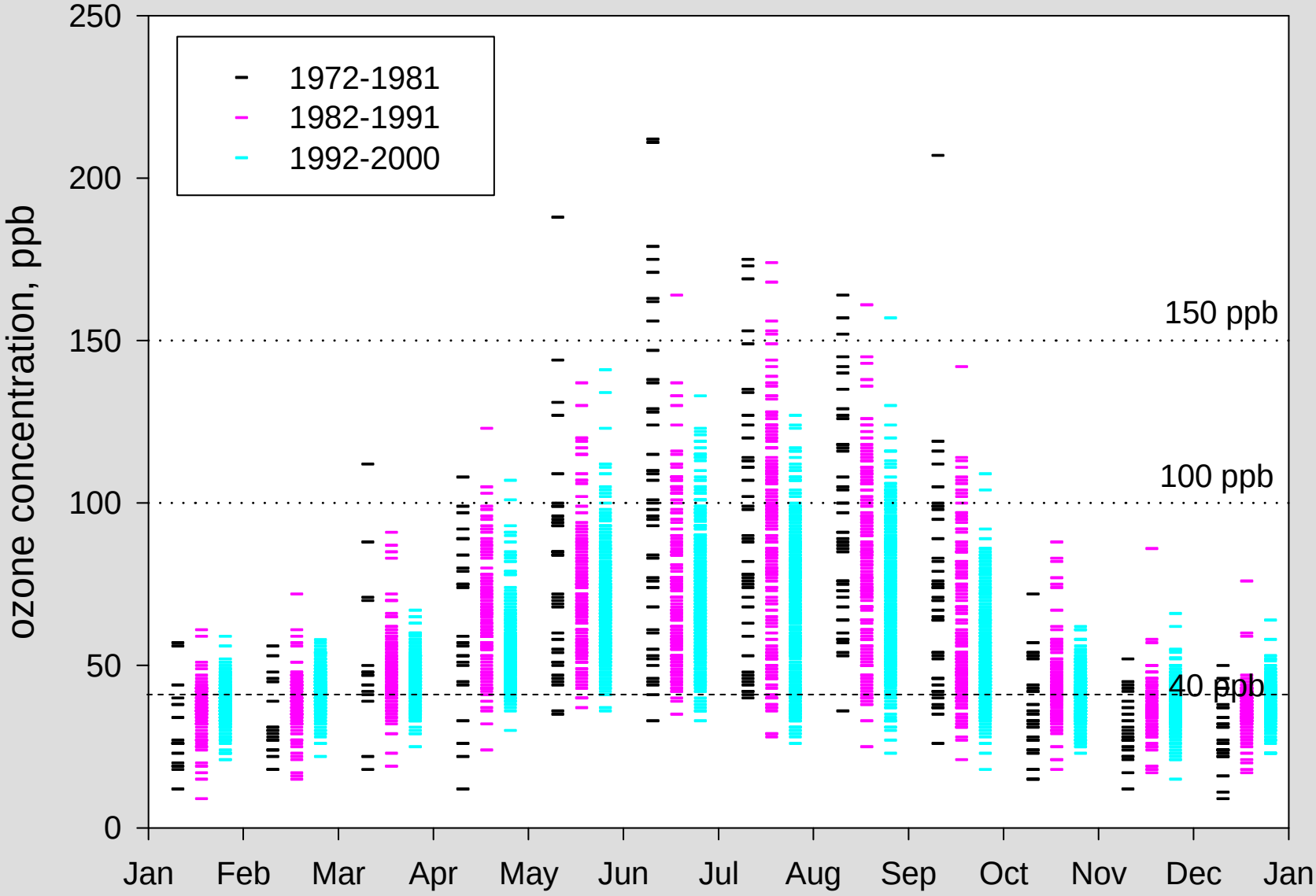
Ozone Concentrations and Altitude



UK Monthly Maximum Ozone Concentrations



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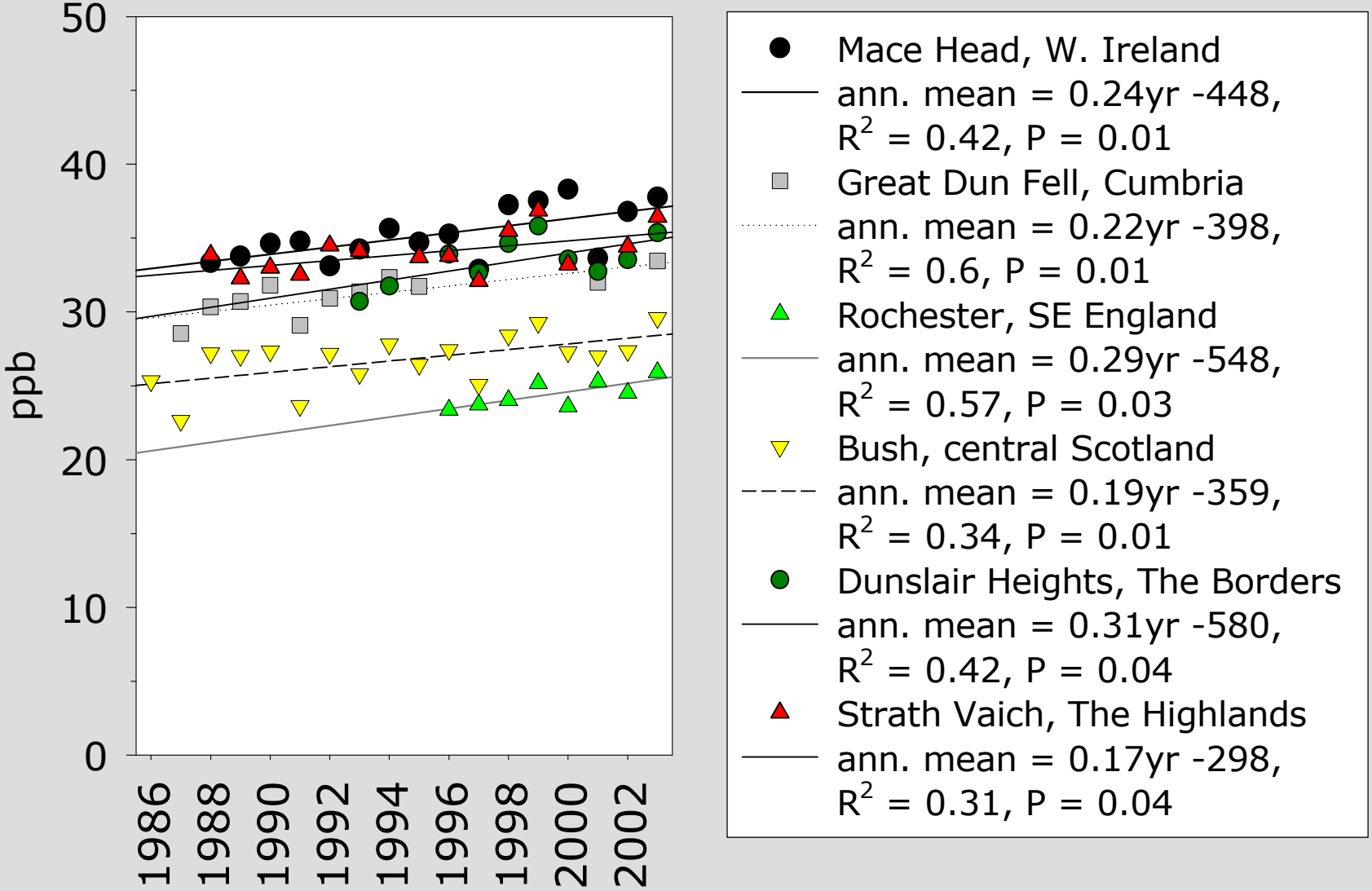
Ozone Concentrations: Trends in the Annual Mean