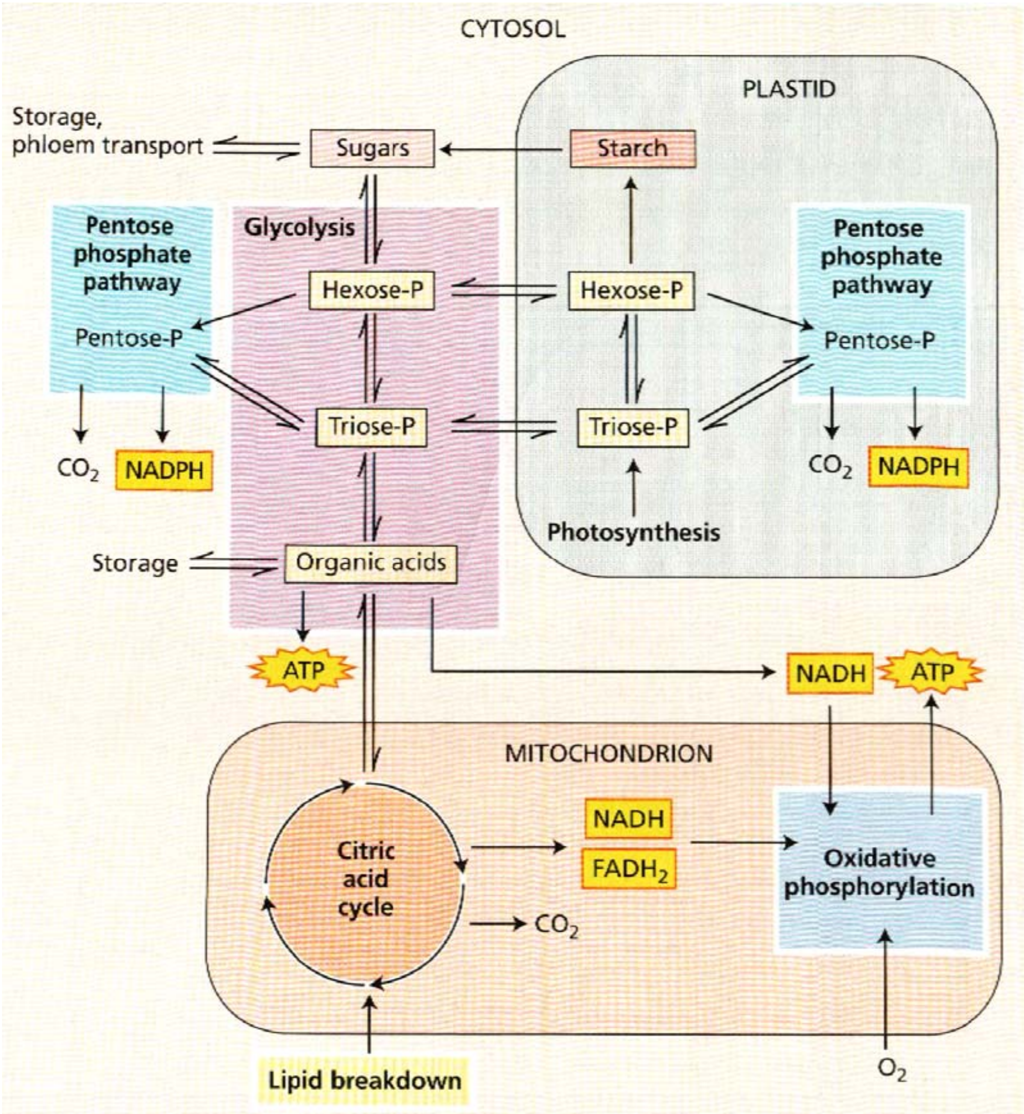
Ecophysiology

Please Note: Some of the slides are Animated and are best viewed as a Slide Show; some slides have attached notes below the slides and these are best viewed in Normal (editing) view.

3. Respiration

Over View of Respiration in Plants



There are 3 Key Pathways

1.Glycolysis

- Cytoplasm
- Plastids

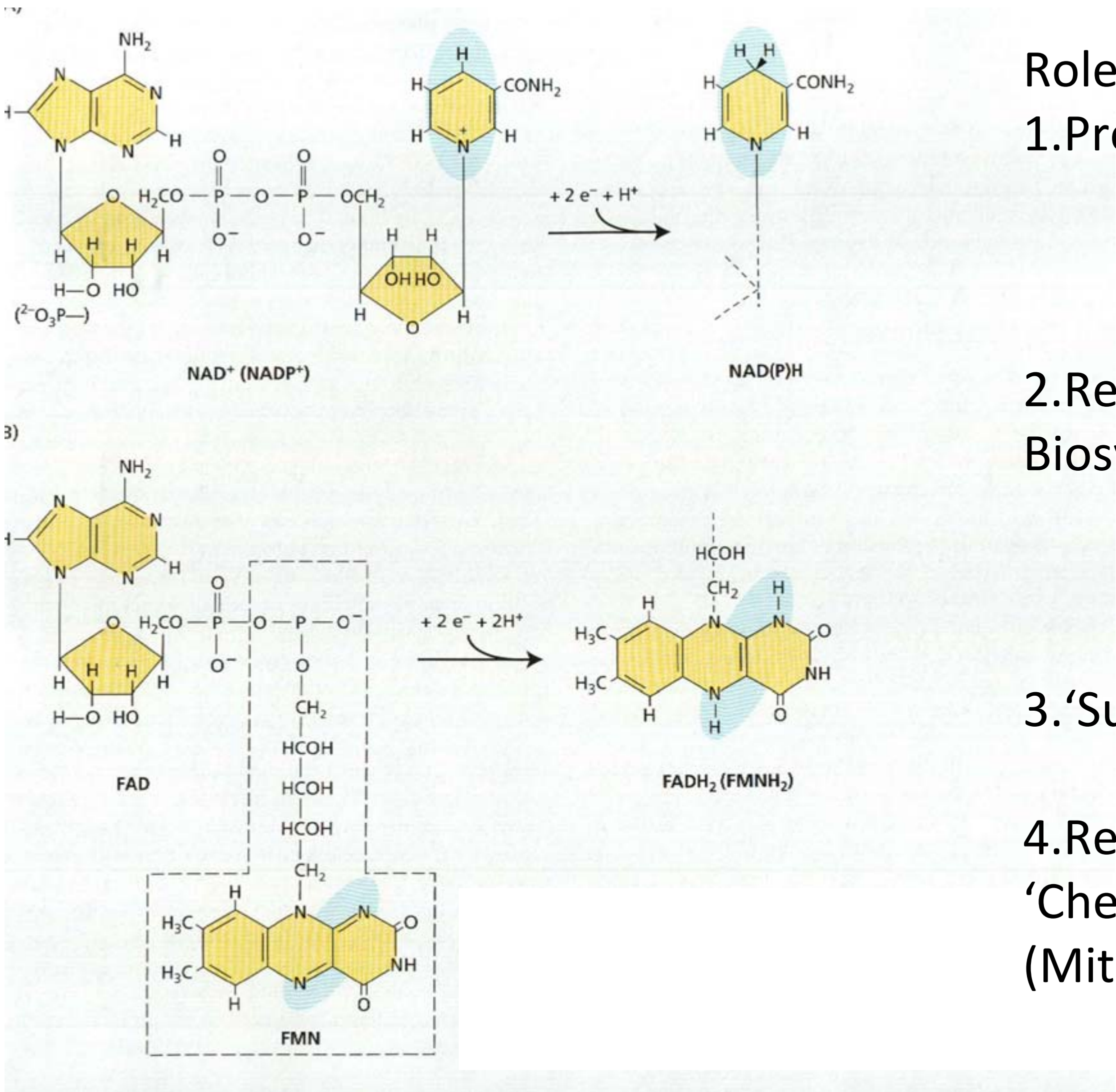
2.Pentose Phosphate Pathway

- Cytoplasm (Oxidative)
- Plastid (Reductive – C3 Cycle Running Backwards)

3.Krebs (TCA) Cycle

- Mitochondria

Respiration Generates Metabolites, Reducing Potential, & ATP



Role:-

1.Precursors for Biosynthesis

- Pentose sugars, amino acids, phenolic compounds, etc.

2.Reducing Potential for Biosynthesis

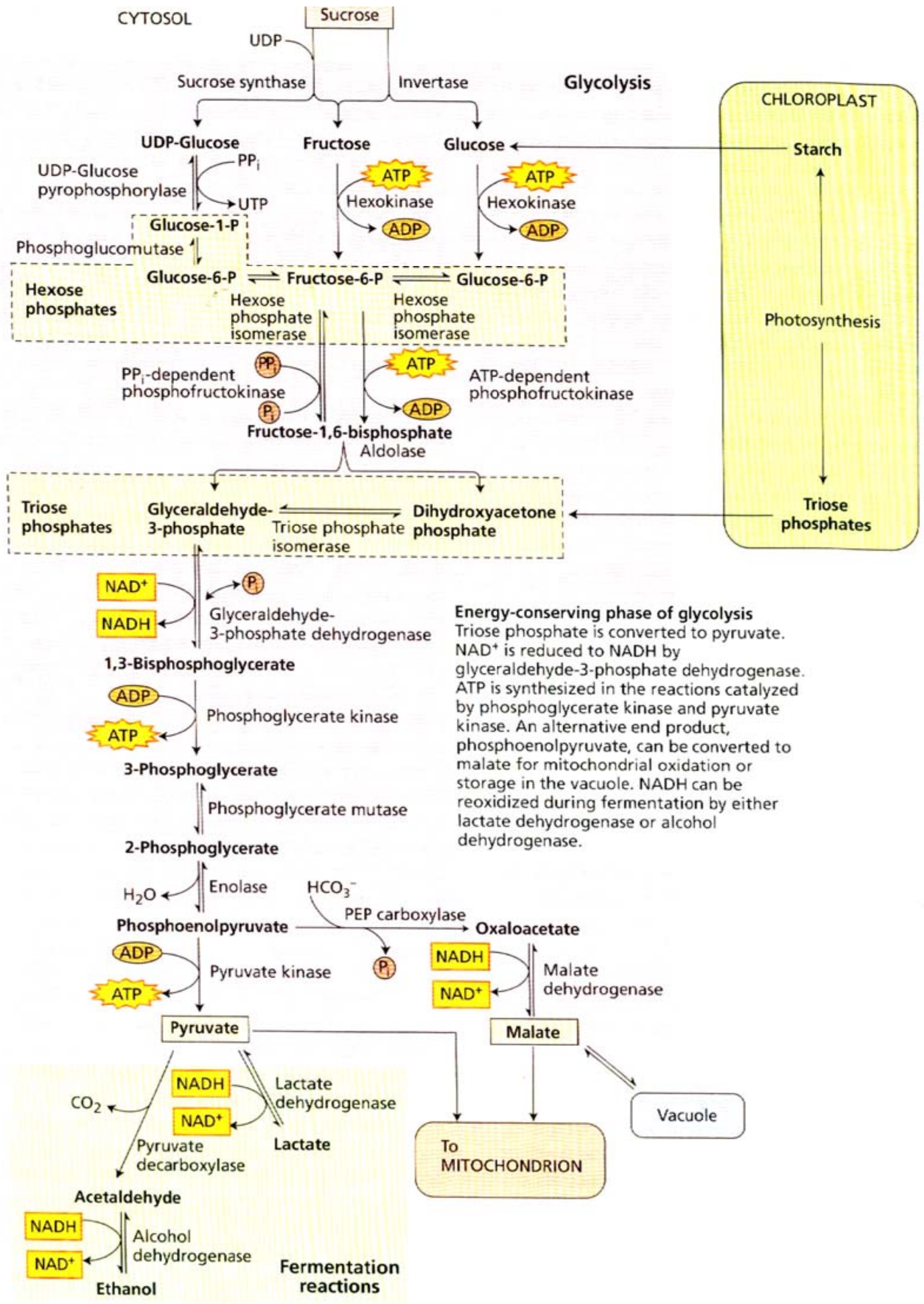
- NADPH from Oxidative PPP, NADH & $FADH_2$ in TCA Cycle

3.‘Substrate Level’ ATP

- Glycolysis & TCA Cycle

4.Reducing Potential for ‘Chemiosmotic’ ATP synthesis (Mitochondria)

- Deatils of Glycolysis
  - Carbon Entry From
    - Hexose
    - Triose Phosphate
  - Hexose
    - 2 ATP Consumed
    - 4 ATP Generated
    - 1 NADH Generated
  - Triose Phosphate
    - 2 ATP Generated
    - 1 NADH Generated



Features of Plant Oxidative Phosphorylation

- **Complex I** (NADH/UQ Oxidoreductase)
  - 4 H^+ Pumped
  - Sensitive to Rotenone & Piericidin
- **Complex II** (Succinate Dehydrogenase, $FADH_2$ / UQ Oxidoreductase)
  - Does Not Pump H^+
- **Complex III** (UQH_2 / Cyctochrome C Oxidoreductase)
  - Pumps 4 H^+
  - Sensitive to Antimycin
- **Complex IV** (Cyt C / H_2O Oxidoreductase)
  - Pumps 2 H^+
  - Sensitive to CO, Azide, CN^-
- **Alternative Oxidase (AO)**
  - Does Not Pump H^+
  - Sensitive to SHAM (salicylhydroxamic acid)
  - Cytoplasmic-side, Ca^{2+}-Dependent NADH and NADPH Dehydrogenase
  - Matrix-side NADH and Ca^{2+}-dependent NADPH Dehydrogenase
- **ATP Synthase**
  - F0/F1 Type enzyme, ~ $3H^+$ / ATP
- **Uncoupling Protein (UCP)**
  - A ‘Protonophore’ that ‘Uncouples’ ATP synthesis from Mitochondrial Electron Transport

Use of Photosynthate in Plants

TABLE 1. Utilization of photosynthates in plants, as dependent on the nutrient supply.*

Item	Utilization of photosynthates % of C fixed	
	Free nutrient availability	Limiting nutrient supply
Shoot growth	40*–57	15–27*
Root growth	17–18*	33*–35
Shoot respiration	17–24*	19–20*
Root respiration	8–19*	38*–52
– Growth	3.5–4.6*	6*–9
– Maintenance	0.6–2.6*	?
– Ion acquisition	–13*	?
Volatile losses	0–8	0–8
Exudation	<5	<23
N_2 fixation	Negligible	5–24
Mycorrhiza	Negligible	7–20

Proportions Vary with Species & Environmental Conditions, but...

- Most Photosynthate is Used for Growth
- Up to ½ Used for Respiration
    - Growth, Maintenance, Transport

RQ & P:O Ratios

- Respiratory Quotient (RQ) Varies
  - CO_2 Released / O_2 Consumed, dependent on
    - oxidation state of substrate – lipids ~ 0.4, hexoses ~1.0
    - Tissues – photosynthetic ~ 1.0, N-fixing roots ~1.6
- P:O ratio
  - ATP Produced / O2 Consumed
    - <1.0 to >2.5

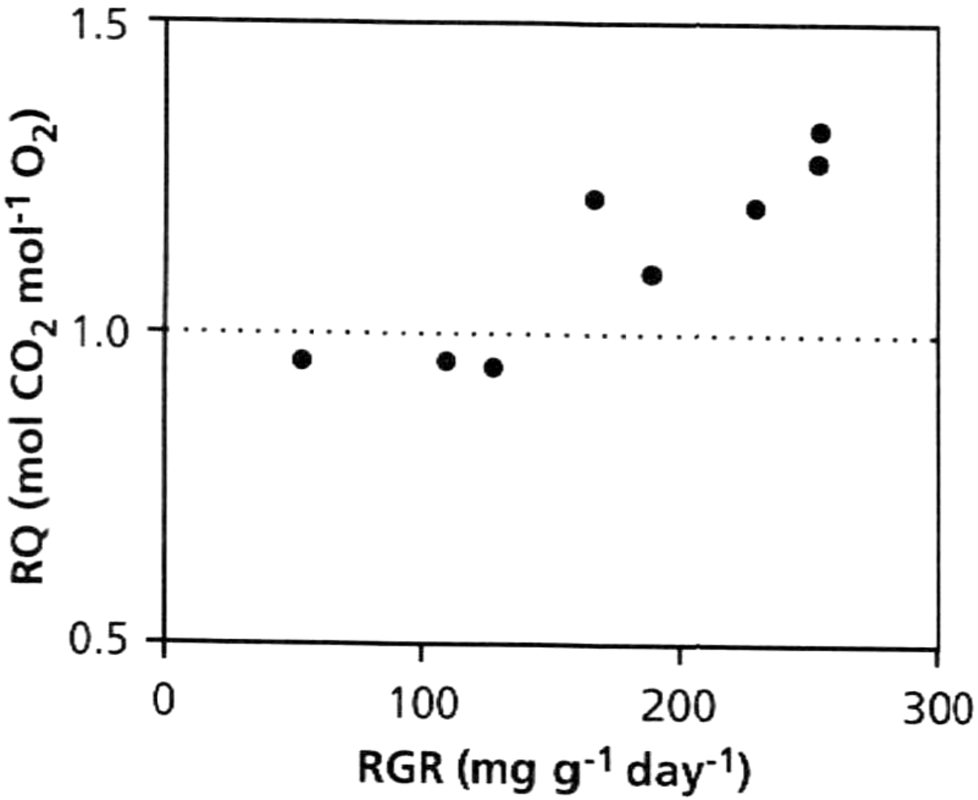
TABLE 2. The respiratory quotient (RQ) of root respiration of a number of herbaceous species.*

Species	RQ	Special Remarks
Allium cepa	1.0	Root tips
	1.3	Basal parts
Dactylis glomerata	1.2	
Festuca ovina	1.0	
Galinsoga parviflora	1.6	
Helianthus annuus	1.5	
Holcus lanatus	1.3	
Hordeum distichum	1.0	
Lupinus albus	1.4	
	1.6	N_2-fixing
Oryza sativa	1.0	NH_4^+-fed
	1.1	
Pisum sativum	0.8	NH_4^+-fed
	1.0	
	1.4	N_2-fixing
Zea mays	1.0	Fresh tips
	0.8	Starved tips

RQ Increases with Growth Rate

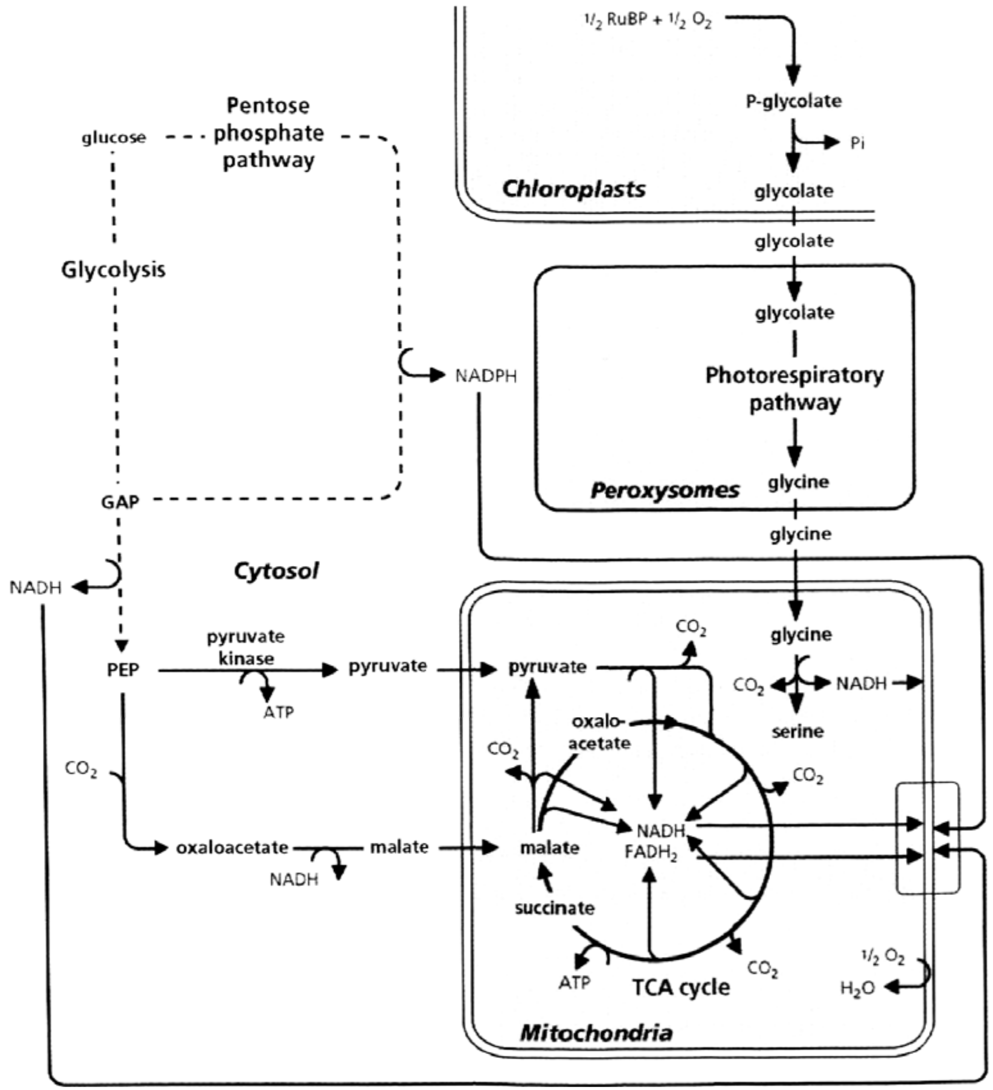
Hexose Respiration for ATP gives RQ ~ 1.0

Photosynthesis Consumes CO_2 so RQ Rises

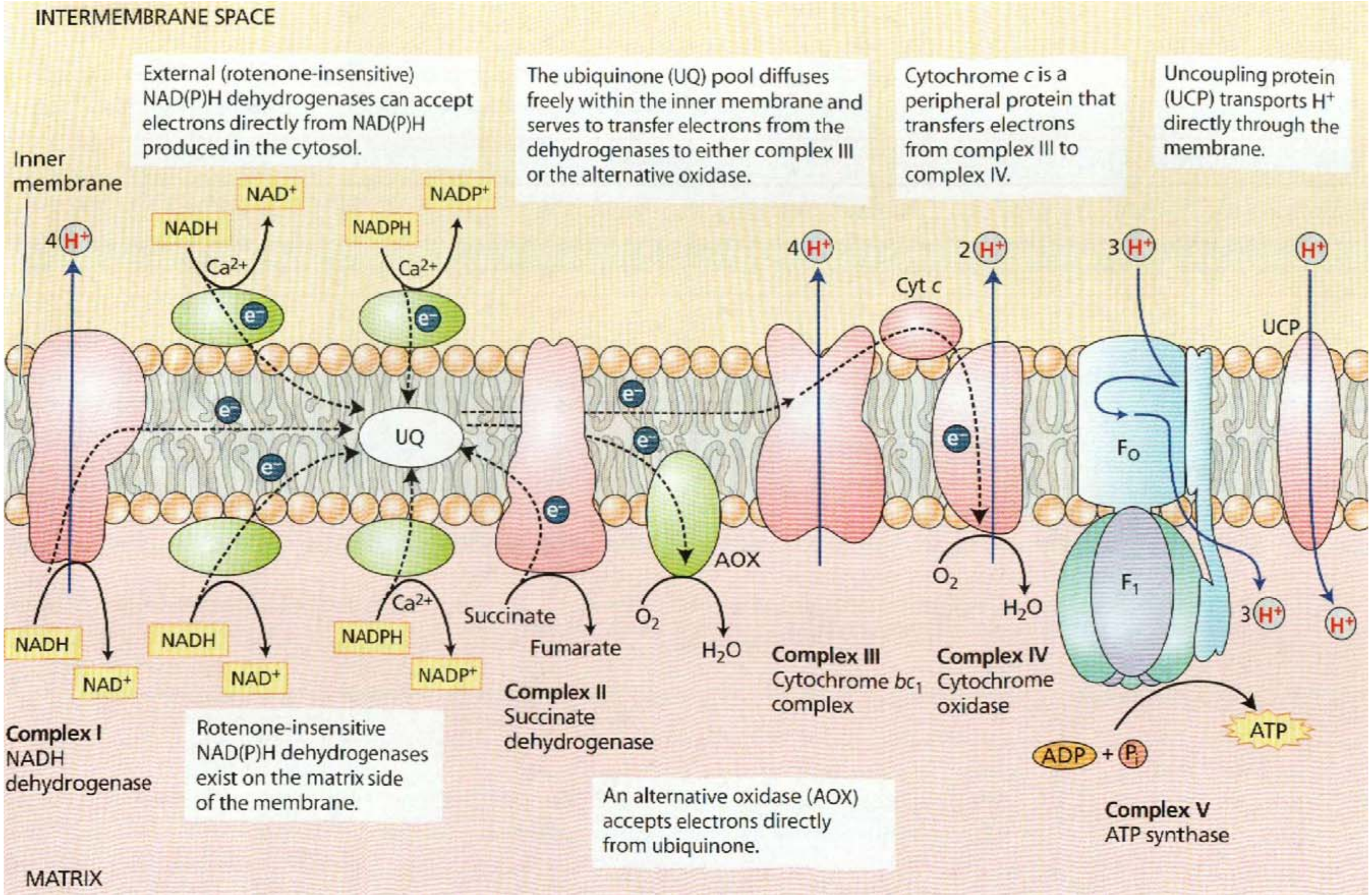


Carbon Enters Mitochondria as Pyruvate, Malate, & Glycine

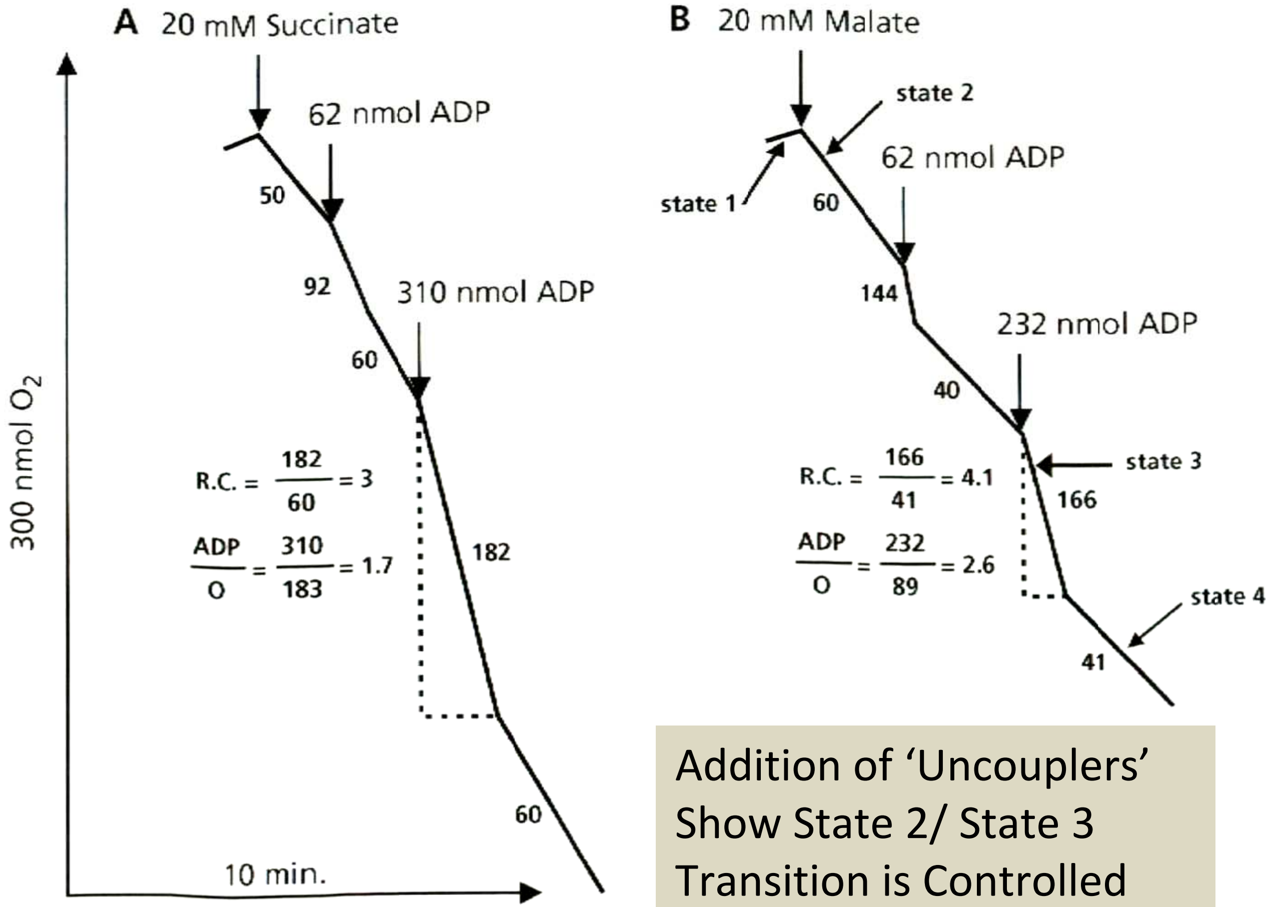
- Malate & Pyruvate are Major Metabolites in non-Photosynthesising (green and non-green) Tissues
- Glycine (from Photorespiration) is the Main Metabolite in Photosynthesising Green Tissues



Products of TCA Cycle Drive Electron Transport



Studies on Isolated Intact Mitochondria Reveal ETR is Under 'Respiratory Control'



Addition of 'Uncouplers' Show State 2/ State 3 Transition is Controlled Mainly by DH^+, not ADP Concentration

State 1

Initially Low ETR as no Substrate for TCA Cycle Present

State 2

Import of TCA Cycle Intermediates Drive Limited ETRs

State 3

Exchange of Matrix – Side ATP for Cytoplasmic-Side ADP Stimulates ETR

State 4

Internal ADP converted to ATP, ETR Stops

ETR Under RC *i.e.*

Demand for ATP

Chemiosmosis: ATP Production by H^+ Gradients

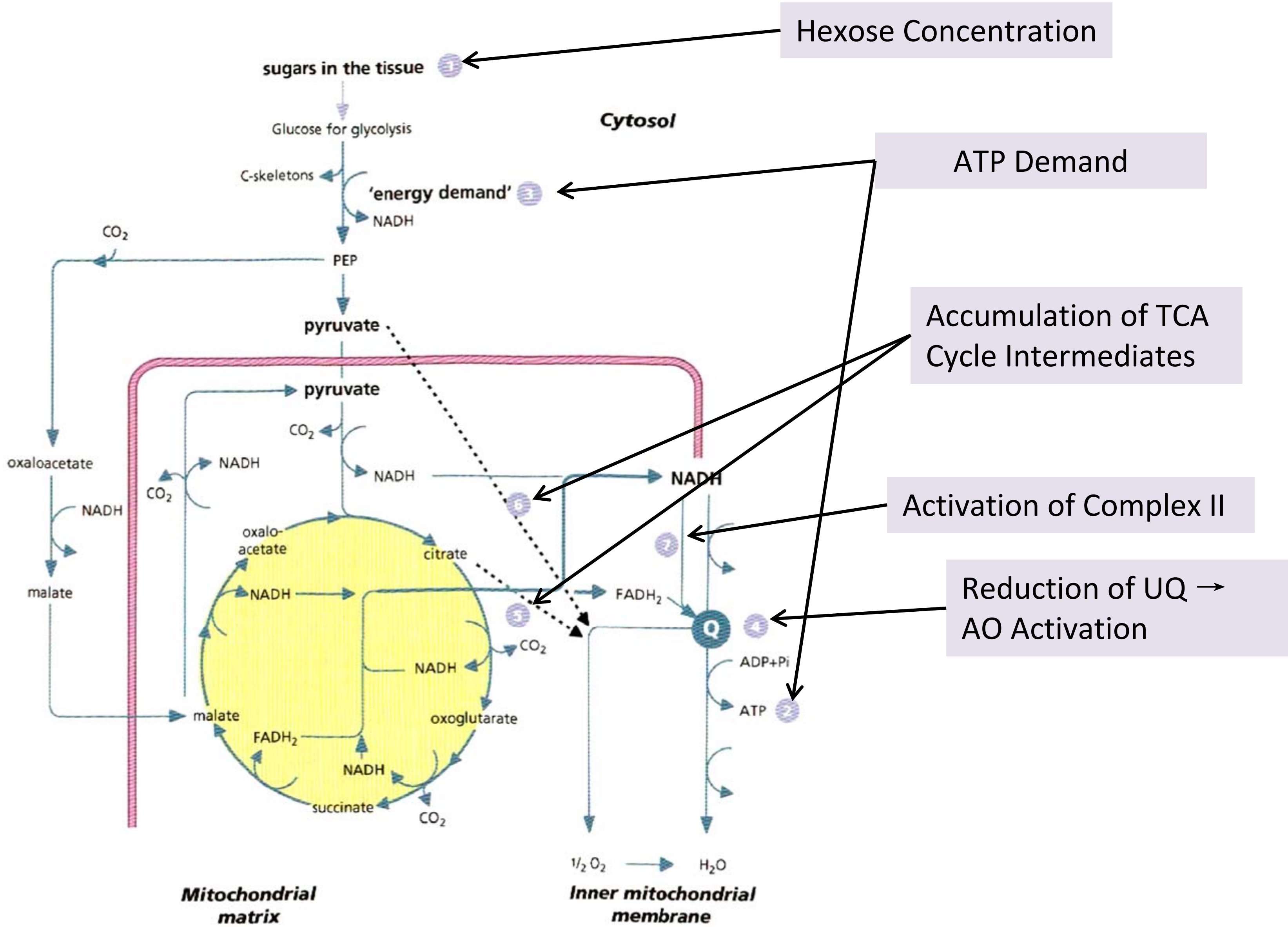
$$pmf = \Delta\mu_{H^+} = \Delta\varphi - \frac{RT}{F}\ln\left(\frac{H^+_{out}}{H^+_{in}}\right)$$

$$pmf = \Delta\mu_{H^+} = \Delta\varphi - 2.303\frac{RT}{F}\Delta pH$$

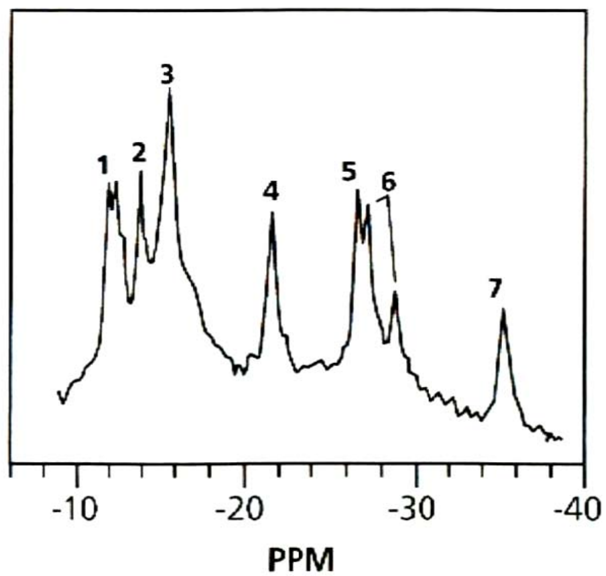
R = Gas Constant (8.32 J mol^{-1} K^{-1})
T = Temperature ° K
F = Faraday's Constant (96,500 J-K .mol-mV^{-1})
Dj = Membrane Potential (mV)
pmf= Proton Motive Force
DmH$^+$ = electrochemical potential for H^+

The cytoplasmic side of the mitochondria is well buffered at ~pH 7.5 so the Dj provides most of the driving force for H^+ movement into the matrix through the ATP Synthase.

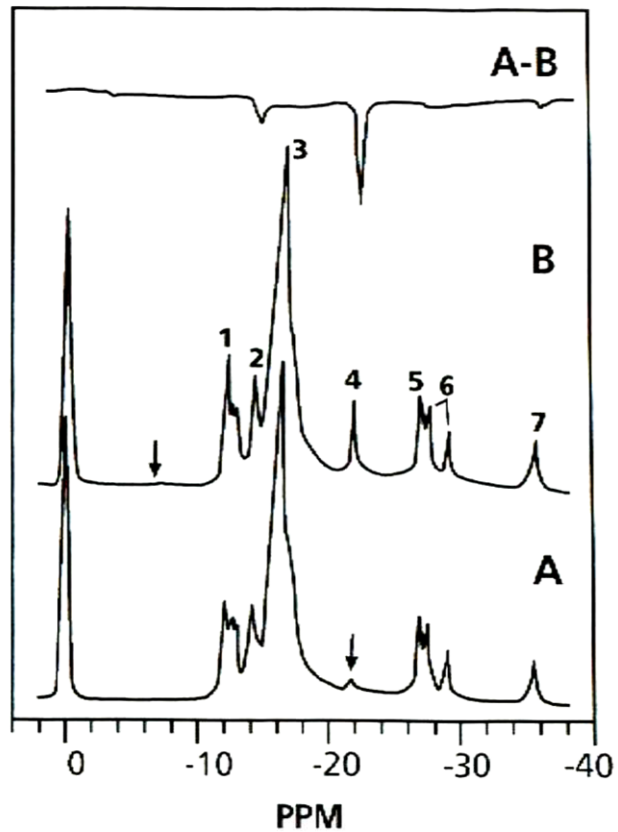
Major Control Points of Respiration in Plants



NMR Spectroscopy can be used to Show ATP Synthesis / Hydrolysis *In Vivo*



Peak 4 is g-ATP Phosphate Atom
Peak 2 is free Phosphate in Cytoplasm



Spectrum A: after microwave saturation in g-ATP band
Spectrum B: control
A –B: difference Spectrum showing spin polarized P atom appearing in Cytosolic fraction

ADP:O Ratios can be Calculated from Concomitant NMR and O_2 Uptake Measurements on Intact Tissues

TABLE 3. The in vivo ADP:O ratios in root tips of *Zea mays* (corn) determined with the saturation transfer ^{31}P NMR technique and O_2 uptake measurements.

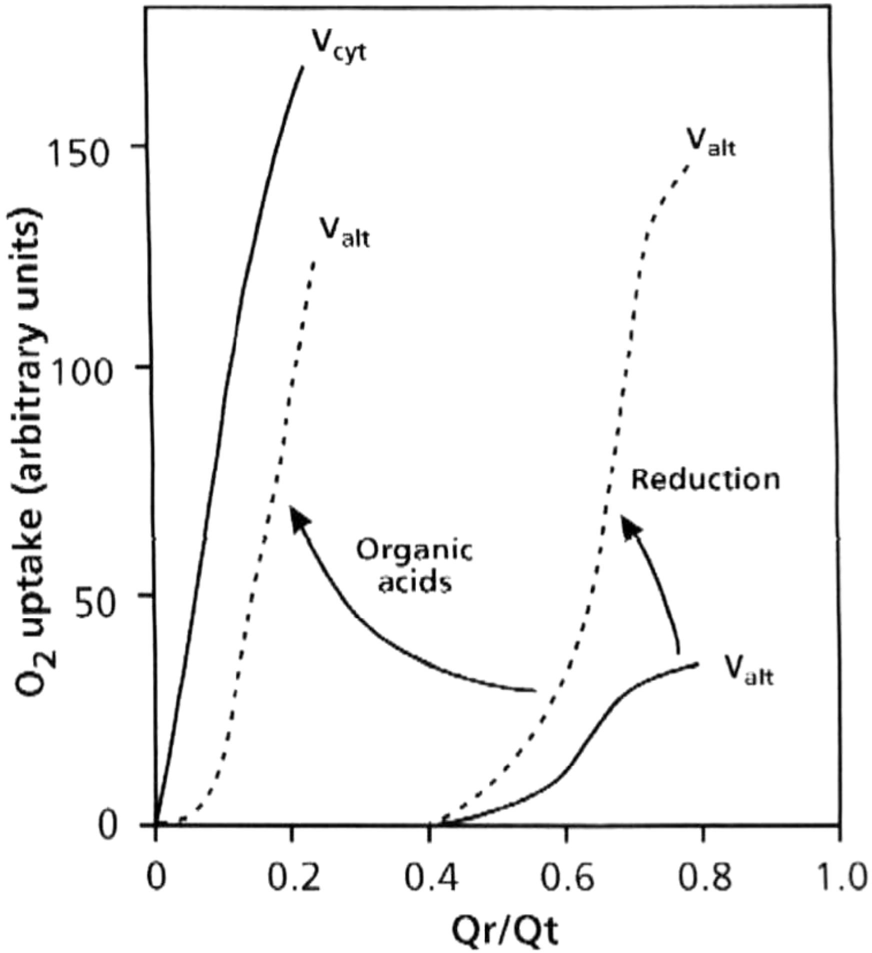
Exogenous substrate	O_2 concentration	Inhibitor	Rate of O_2 uptake	Rate of ATP production	ADP:O ratio
Glucose	100	None	22	143	3.2
Glucose	0	None	0	<20	–
None	100	None	15	93	3.0
Glucose	100	KCN	14	26	1.0
Glucose	100	KCN+SHAM	4	<20	–
Glucose	100	SHAM	21	137	3.2

The Rate of ET Through the ‘Cytochome’ & AO Pathways is Regulated

ETR Through Cyt Pathway (V_{cyt}) is Triggerec By Low Reduction State of UQ Pool (Qr/Qt)

ETR Through AO Pathway (V_{alt}) is Triggered by High Reduction State of UQ Pool (>40%)

Addition of TCA Cycle Organic Acids ‘Converts’ AO to Form Activated by Low UQ Pool Reduction State



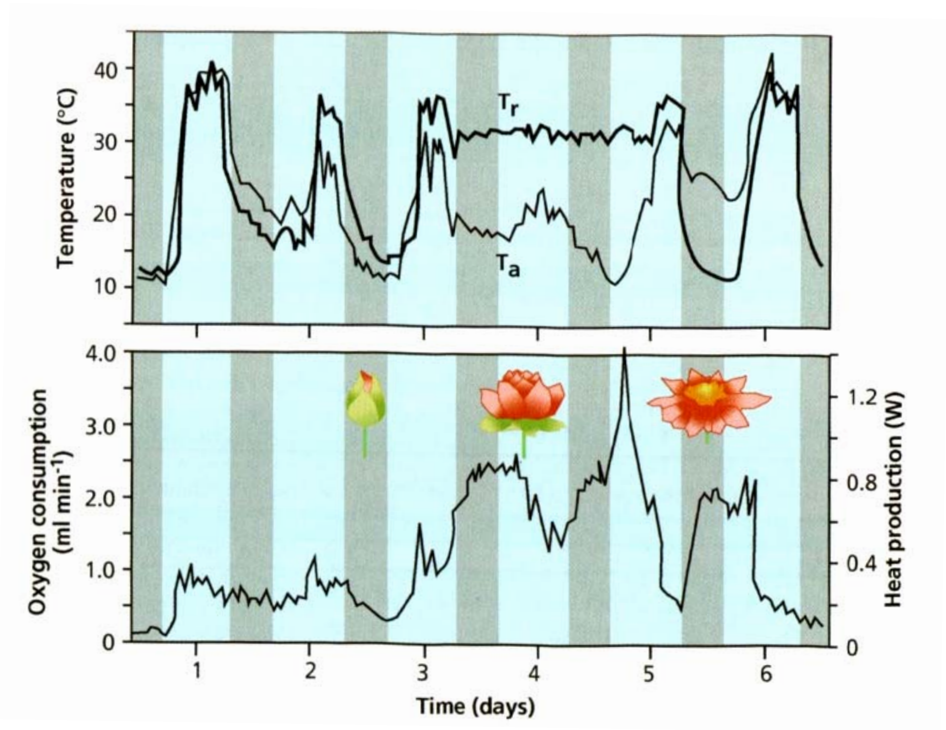
Electron Flow through two Pathways is Regulated by

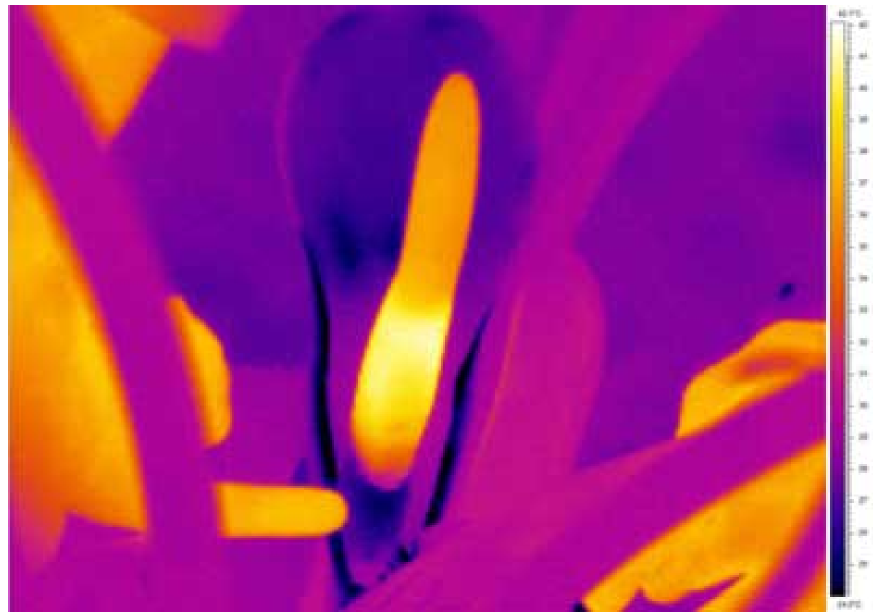
Redox State of UQ Pool

AND

Concentration of TCA Cycle Intermediates

What Role Does the AO Pathway Play?





Thermogenesis:

Rise of >15C ambient in some specialized tissues
e.g. Spadex of

Sacred Lotus (*Nelumbo nucifera*)

Skunk Cabbage (*Simplocarpus foeitidus*)

Heart-leaf Philodendron (*Philodendron selloum*)

Seymour & Schultz-Motel 1996 Nature 238: 305

Thermogenesis Appears to be a Reproductive Strategy

There is no evidence that Thermogenesis can alleviate cold stress in plants

What Role Does the AO Pathway Play?

- Energy Overflow
  - When UQ is Highly Reduced, ROS can be Generated. Activation of the AO when TCA Cycle Intermediates are High Will Decrease the Redox State of UQ and Prevent ROS Generation
- Uncouples ATP Synthesis from C-Skeleton Demand
  - When C-Skeleton Demand is High (*e.g.* Citrate excretion) ATP Requirement is Low – Uncouples ATP Synthesis
- Decreases Redox State of Chloroplast
  - SHAM and KCN decrease Photosynthetic O_2 Evolution. AO may oxidise NAD(P)H Excreted from the Chloroplast in High Light to Prevent 'over-reduction'.
- Allow Limited Oxidative Phosphorylation When Cyt Pathway is Inhibited
  - Plants produce inhibitors of Cyt Pathway (CN-, sulphide, CO_2,); AO may provide limited ATP synthesis under these conditions.
- High Rates of ROS Production Induce Transcription of AO Genes
  - Role in prevention of ROS generation

Environmental Effects on Respiratory Processes

- Flooding, Hypoxia, & Anoxia
  - Diffusion of Gasses in Liquid ~ 10,000 Slower than in Air
  - O_2 Concentration is water is ~40x Lower than in Air
  - Leads to Anaerobic Respiration in Roots of Plants in Flooded / Clay Impacted soils → Low ATP → Transcriptional Changes → e.g. ADH
- Fermentation
  - Accumulation of Lactate → Acidosis →inhibits Lactate Dehydrogenase→ Ethanol Accumulation →Inhibits normal PDC Acetyl Co-A Activity → Accumulation of ***Acetaldehyde*** (Very Toxic)

TABLE 7. The effect of supplying ethanol in aerobic and anaerobic nutrient solutions to the roots of *Pisum sativum* (garden pea) at a concentration close to that found in flooded soil (i.e., 3.9 mM) or greater than that.

	Aerobic control	Aerobic + ethanol	Anaerobic control	Anaerobic + ethanol
Ethanol in xylem sap (mM)	37	540	90	970
Stem extension (mm)	118	108	94	74
Final fresh mass (g)				
shoot	11.9	11.9	10.7	11.4
roots	7.8	9.7	5.7	6.1

Source: Jackson et al. (1982).

Ethanol is Toxic to Humans but not to Plants!

Strategies for Avoiding Hypoxia

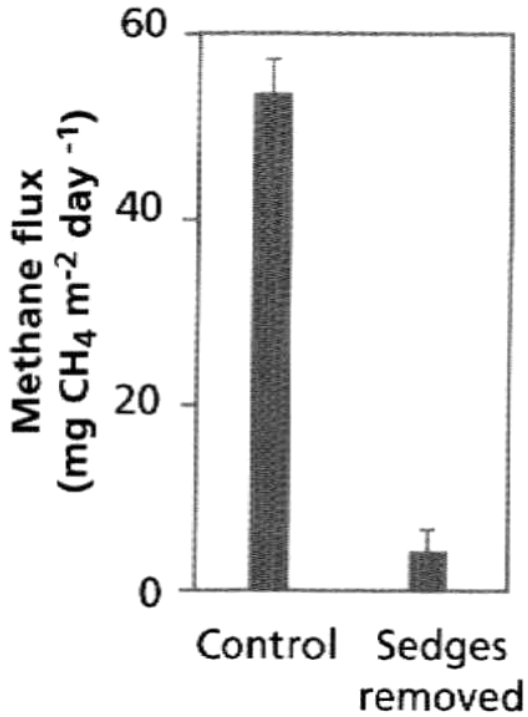
Aerenchyma

Pneumatophores (in Mangrove)

Lenticels

The above rely on Increasing O_2 Diffusion to Roots Through Structural Modifications (& Accelerated Methane Loss to Atmosphere?)

May be a Role for Pressure Driven O_2 Flow from Shoot to Root?



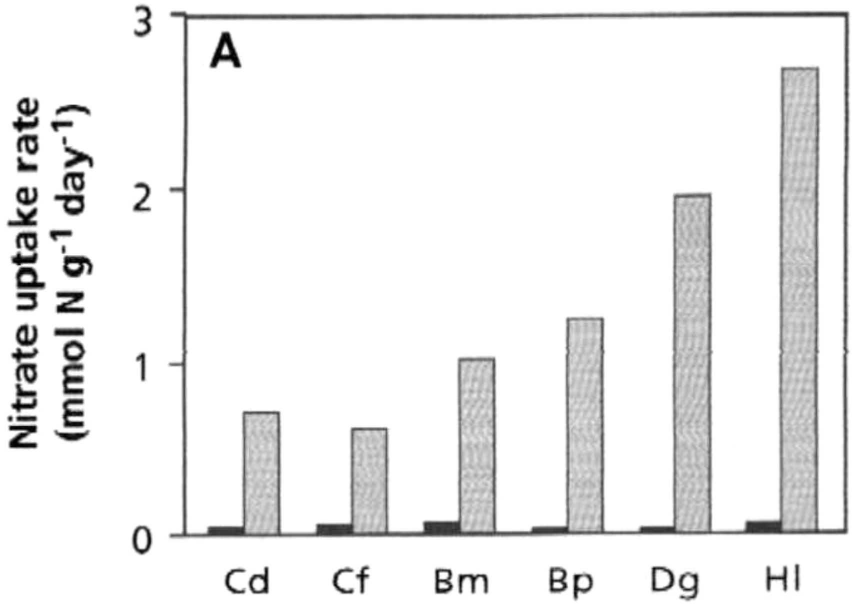
Respiration Is Affected By Stress & RGR

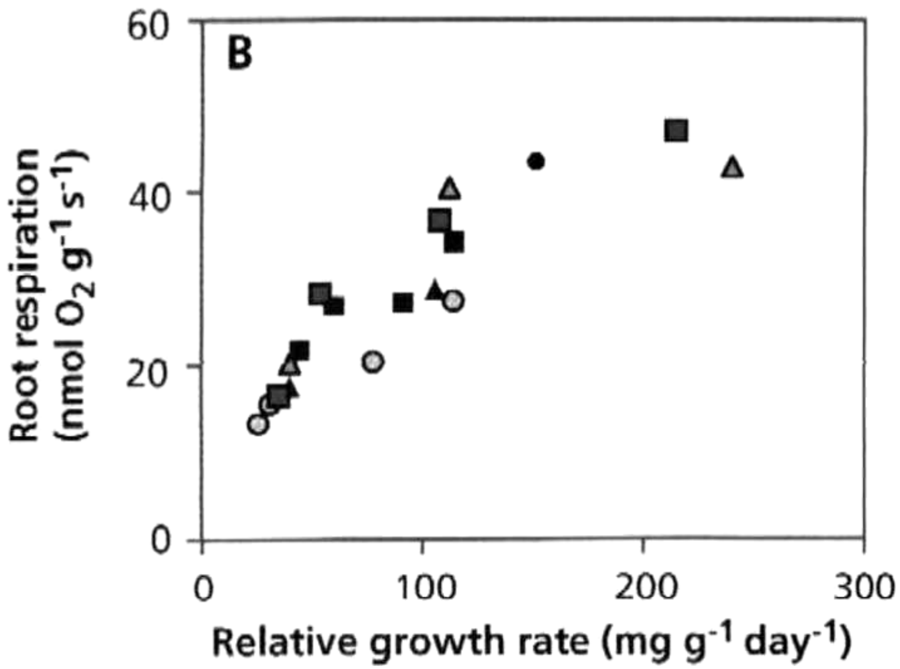
Salinity & Drought Stress Cause an Initial Rapid Increase in Respiration

In Soybean seedlings Drought causes rapid decrease in CO_2 Assimilation but no Change in Respiratory O_2 Uptake *BUT* an Increase in AO pathway ETR Occurs

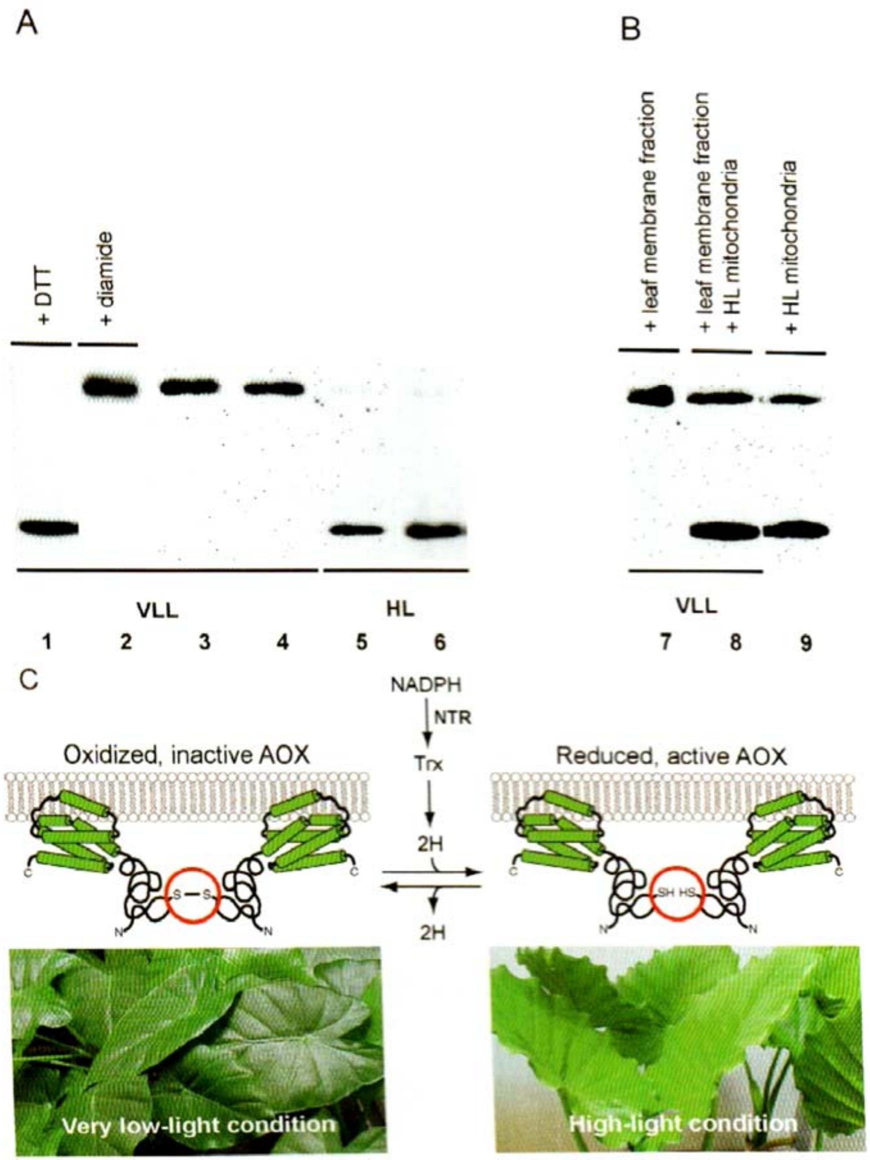
In longer term Compatible Solutes are Synthesized; Energy required for this Matches the Observed Decline in Respiration.

Manipulation of N-supply shows Growth Rates Correlate well with Respiration





Respiration Is Affected By Stress & RGR – Irradiance



- Transfer to Low Light → Decreased Respiration

Addition of Sucrose → NO INCREASE in Respiration
due to
REDUCED DEMAND for Energy
NOT
REDUCED SUPPLY of Photosynthate

- Shade Plants Show Low AO Activity (*Alocaia*)
  Sun Plants Show High AO Activity (*Spinacia*)
  AO Involved in Excess Energy Dissipation?
- AO Activity is Controlled by Redox Status of Mitochondria

Active AO is Monomer, Inactive AO is a Dimer

Respiration and Photosynthesis Rates Are Co-Regulated by Irradiance

TABLE 8. The daily carbon budget (mmol g^{-1} day^{-1}) of the leaves of *Spinacia oleracea* (spinach), a sun species, and *Alocasia odora* (giant upright elephant ear), a shade species, when grown in different light environments.*

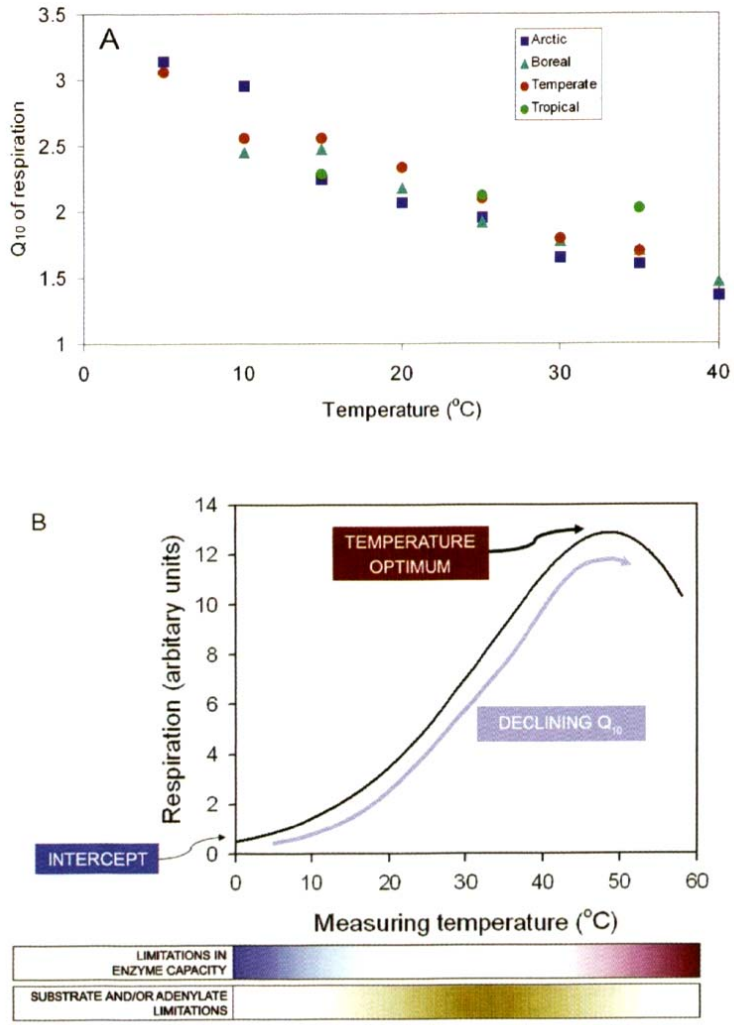
	Photosynthesis		Leaf respiration		Net leaf carbon gain	
Irradiance	*Spinacia oleracea*	*Alocasia odora*	*Spinacia oleracea*	*Alocasia odora*	*Spinacia oleracea*	*Alocasia odora*
500	26	nd	3.4 (13)	nd	23 (87)	nd
320	21	11	2.4 (12)	1.1 (10)	18 (88)	9.4 (90)
160	15	9	1.7 (11)	0.82 (9)	14 (89)	8.2 (91)
40	nd	4.5	nd	0.76 (17)	nd	3.7 (83)

Source: Noguchi et al. (1996), K. Noguchi, pers. comm.

* Irradiance is expressed in μmol m^{-2} s^{-1}. Percentages of the photosynthetic carbon gains have been indicated in brackets; nd is not determined; in the original paper the species name is erroneously given as *Alocasia macrorrhiza*.

Temperature Effects on Respiration

- Respiration Increases With Temperature
- Q10 Response Greatest in Low Temperature-Acclimated Plants Regardless of Their Origin
- Plants Adjust Respiration Rates to Achieve Equivalent Rates Regardless of Growth Temperature



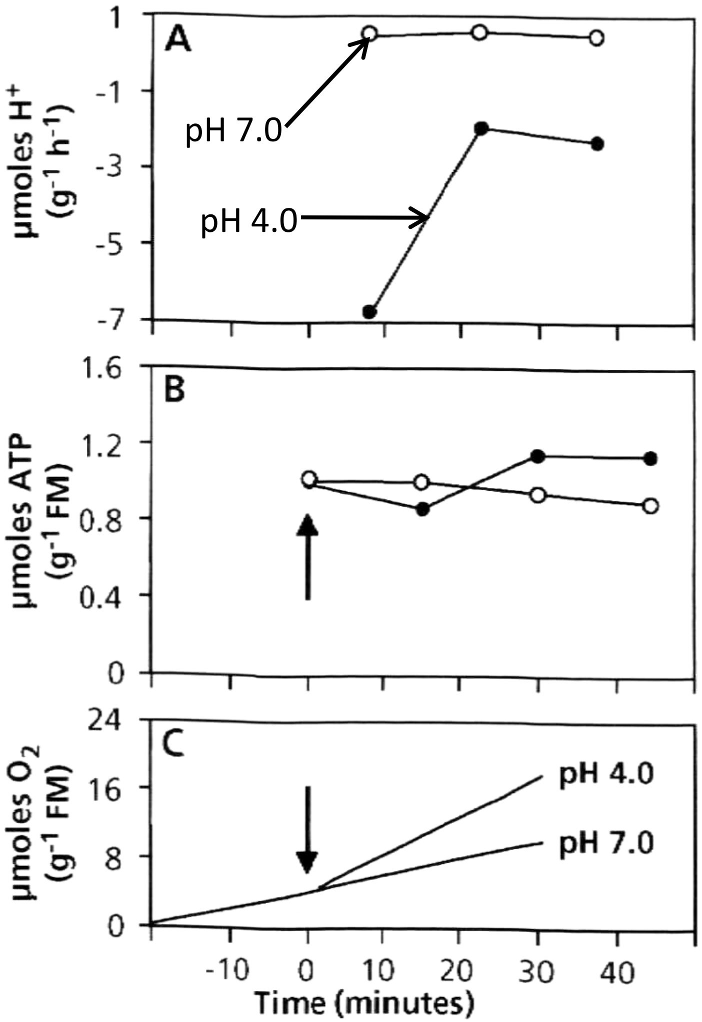
Respiration is Thermodynamically Limited at Low Temperatures

Plants respond by Increasing the Abundance of Respiratory Proteins

Subsequent Transfer to Warm Conditions Shows Large Increase in respiration (Q10 >2.5)

Respiration Increases With Rhizosphere Acidity

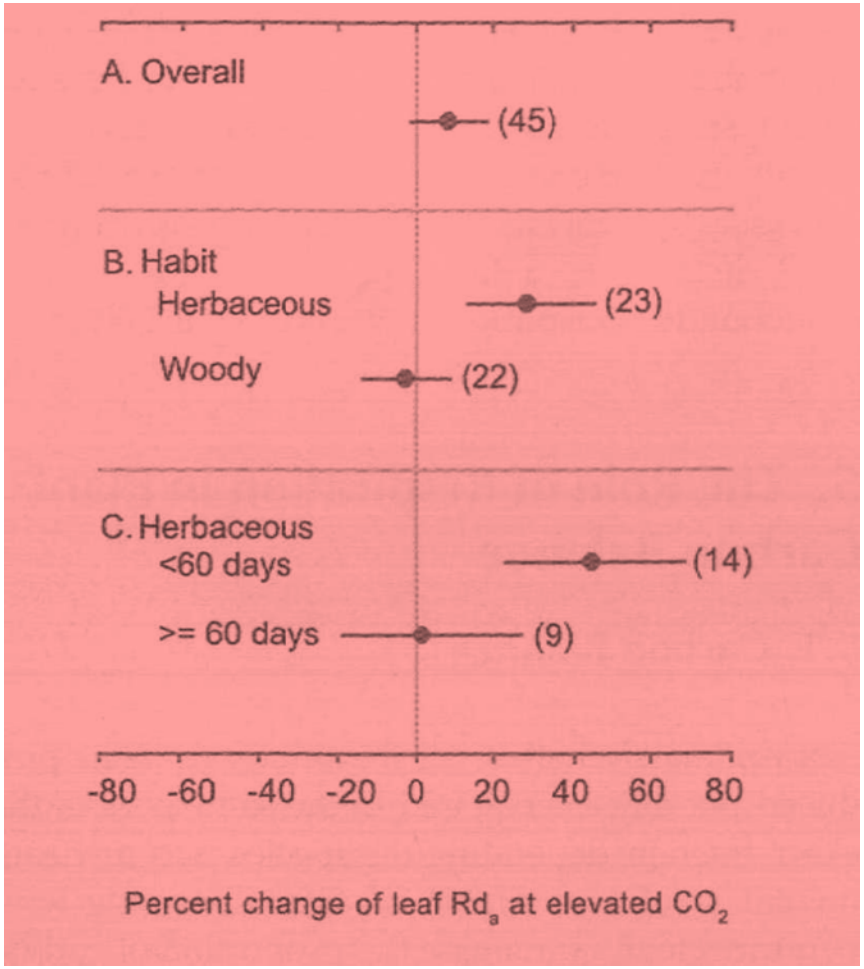
Zea mays Seedlings



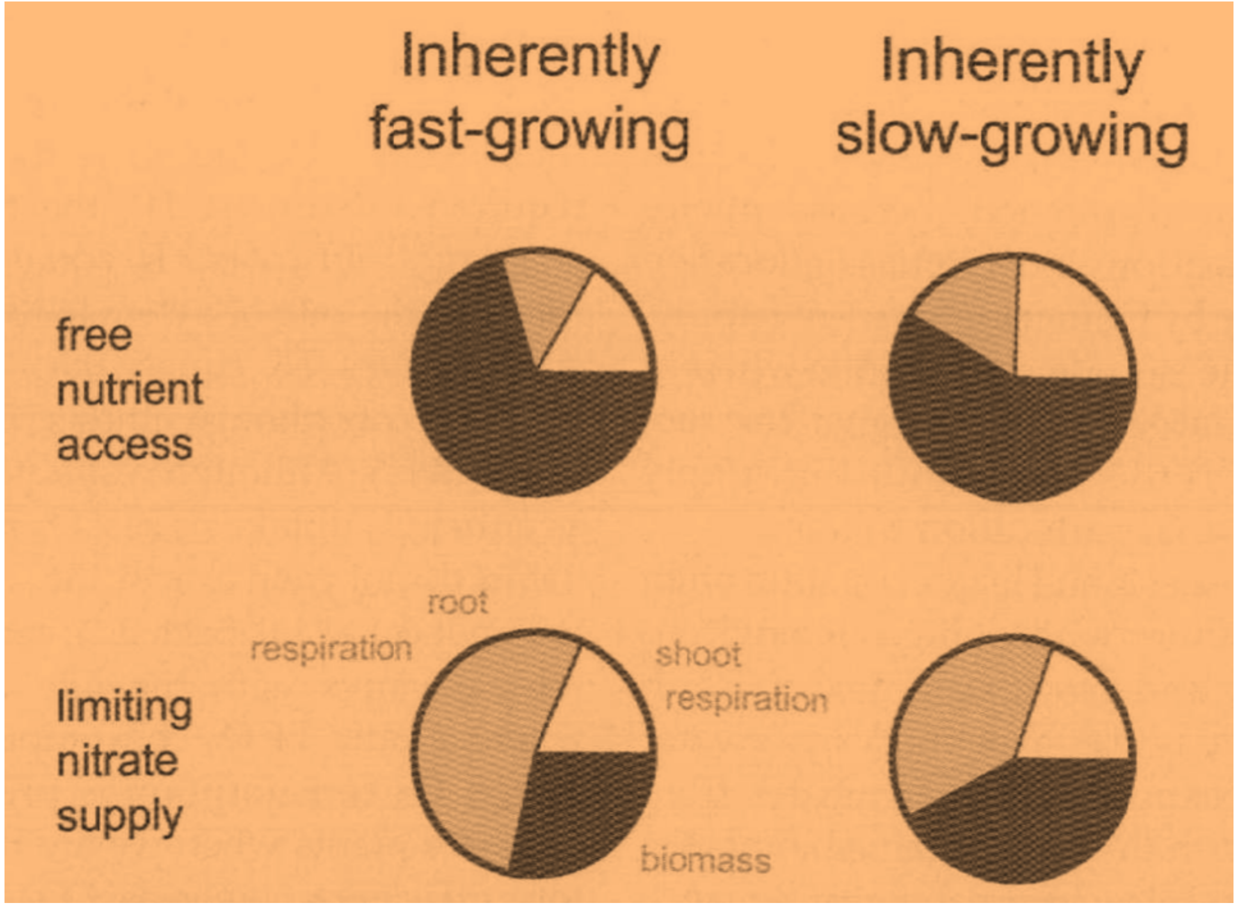
It is Difficult to Assign this Respiratory Burst to Any Mechanism

Although the Plasma Membrane P-Type H^+ pumping ATPase has been implicated

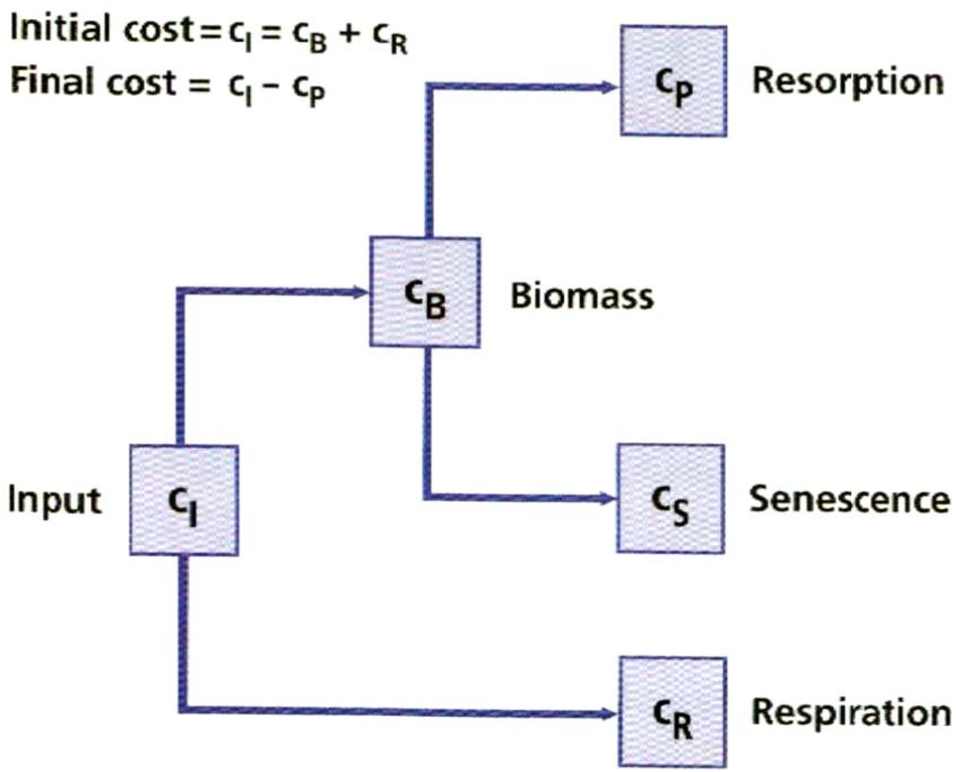
Respiration May Increase in Some Species With Increased Atmospheric CO_2 Concentrations



Root And Shoot Respiration Changes With Growth Rate



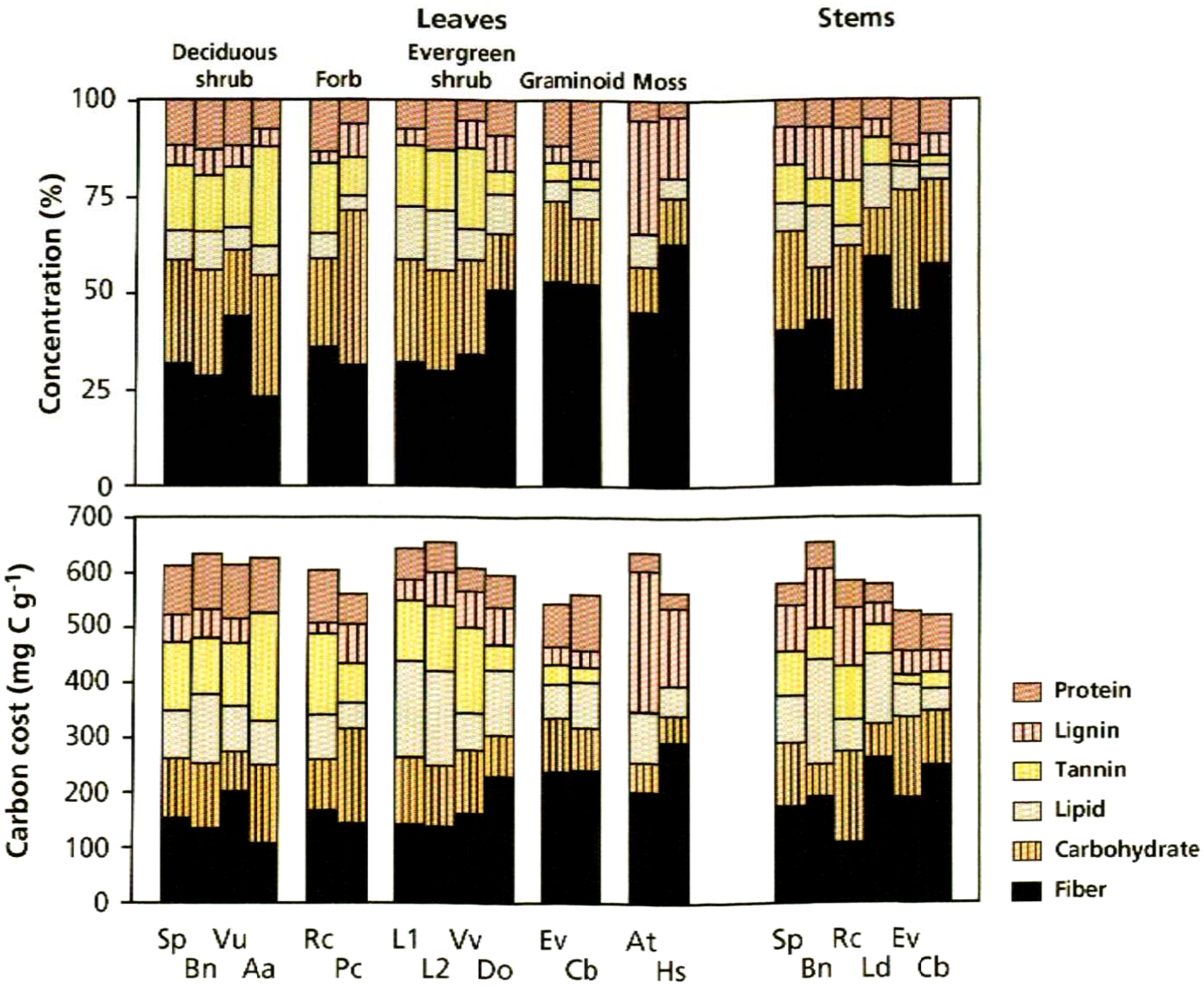
Model for Carbon Utilization During Growth



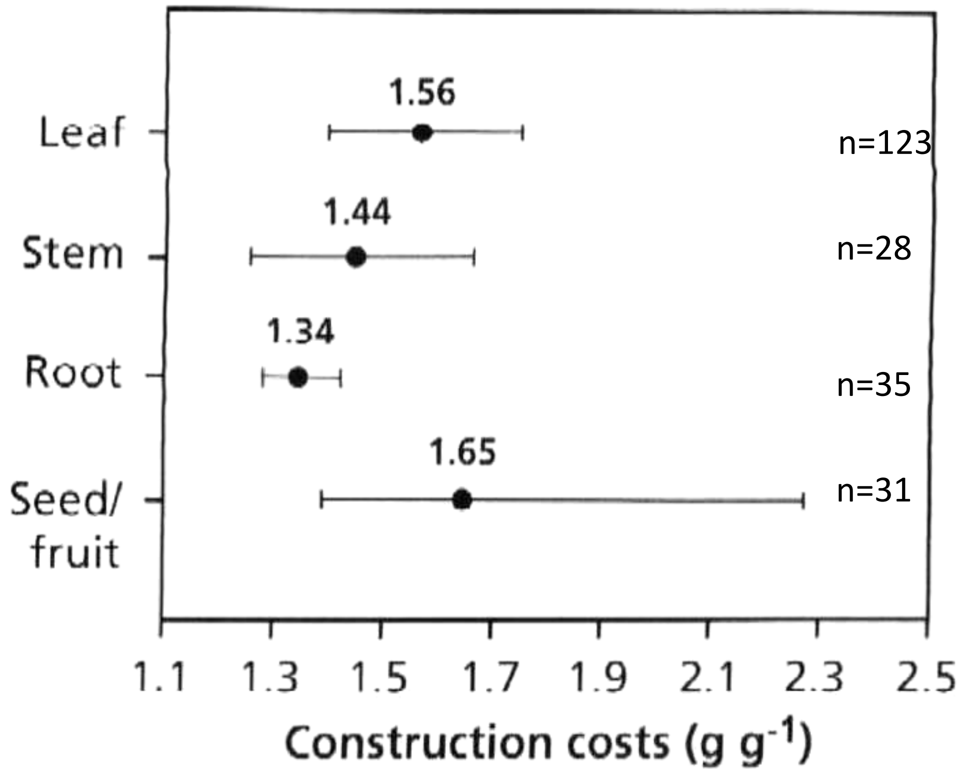
Cs represents the fraction of Biomass that is not Recoverable, *e.g.* Ligno-cellulose

Cr represents the carbon respired for generating the ATP & NAD(P)H Required for biomass production

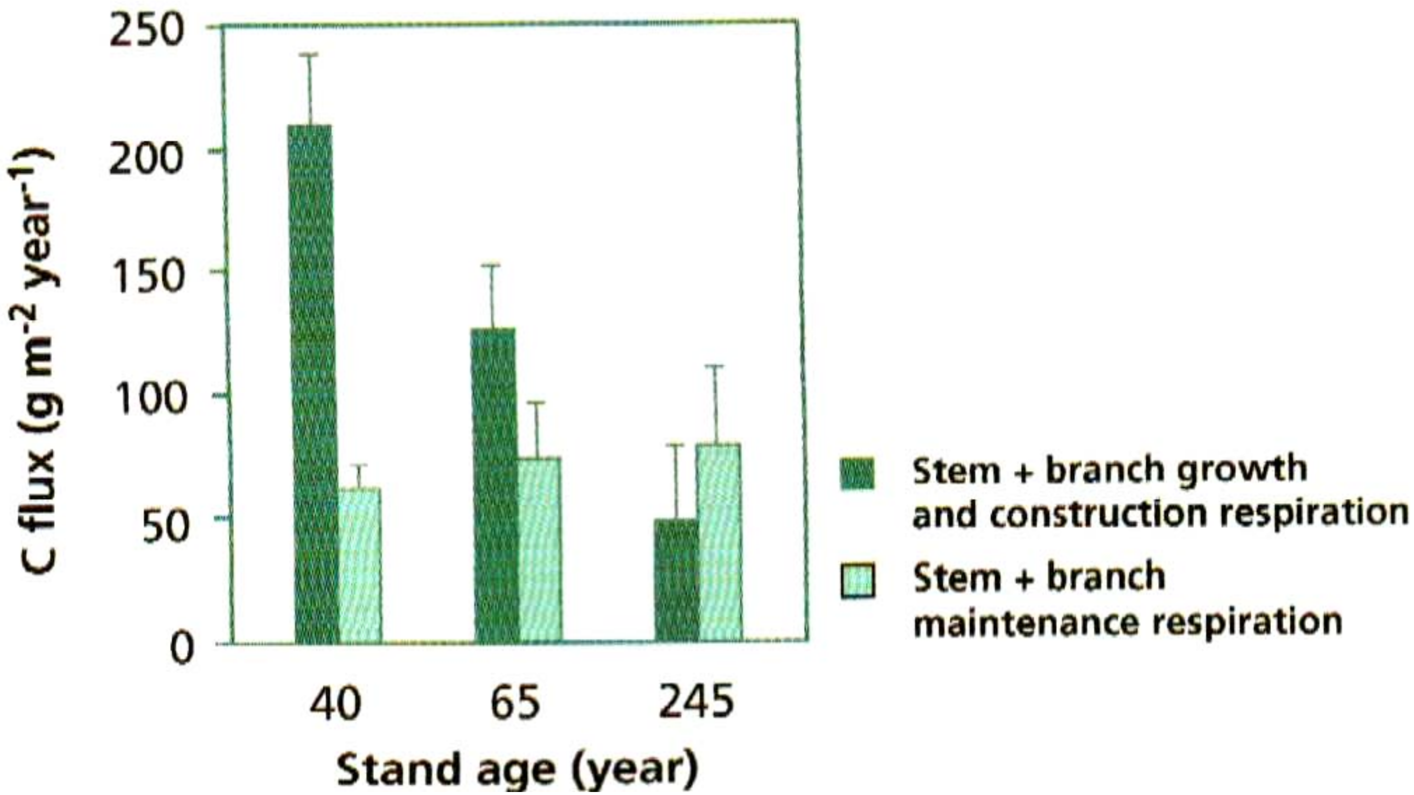
Estimates of the Partitioning of Carbon into the Molecules of Life



Costs of Construction of Plant Organs

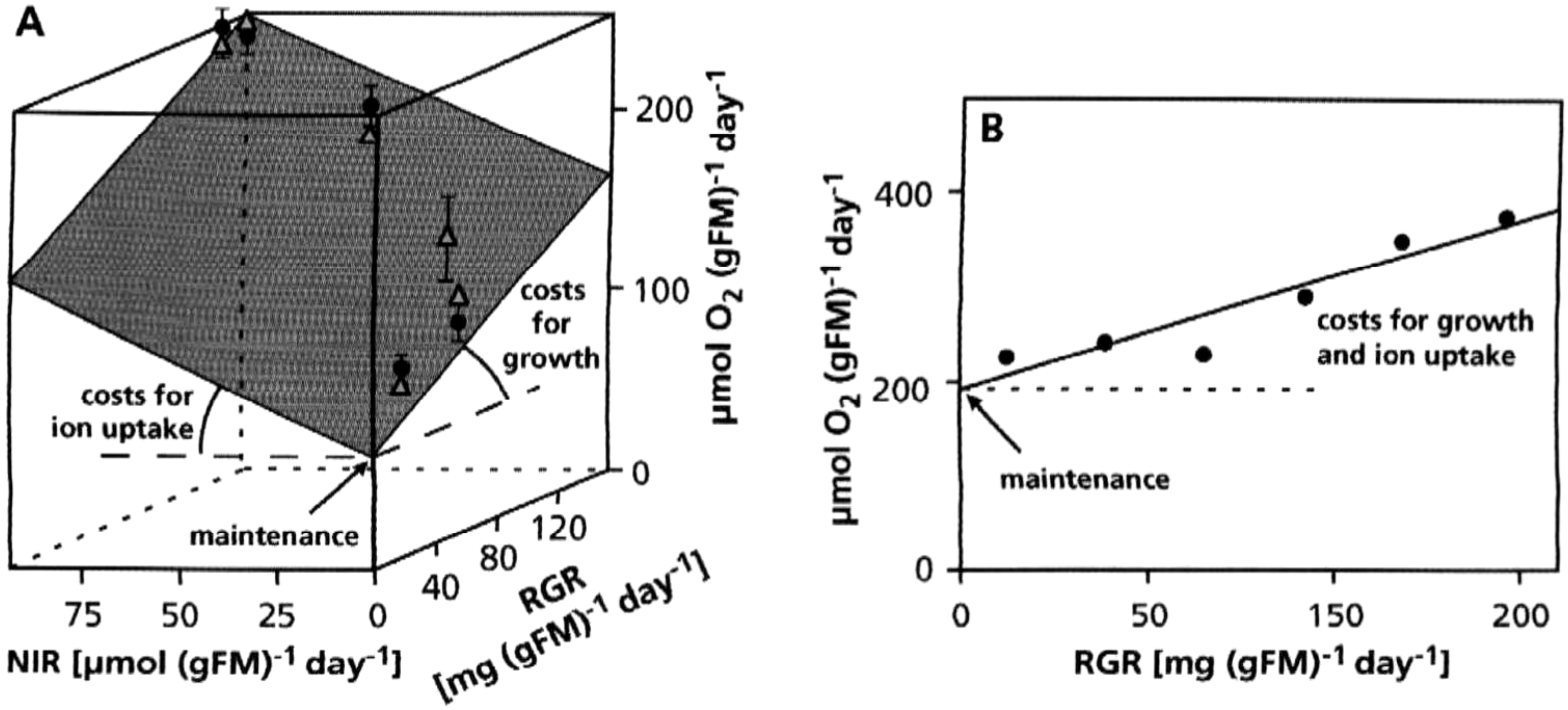


Maintenance Respiration Does Not Change With Plant Age But Growth Respiration Does



Multiple Regression Analyses Reveal 3 Major Respiratory 'Sinks'

- Growth Respiration – (synthesis of new compounds for biomass accumulation)
- Maintenance Respiration – (repair and 'housekeeping' metabolism)
- (Ion) Transport Respiration - (maintaining ion and pH gradients (homeostasis) and nutrient ion acquisition)



Comparisons of Respiratory 'Sinks' in Different Plant Species

A.

	Carex	*Solanum*	*Zea*
Growth, mmol O_2 $(g\,DM)^{-1}$	6.3	10.9	9.9
Maintenance, nmol O_2 $(g\,DM)^{-1}\,s^{-1}$	5.7	4.0	12.5
Anion uptake, mol O_2 $(mol\,ions)^{-1}$	1.0	1.2	0.53

B.

	Dactylis	*Festuca*	*Quercus*	*Triticum*
Growth + ion uptake, mmol O_2 $(g\,DM)^{-1}$	11	19	12	18
Maintenance, nmol $(g\,DM)^{-1}\,s^{-1}$	26	21	6	22

Conclusions

•There is a Good Correlation Between:

Growth Respiration & RGR

Transport Respiration & RGR

Maintenance Respiration & RGR

Model of 3 Major Respiratory Sinks Fits Data Well

•Partitioning of Respiratory carbon between these sinks varies with species and habitat

•Plants have more complex respiratory mechanisms than animals (AO, Protein Uncoupler, etc.) but neither the regulation nor the function is fully understood