

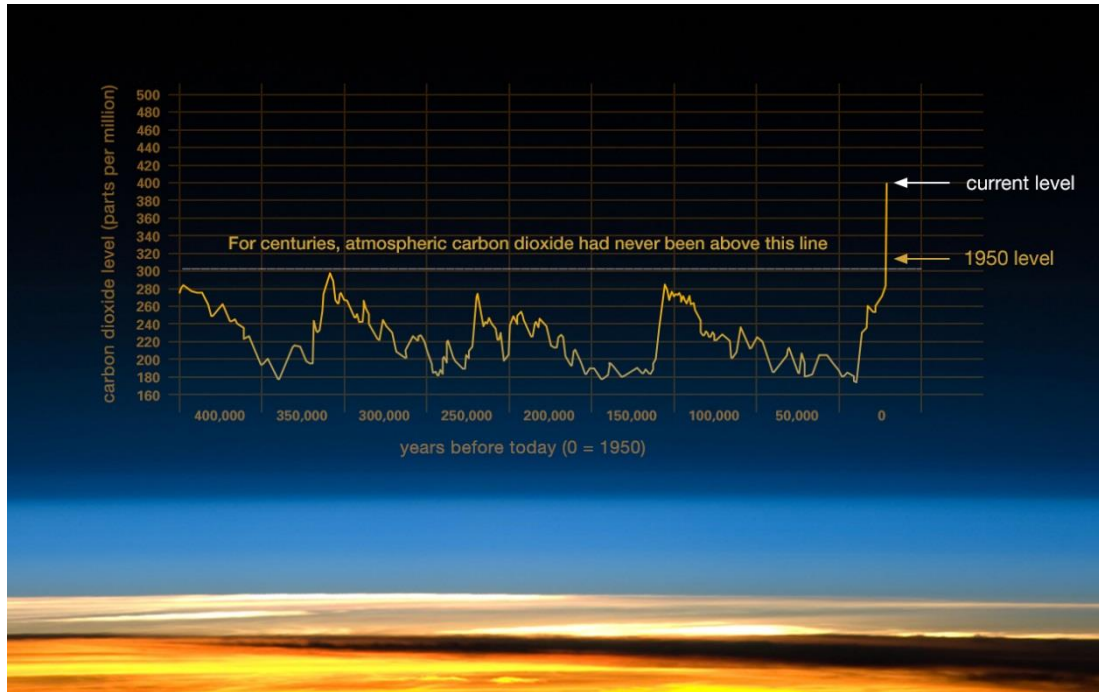
Role of Atmospheric Carbon Dioxide on Plant Metabolite Responses to High and Low Temperature Stress

ACSL





Factors Responsible for Climate Change

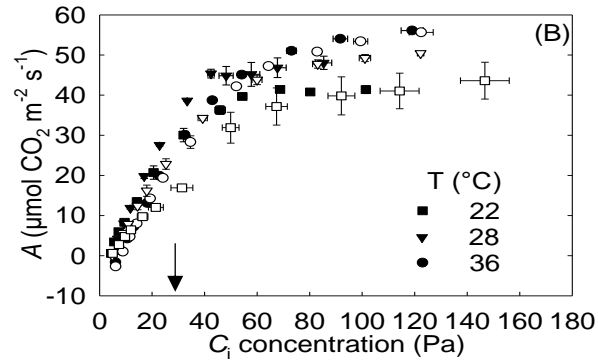


Atmospheric Component	Pre-1750	2015	Warming Potential	Atmospheric Lifetime-years
Carbon dioxide (CO ₂), ppm	~280	399.5	1	100 -300
Methane (CH ₄), ppb	722	1834	28	12.4
Nitrous oxide (N ₂ O), ppb	270	328	265	121
Tropospheric ozone (O ₃), ppb	237	337	n.a.	< 0.01



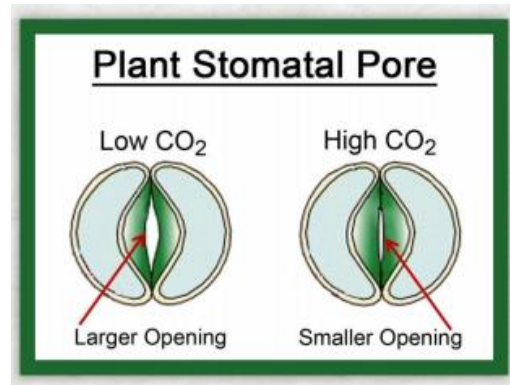
Effects of Carbon Dioxide Enrichment on Plants

Enhanced Photosynthetic Rates, A



Increased Biomass

Decreased Stomatal Aperture



Increased WUE

Nutritional Deficiencies

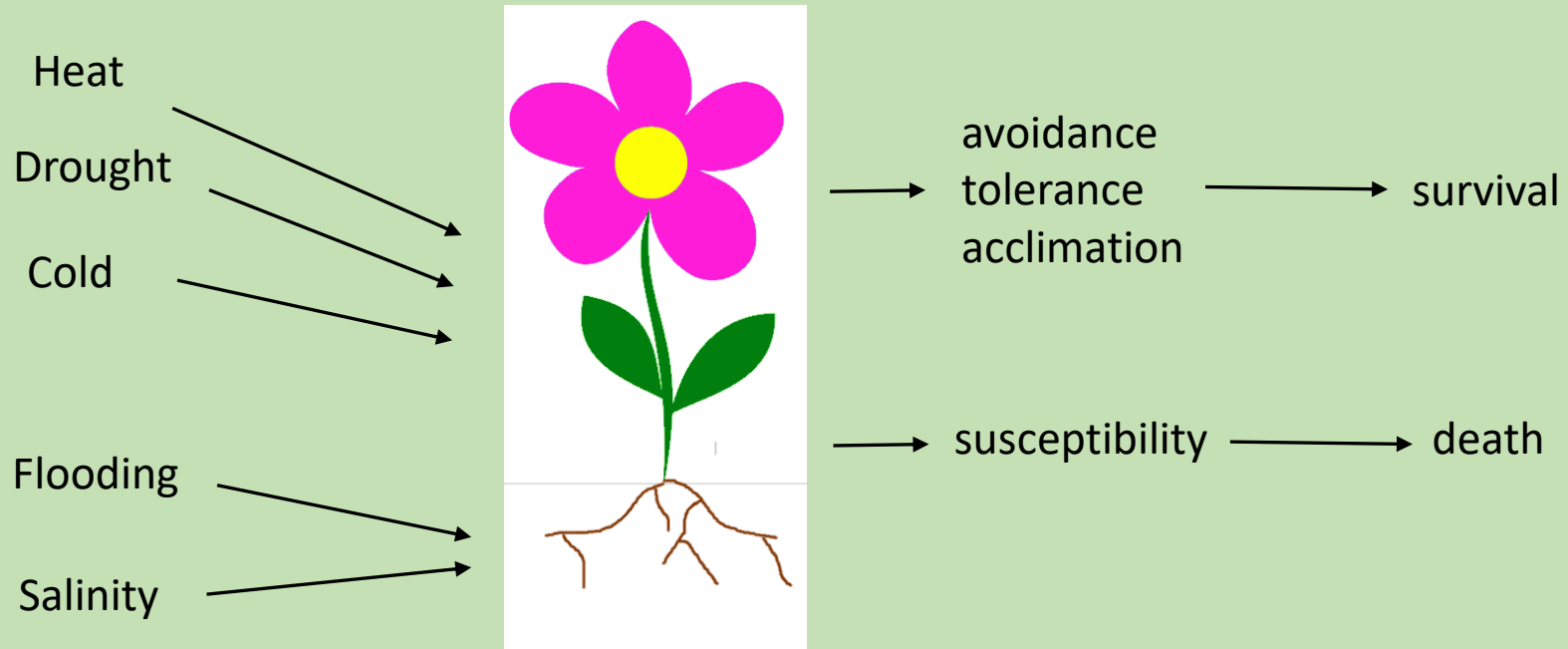
Increased Starch and Soluble Sugars
Decreased Protein and Total N Levels



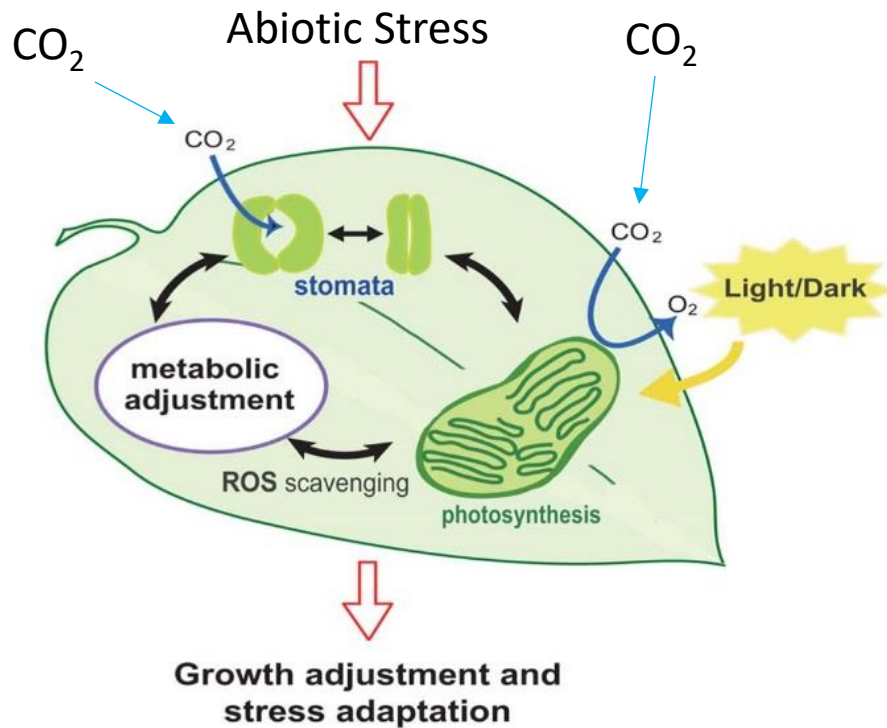
Increased C/N Ratio

Abiotic Stress Effects on Plants

How does CO₂ enrichment alter this model?

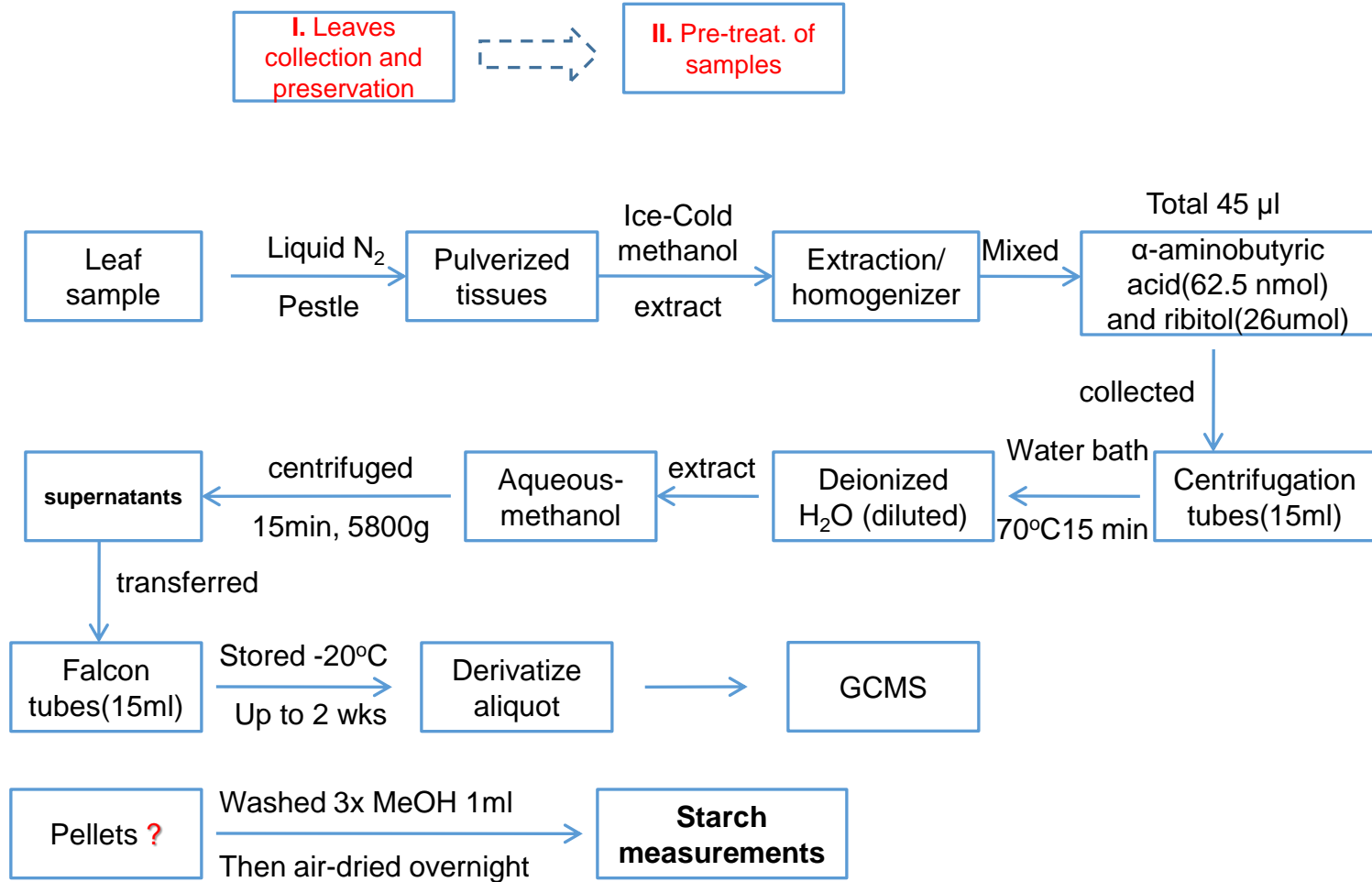


Foliar Metabolites Respond to Abiotic Stress



Atmospheric CO_2 alters metabolite adjustments to abiotic stress

Metabolite analysis

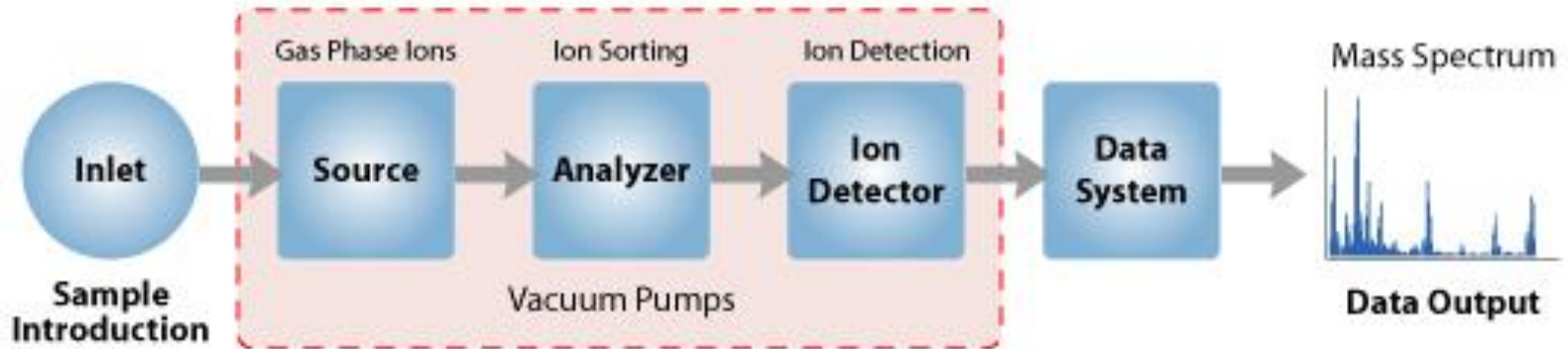
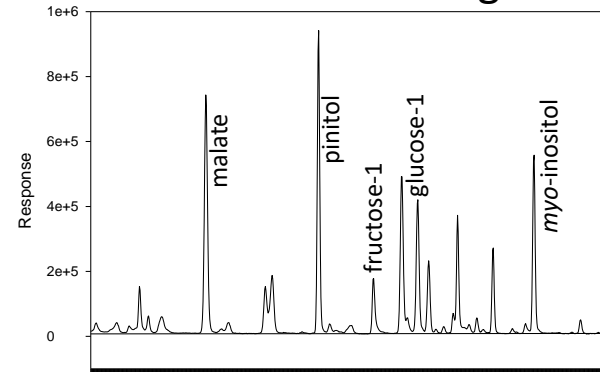


Data Collection and Analysis

GCMS



Total Ion Chromatogram



Metabolite Responses to Chilling Temperatures

About 60% of the Earth's surface is exposed to freezing temperatures annually.

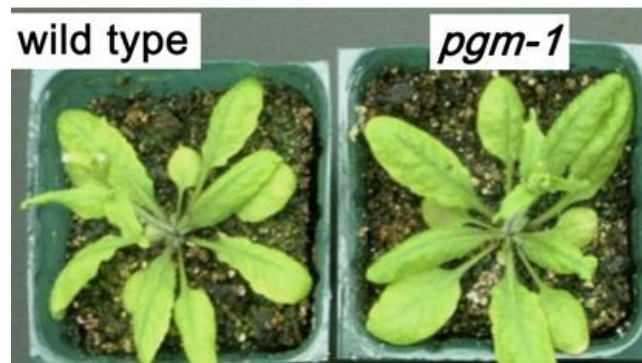


Chilling tolerance
Frost hardiness
Cold acclimation

Plants have mechanisms to survive frost.



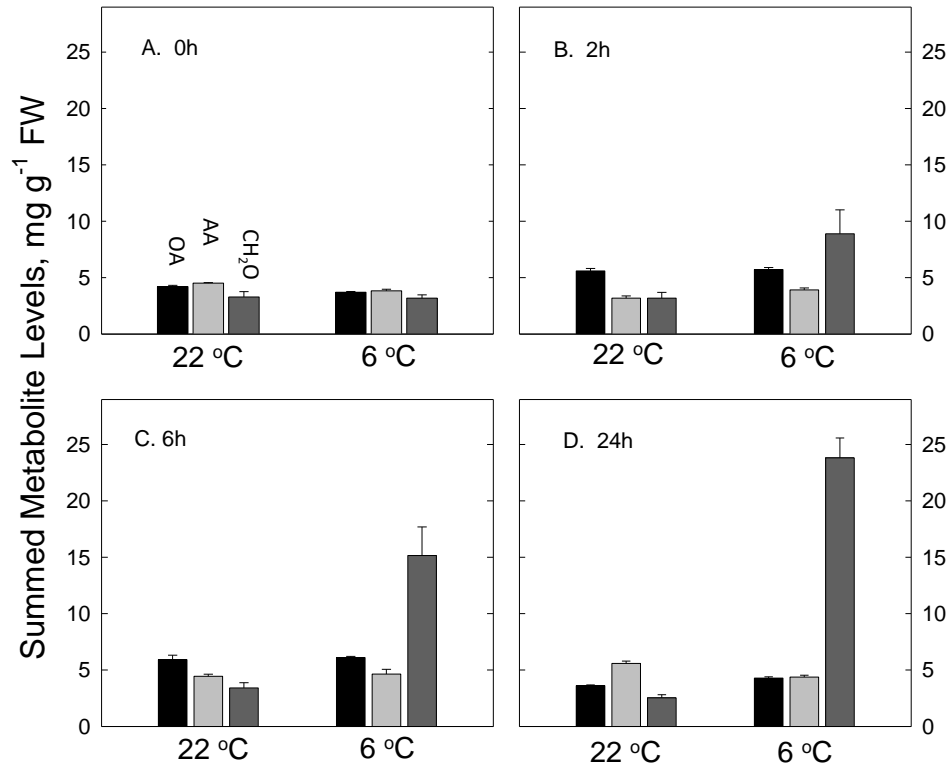
Arabidopsis thaliana



Net gas exchange rates of excised WT *Arabidopsis* leaves were measured in response to a chilling temperature treatment. Net CO₂ assimilation rates (*A*), stomatal conductance (*g_s*), and intercellular CO₂ (*C_i*) of *Arabidopsis* leaves were determined at 22 and 6 °C using a CO₂ partial pressure of 38 Pa and an irradiance of 125 μmol m⁻² s⁻¹

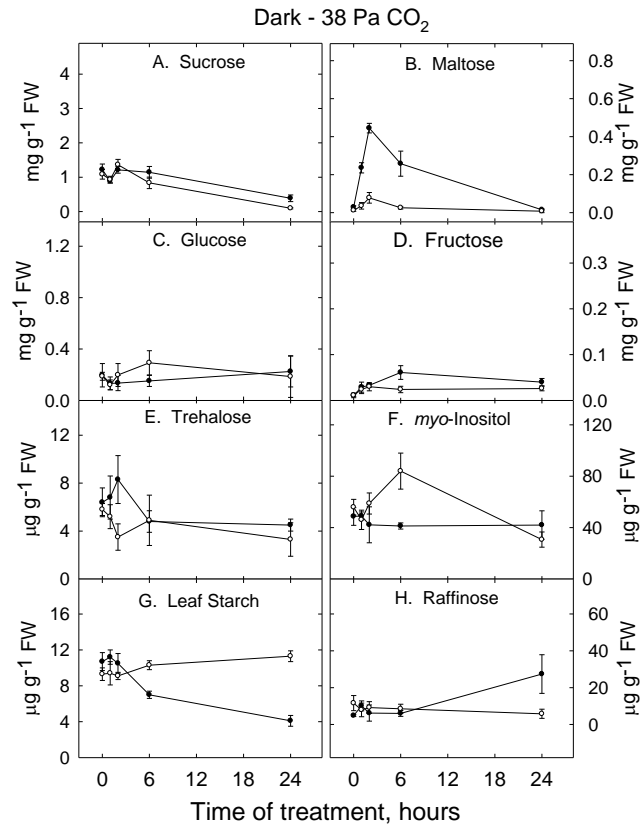
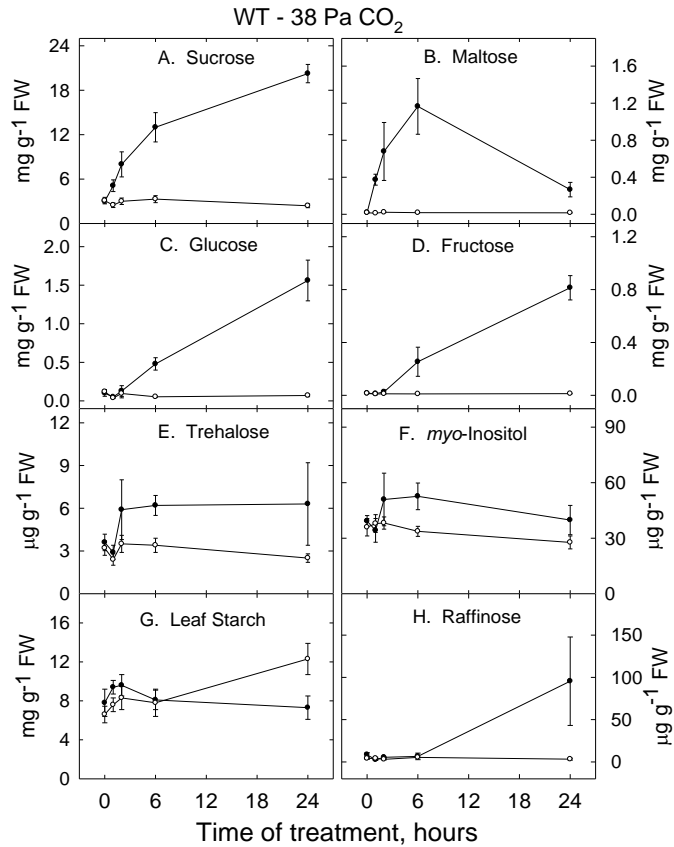
parameter	WT		<i>pgm1</i>		P		
	22 °C	6 °C	22 °C	6 °C	G	T	G x T
<i>A</i> , μmol m ⁻² s ⁻¹	4.7 ± 0.1	5.5 ± 0.2	4.6 ± 0.2	5.3 ± 0.1	ns	**	ns
<i>g_s</i> , mmol m ⁻² s ⁻¹	819 ± 54	897 ± 139	1368 ± 102	1456 ± 139	**	ns	ns
<i>C_i</i> , mmol m ⁻²	370 ± 1	353 ± 3	34 ± 15	32 ± 19	**	ns	ns

Low Temperature Effects on Metabolites in Wild Type Arabidopsis Leaflets

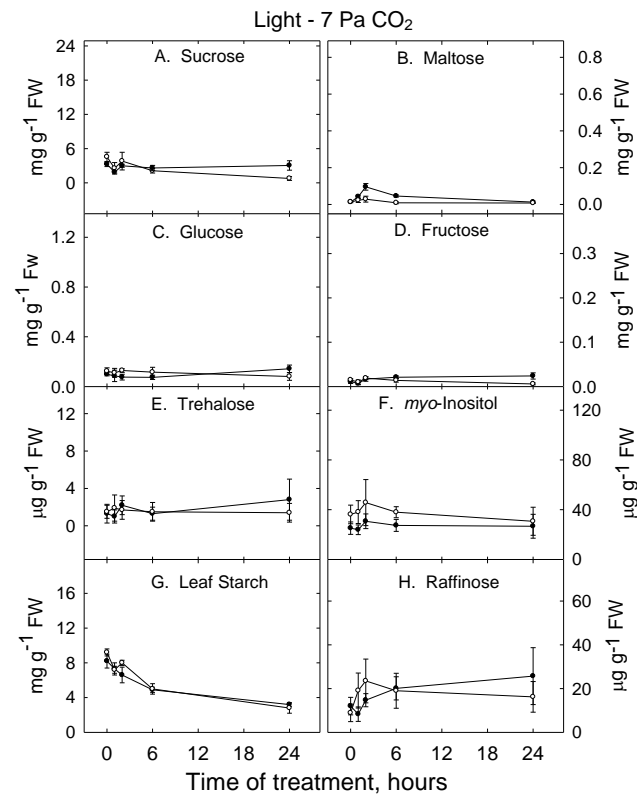
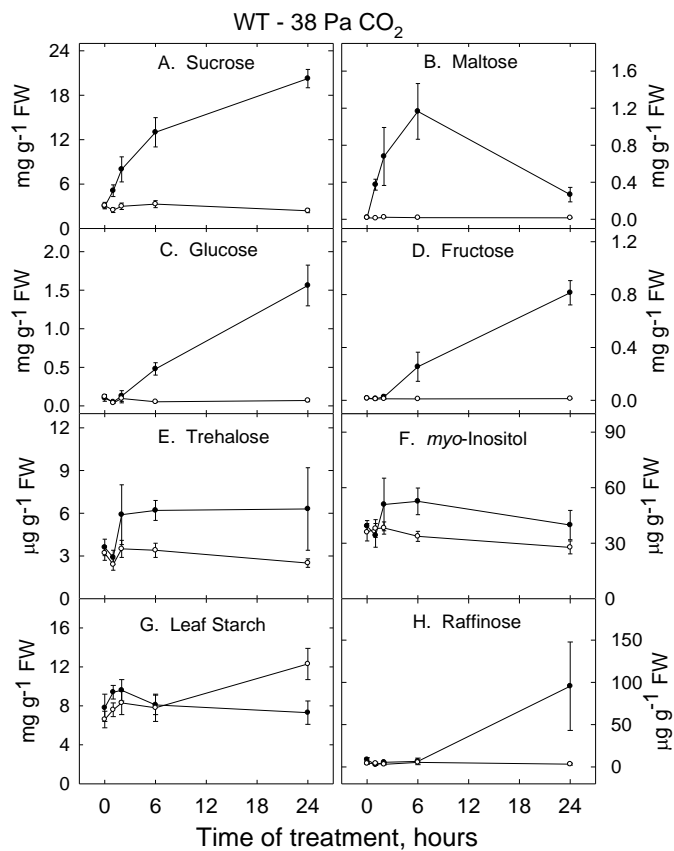


CH₂O increased
~ 6-fold in 24 h

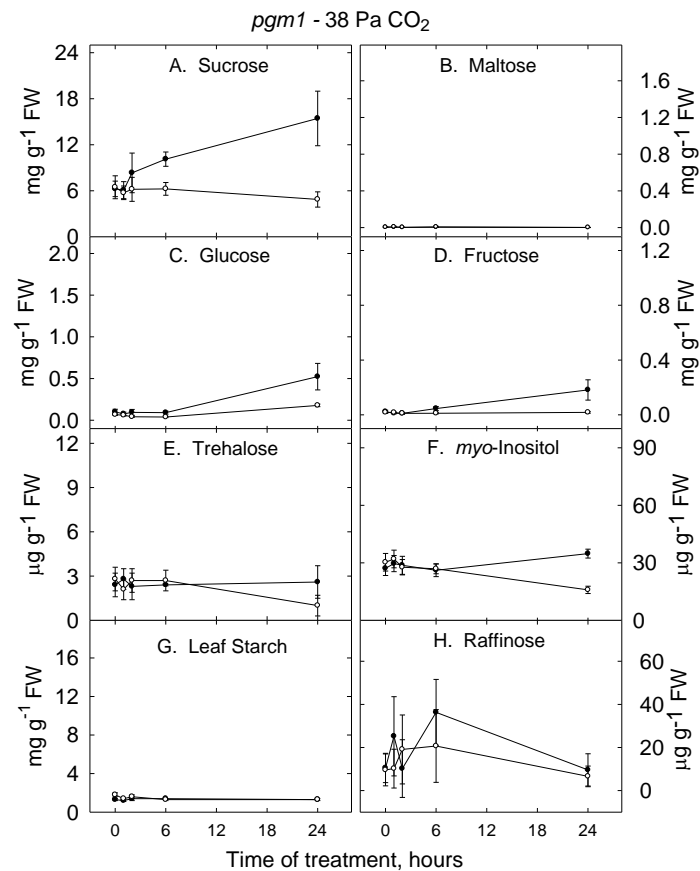
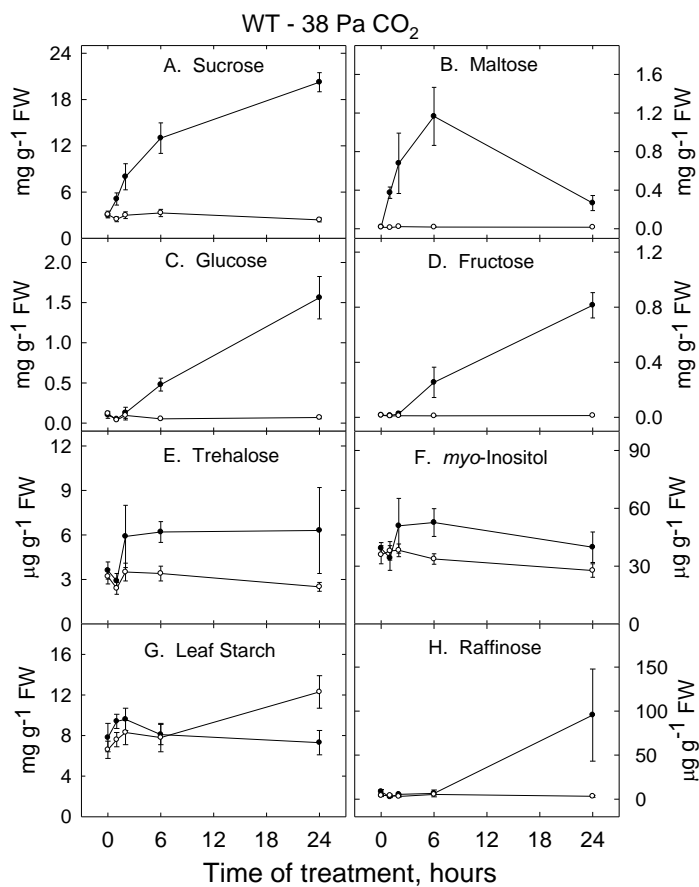
Light/Dark effects on CH₂O Accumulation in WT Arabidopsis Rosettes



Subambient CO₂ effects on CH₂O Accumulation in WT Arabidopsis Rosettes



Starch effects on CH₂O Accumulation in Arabidopsis Rosettes



Carbon Balance Calculations

Pathway of Leaf Starch Degradation

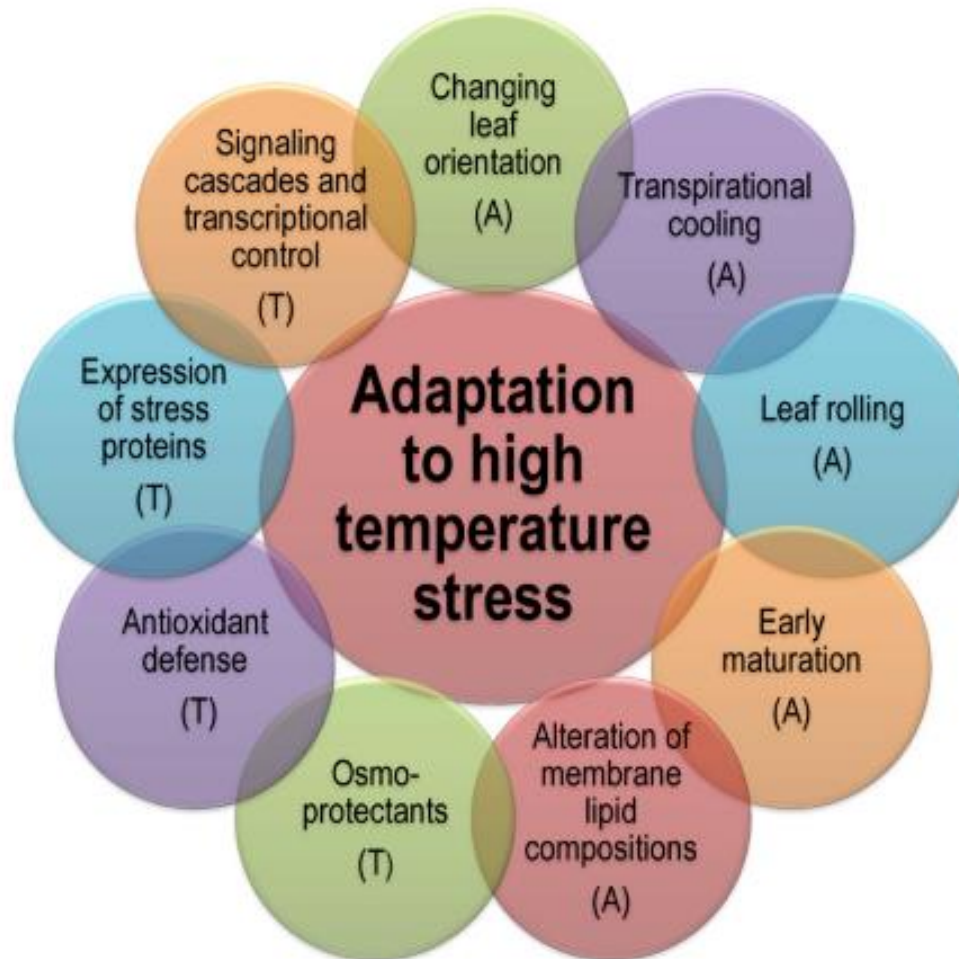
Polysaccharide → Oligosaccharide → Maltose → Hexose

Net gain from P_N ,	$5.7 \mu\text{g C m}^{-2}$
Net gain in Suc, Glu, Fru,	$2.4 \mu\text{g C m}^{-2}$ (42% in 24h)
Total C in Starch,	$0.2 \mu\text{g C m}^{-2}$

Conclusions

1. Foliar soluble carbohydrates accumulate 6- to 8-fold in response to chilling temperatures in 1 day. [compatible solutes, osmolytes and ROS protection]
2. This process mostly depends on energy and carbon from photosynthesis.
3. Maltose, hexose and trehalose accumulation were reduced in a starchless mutant compared to the WT.
4. Although the total amount of carbon stored in starch is small, leaf starch may contribute to carbohydrate synthesis during cold acclimation.
5. Sub-ambient CO₂ prevented carbohydrate accumulation due to chilling temperatures.

High Temperature Stress on Soybean Leaflets

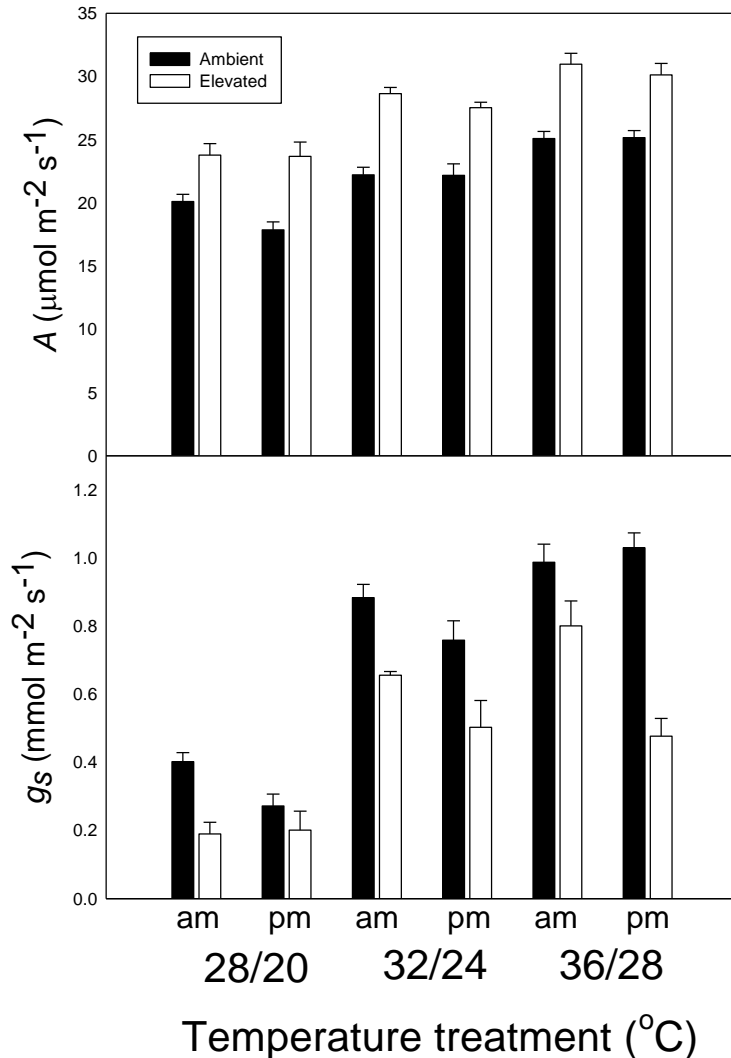


Experimental Protocol

1. Grew soybean plants in matching environmental chambers at three temperatures and two CO₂ treatments.
2. Leaf samples were harvest at 1, 5 and 10 h after the start of the photoperiod between 20 and 24 DAS.
3. Gas exchange was measured with a Li-Cor 6400 on the same day leaflets were sampled for metabolite analysis
6. A total of 42 leaf metabolites and leaf starch were quantified by GCMS, UPLC and coupled enzyme analysis.

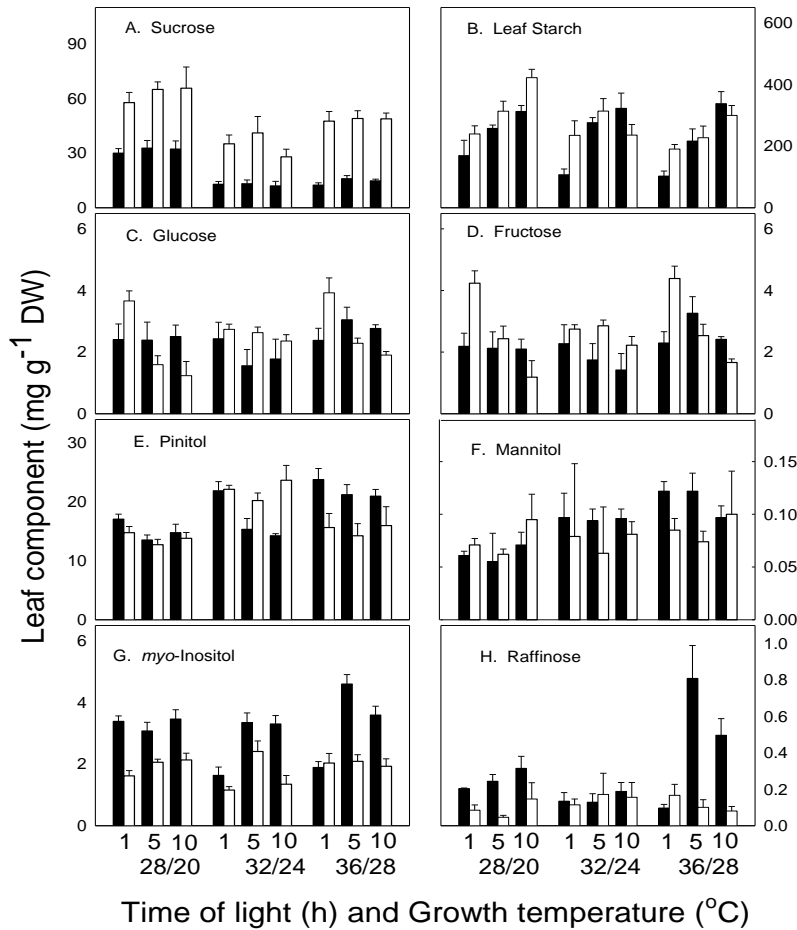


Growth Temperature Effects on Soybean Leaflet P_N Rates



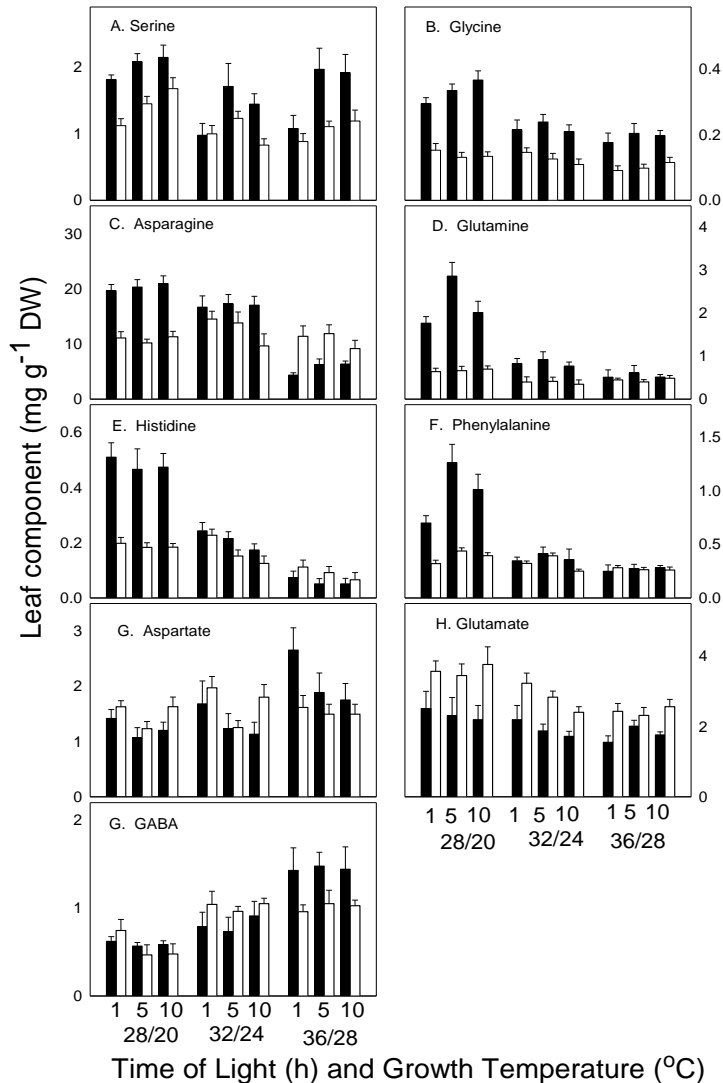
1. A increased with growth temperature and with CO_2 enrichment. No diurnal effects observed.
2. g_s increased with growth temperature but decreased with CO_2 enrichment.

Soy leaflet nonstructural carbohydrates



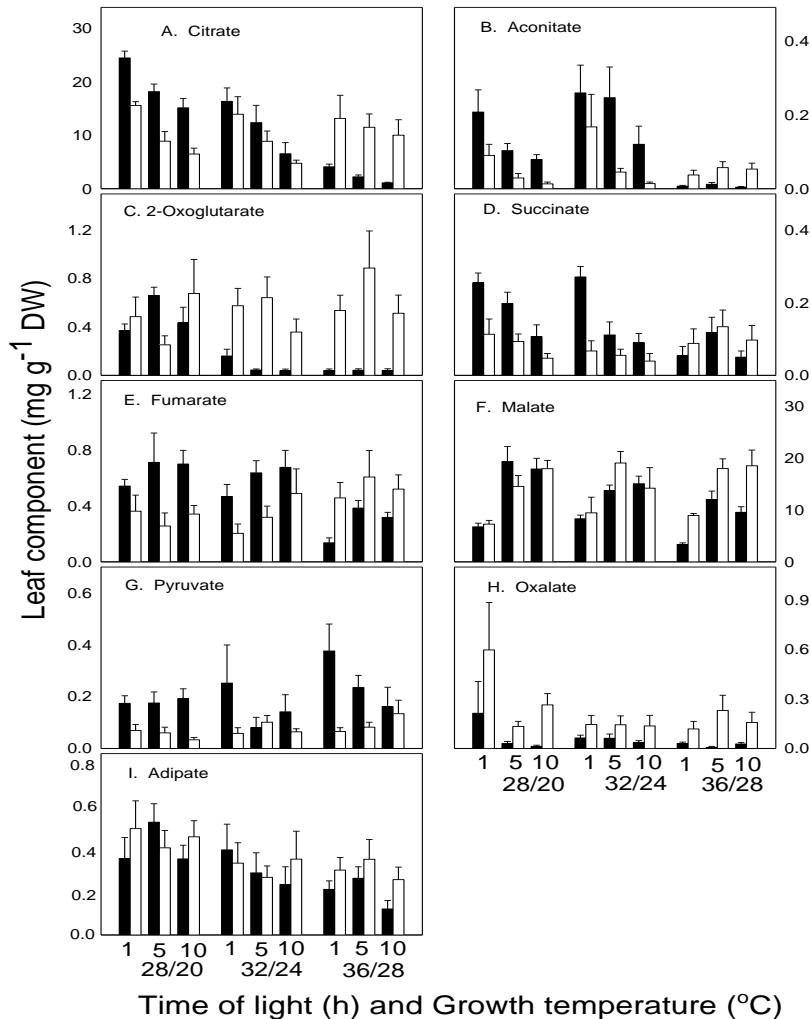
1. Sucrose decreased with increased growth temps.
2. Polyols and raffinose increase with growth temps under Ambt CO_2 .
3. Polyols increase in lieu of sucrose.

Soy leaflet amino acids



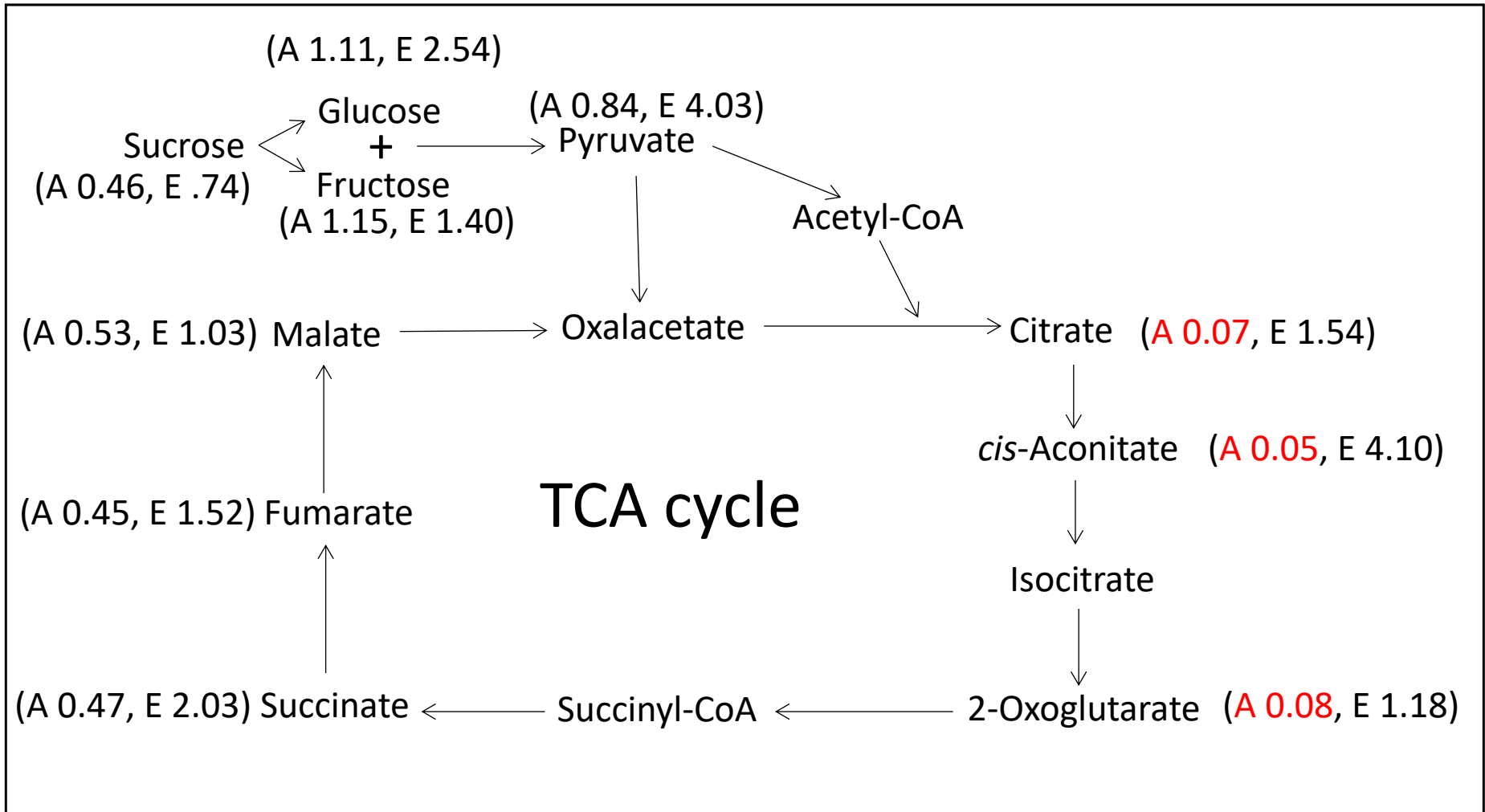
1. Most AAs decreased with growth temperature but GABA and ASP increased.
2. Elevated CO₂ did not reverse temp effects on Phe and Glu.
3. CO₂ effects on Ser and Gly were due to inhibition of PR.

Soy leaflet organic acids



1. Citrate decreases and malate increases diurnally.
2. Elevated growth temperatures decrease some organic acids up to 90%.
3. Ambient (black fill) and elevated (white fill) CO₂ treatments differ.
4. Elevated CO₂ partially reverses high temperature effects.

Effects of Elevated Growth Temperatures on Organic Acids in TCA Cycle



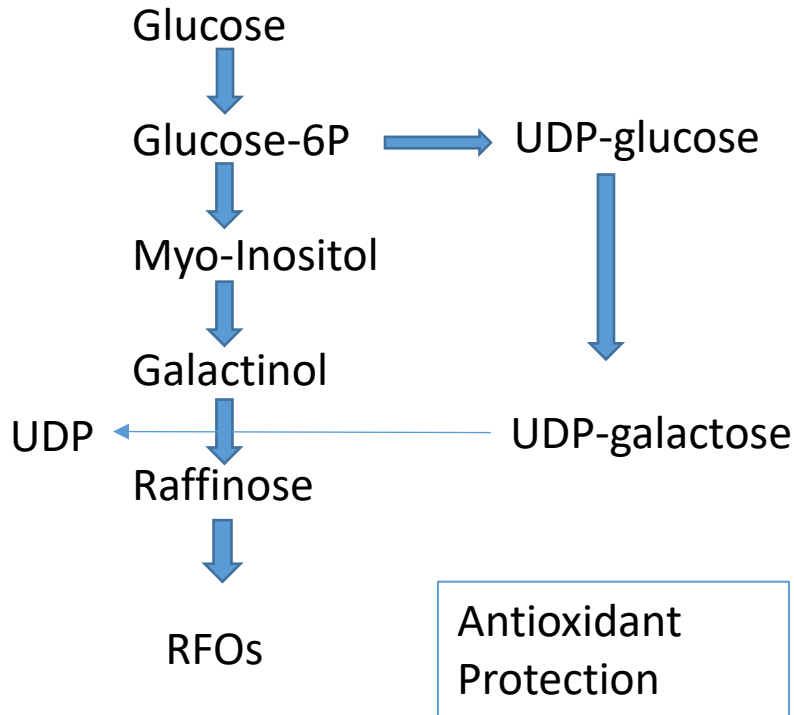
Metabolite ratios comparing 36/28 vs 28/20 °C measured late in photoperiod.
A = ambient; E = elevated CO₂.

Conclusions

1. Elevated growth temperatures inhibited conversion of sugars to organic acids found in mitochondria.
2. Polyols, raffinose and GABA increased
3. 28 of 43 metabolites were altered in the 36 compared to 28 °C growth temperature treatment.
4. CO₂ enrichment mitigated many of the effects of elevated growth temperatures on metabolites in soybean leaflets.

Do Metabolite Responses to Hi and Lo Temperature Stress Overlap?

1. Starch decreases.
2. Polyols, raffinose increase.



Acknowledgments

Gas Exchange/WUE

Jim Bunce
Shardendu Singh

Hyperspectral Imaging

Moon Kim
Byung-Kwan Cho

Statistics

Bryan Vinyard

Phenolics/LCMS

Dave Luthria