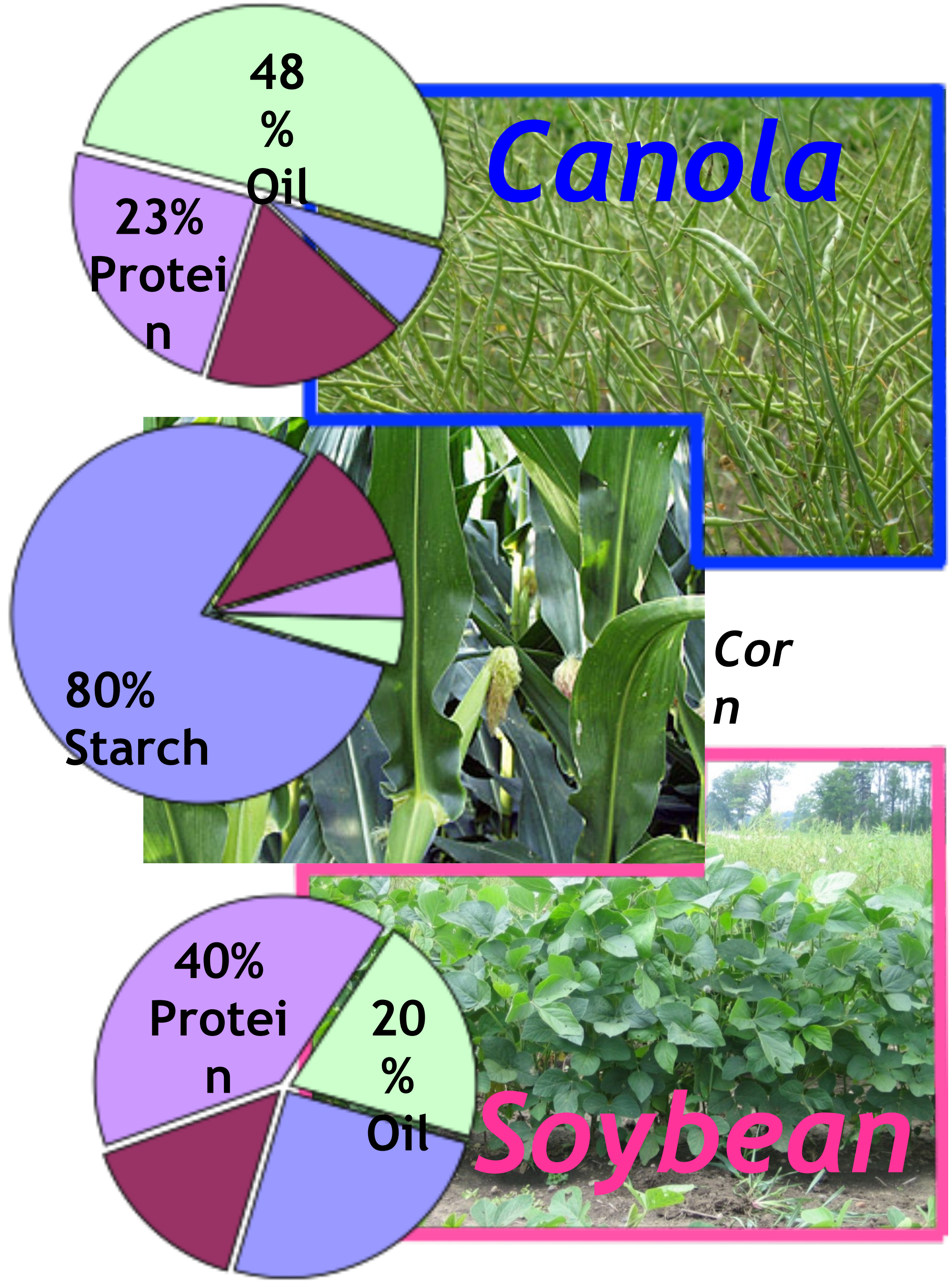
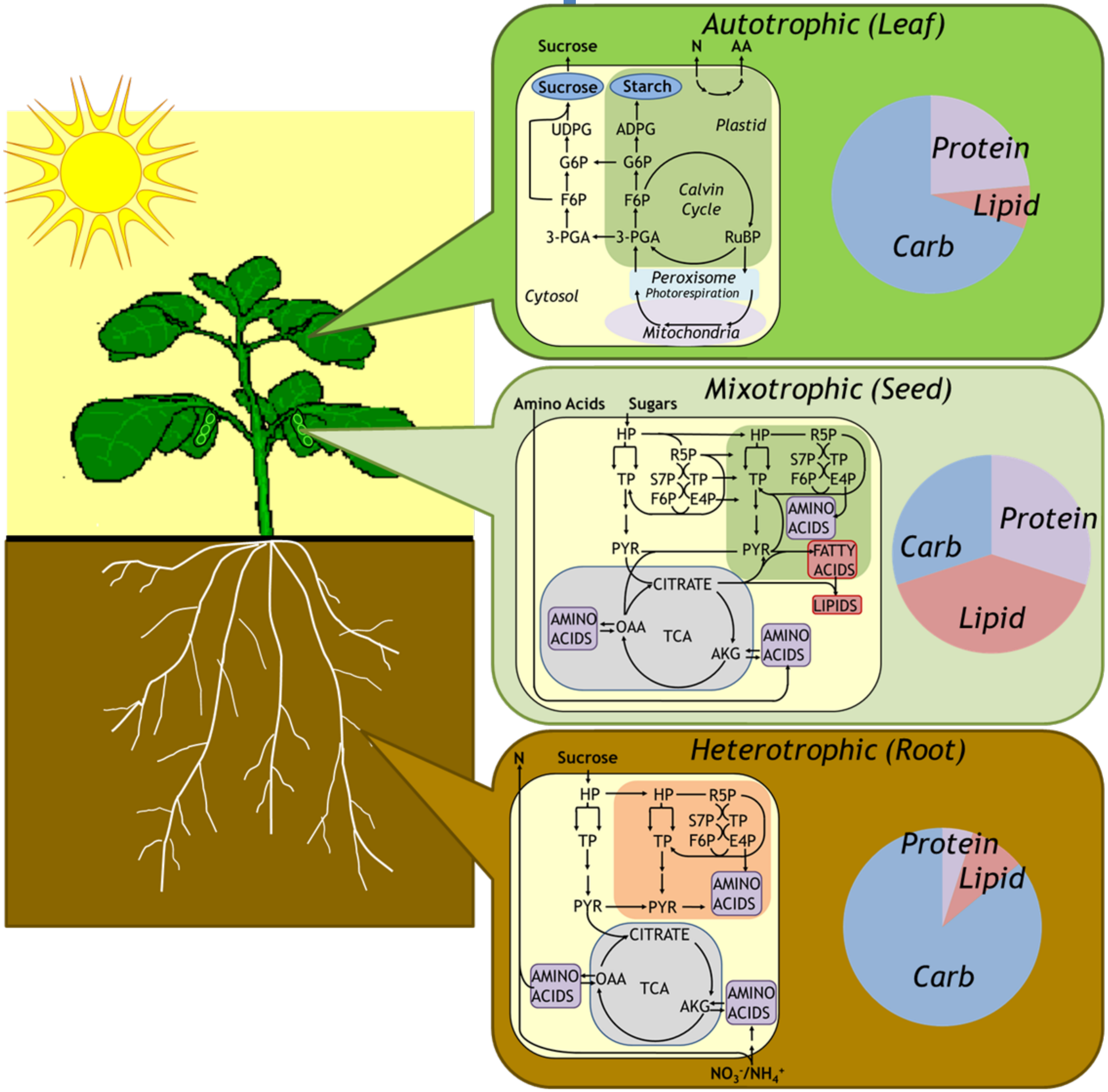

USDA
ARS
United States Department Of Agriculture
Agricultural Research Service
Opportunities to Redefine Carbon Partitioning in [illegible] Soybeans
Doug K. Allen
February 16, 2017
DONALD DANFORTH
PLANT SCIENCE CENTER
DISCOVERY | COMMUNITY | IMPACT

Rationale

- Crop composition varies
- Storage reserves are generated by central carbon metabolism
- Carbon and energy partitioning varies among species
- "Engineering" benefits (petroleum replacements & improved nutrition)



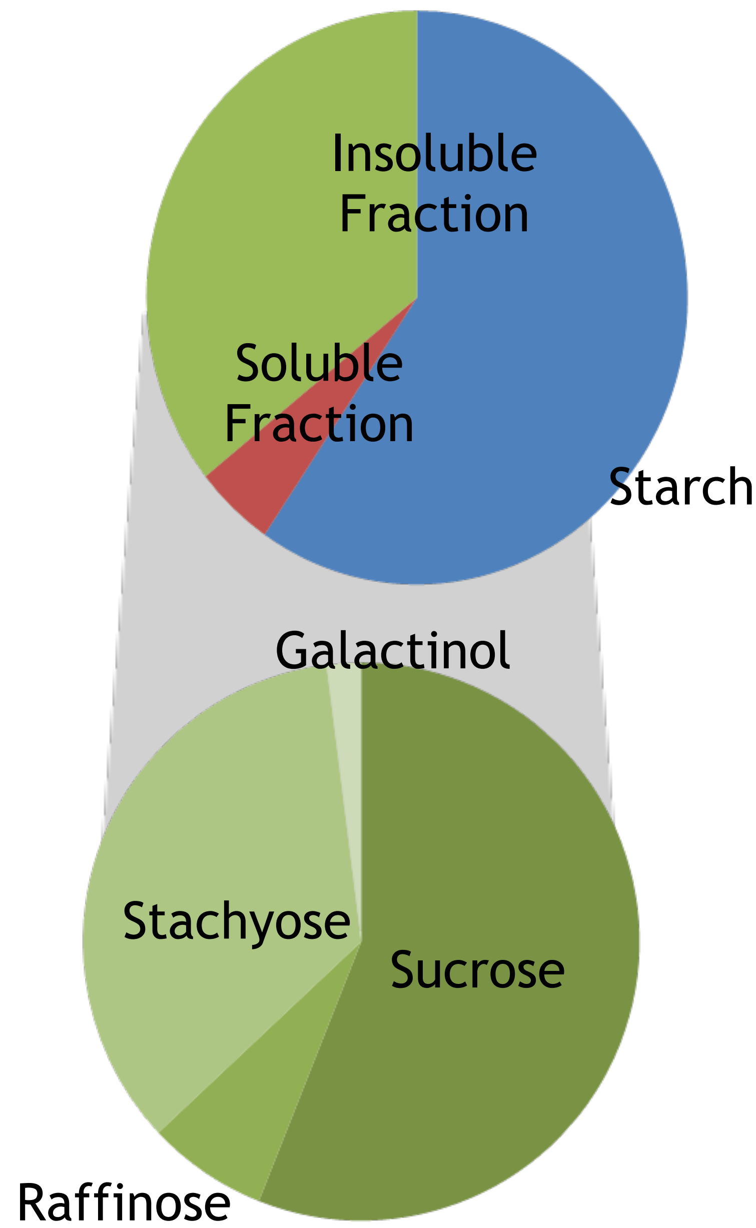
Cells Operate Differently



- Different compositions
- Different metabolism
- Many of the same enzymes
- Enzyme operation is specific to

Carbohydrate Profile

- Cell wall/insoluble fiber → 20-25%
- Starch (decreased at maturation) → <4%
- Soluble carbohydrates → ~10-15%
  - sucrose/galactinol
  - oligosaccharides: raffinose, stachyose



Reducing Oligosaccharides

(Bilyeu, Dierking, Wiebold, Fehr, Schillinger, Obendorf some work sponsored by USB)

- Impact metabolizable energy negatively
- Considered as anti-nutritional factors
- Soluble carbohydrates not critical for desiccation tolerance (no reduction in germination/emergence) (Obendorf 1997; Schillinger et al Patent 8471107, Neus etal 2005)
- Variant alleles with altered genes (e.g. RS2) produce less oligosaccharides,

  →more sucrose (up to 85% of soluble carbohydrate)

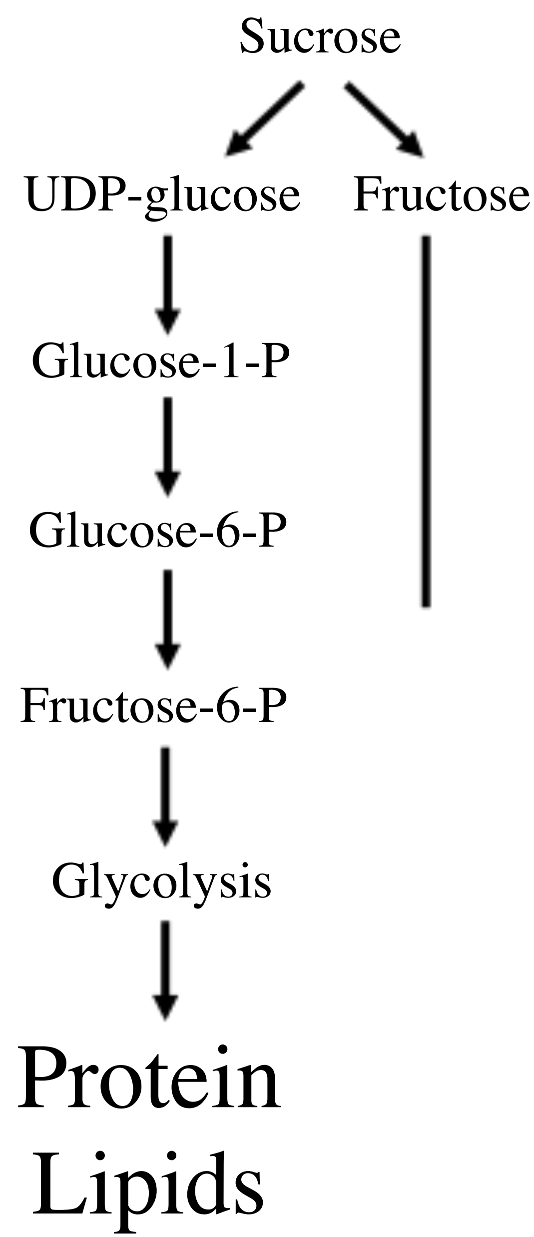
  (Hageley, Palmquist and Bilyeu 2013)

  →higher metabolizable energy in soy meal

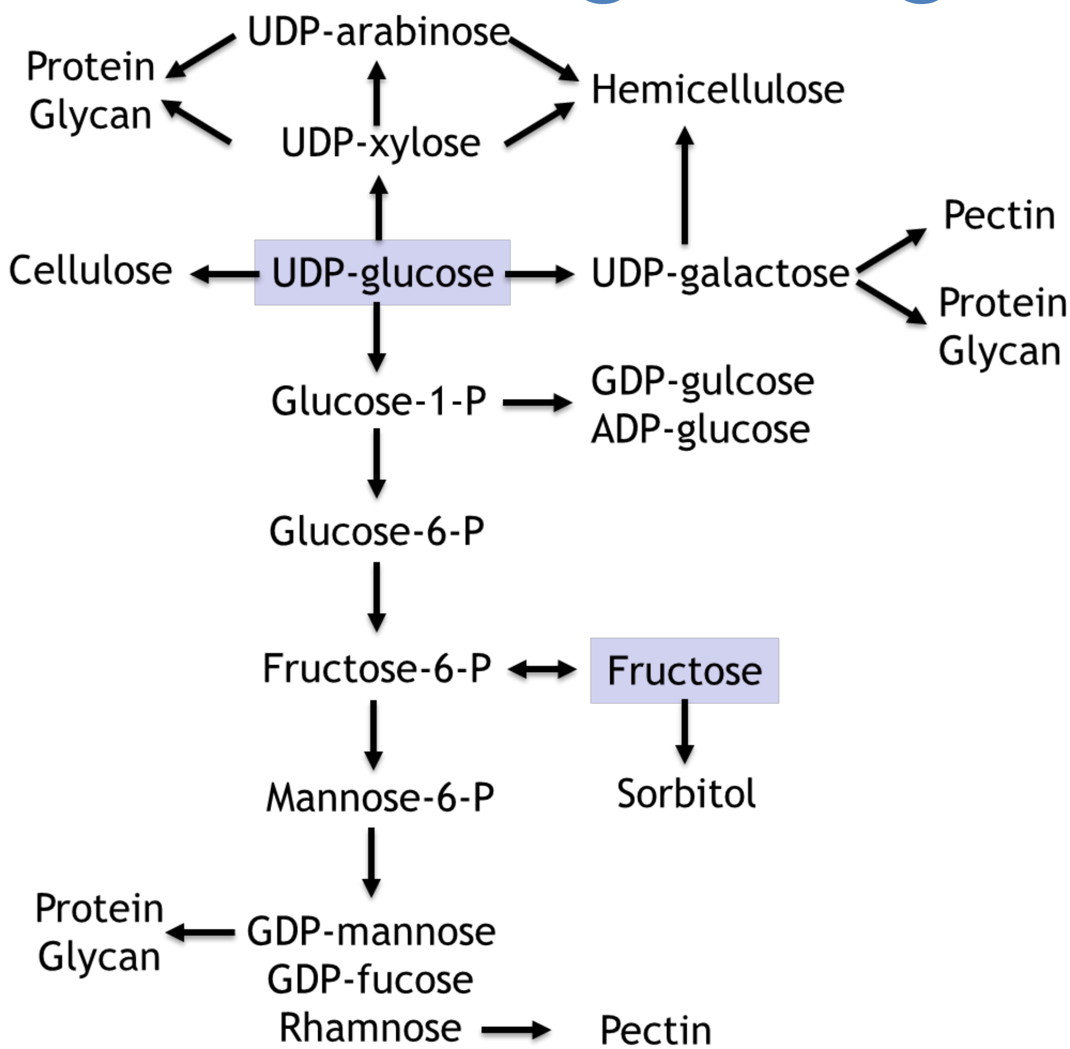
What *FURTHER* can be done?

- Can insoluble components be reduced?
  →UDP-glucose metabolic partitioning
  →sucrose is imported in seeds

- Can sucrose be converted to something of higher value?
  - protein?
  - oil?
  - transcription factors such as *Wrinkled 1*



Wall Biosynthesis from Glycosides including UDP-glucose & Fructose

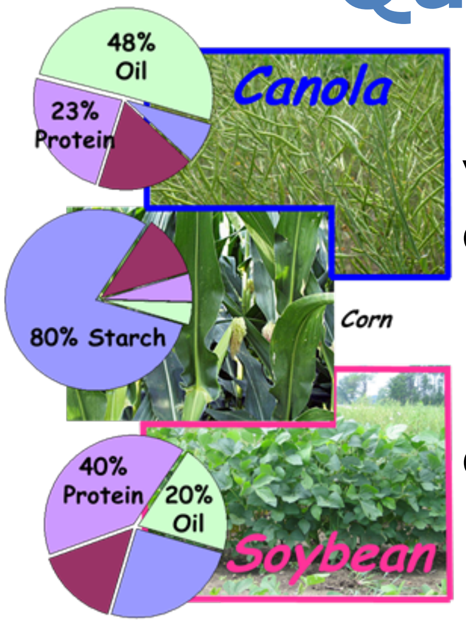


- Reduced glycosyltransferase mutants indicate 20% reduction in arabinose content (Egelund et al 2007)
- Structural and protective functional features of cell wall limit complete removal
- Cell wall biosynthesis is still unknown, though composition can be highly variable
- Removing recalcitrant wall

Storage Reserve Generation

- Soybeans require a lot of nitrogen for protein biosynthesis
- Balance of amino acids with sugars
  - →Amino acid transporters impact seed protein levels (Sanders et al. 2009)
- Protein and Oil and Protein and Yield are inversely related → different inputs from maternal plant
- Mechanisms determining differential partitioning of seed reserves remains unknown

Quantify Cellular Metabolism

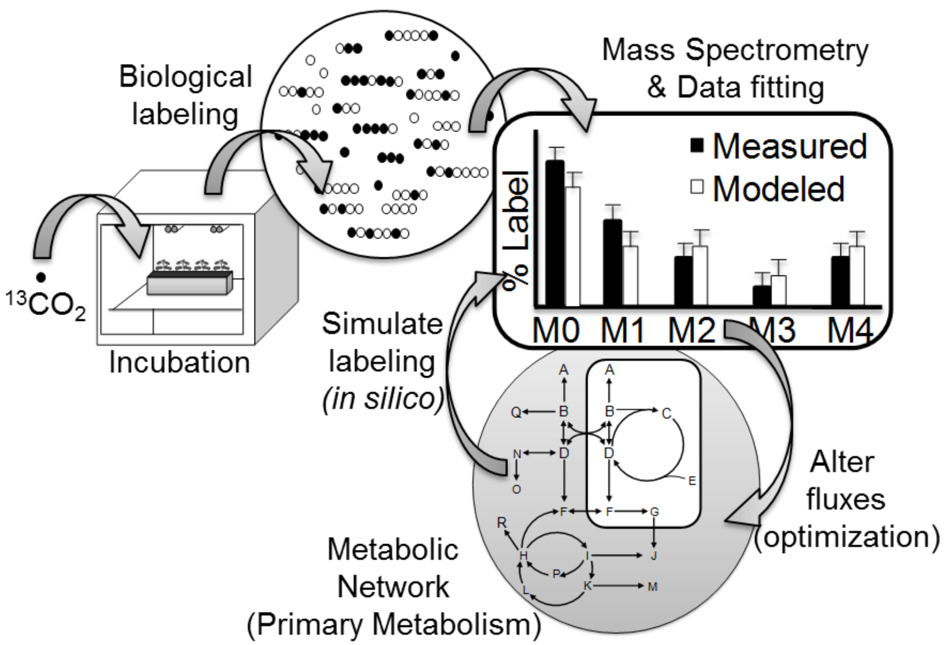


Plant seed composition varies: oil, protein and carbohydrate

Engineering nutrition & chemical feed stocks

Different metabolic functions:

Seeds = storage or germination
Leaves = sink then photosynthesis



Isotopic labeling & computational metabolic flux analysis: central carbon, lipid and protein metabolism

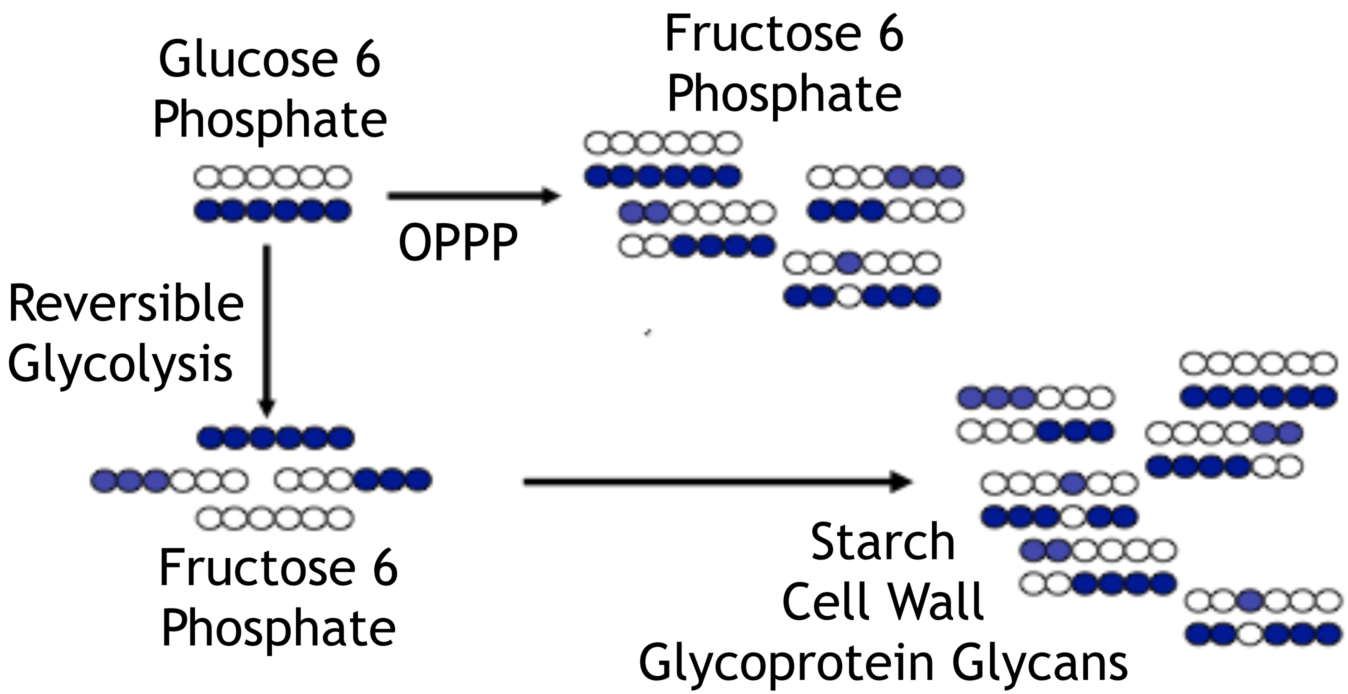
Questions/Aims

- What are *ROUTES* & *RATES* of carbon flow?
- What are the *SOURCES* of energy & redox for storage reserve production?
- What is the impact of environmental or transgenic perturbations

Approaches

- Measure uptake/export rates & composition
- Balance energy & cofactor supply & demand
- Perform isotopic labeling experiments and measure metabolite enrichment
- Develop flux maps with computational tools

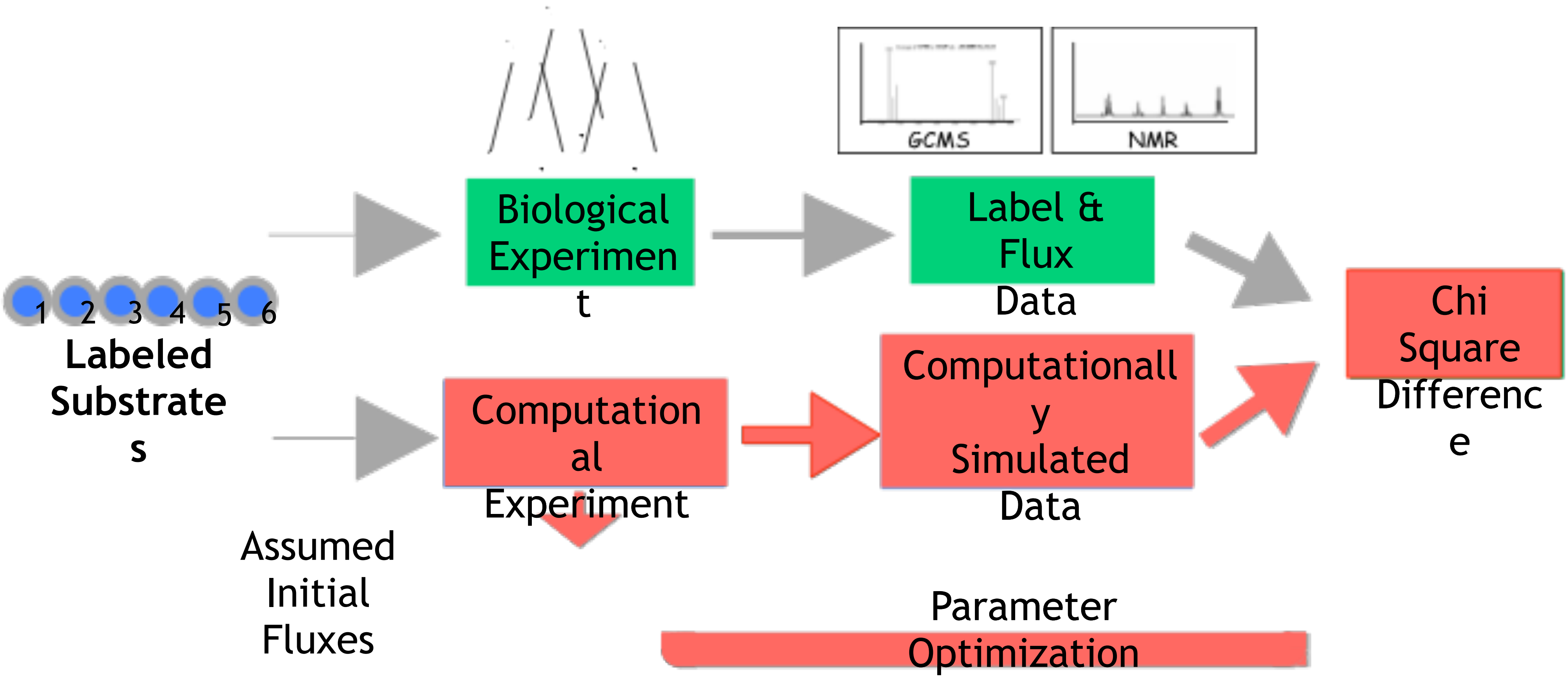
Isotopic Tracers



Flux analysis allows quantification of different pathways

Fluxes determine the balances of redox, energy and elements

Determining Flux Values



Model calculates labeling from flux values

Forward problem

Compare measured and simulated labeling patterns and minimize differences to obtain best fit flux map

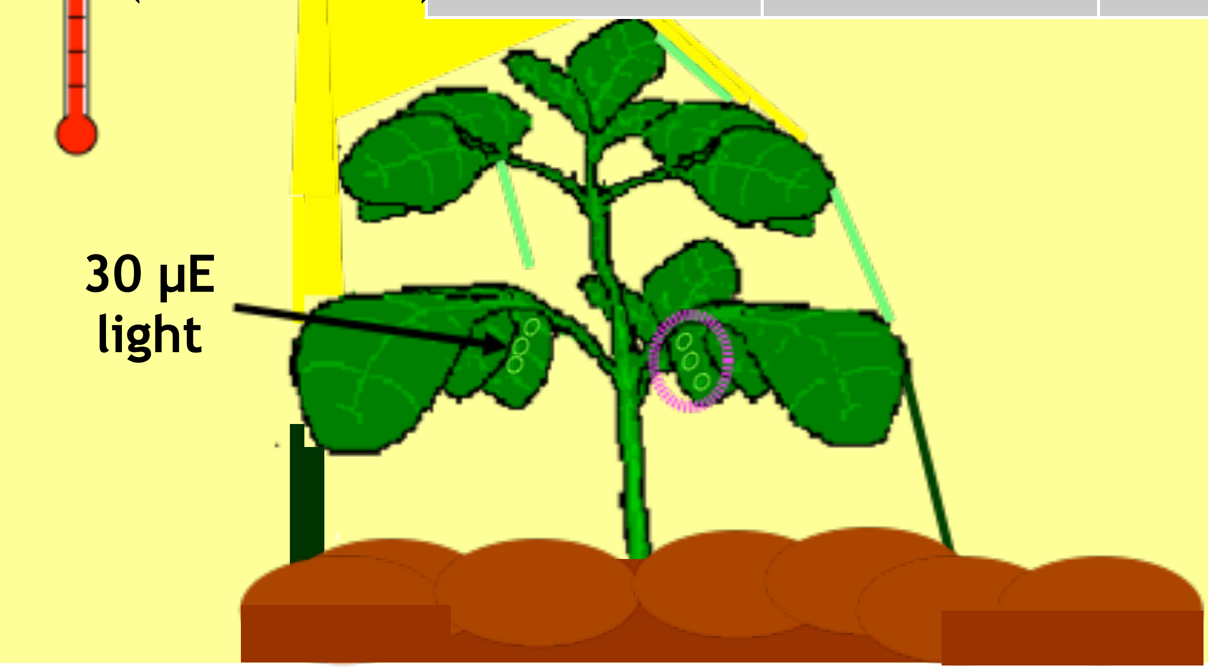
Inverse problem

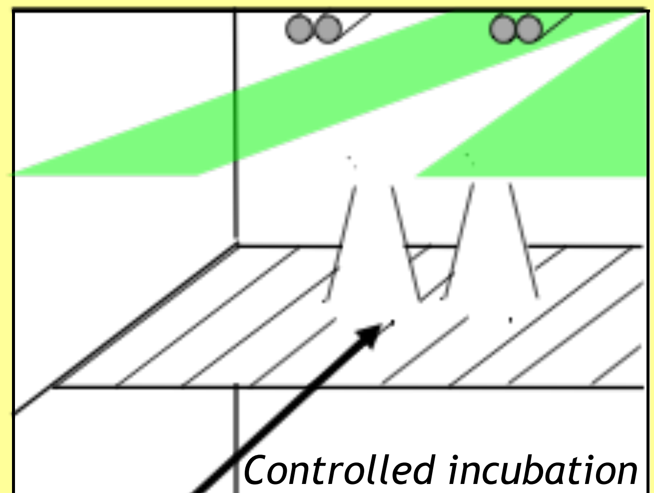
Inspired by Wiechert 2001

Environmental Variables & Culturing Conditions

C:N	Sucrose	Glucose	Glutamine	Asparagine
6	90	45	140	50
13	140	70	70	25
21	150	75	45	16
37	170	85	25	12
91	180	90	11	4

26-27°C
(78.8-80.6°F)



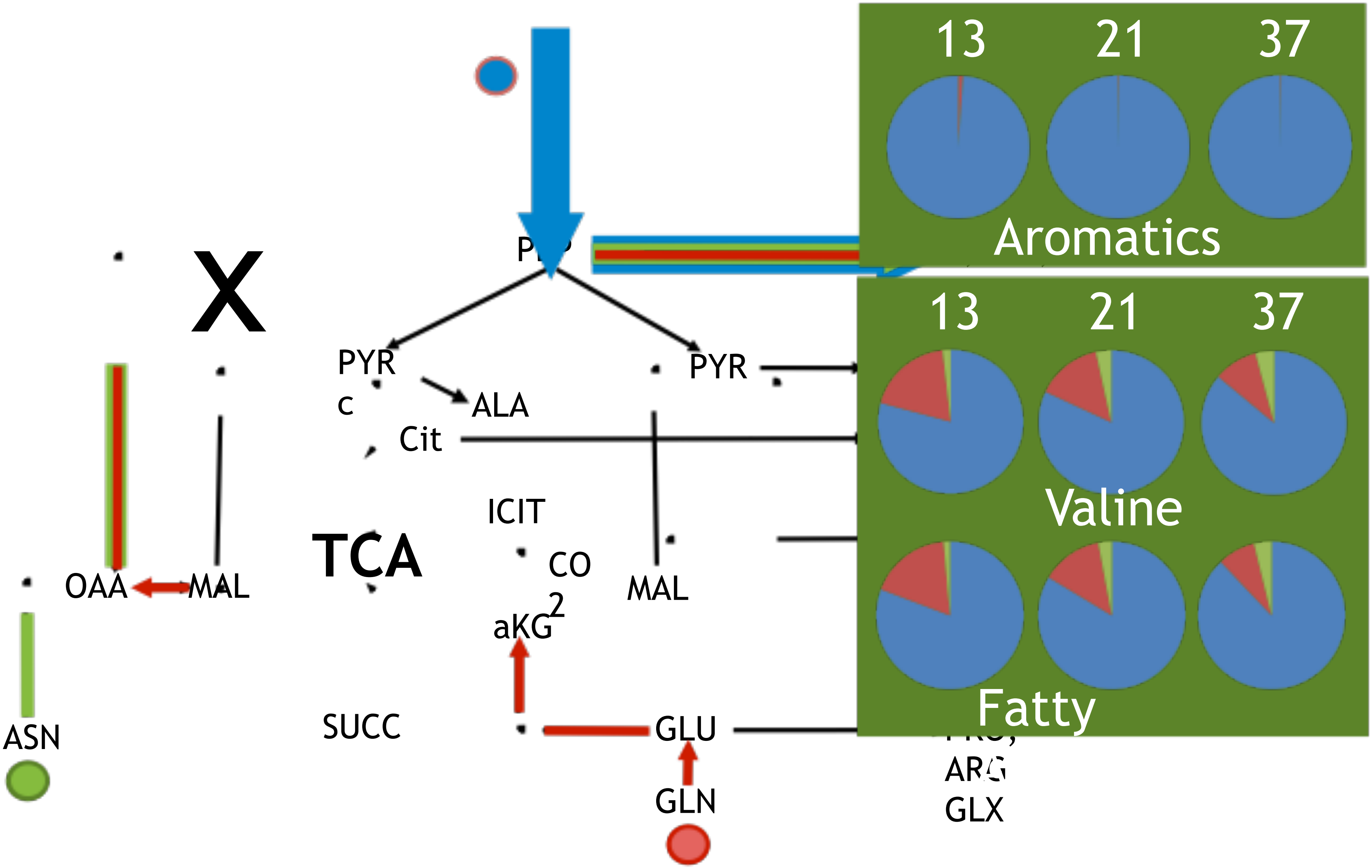


Starting Fresh
Weight: 25-40mg

14 day culture experiments

Allen and Young 2013, Plant Phys.
Allen et al. 2009, Plant J.

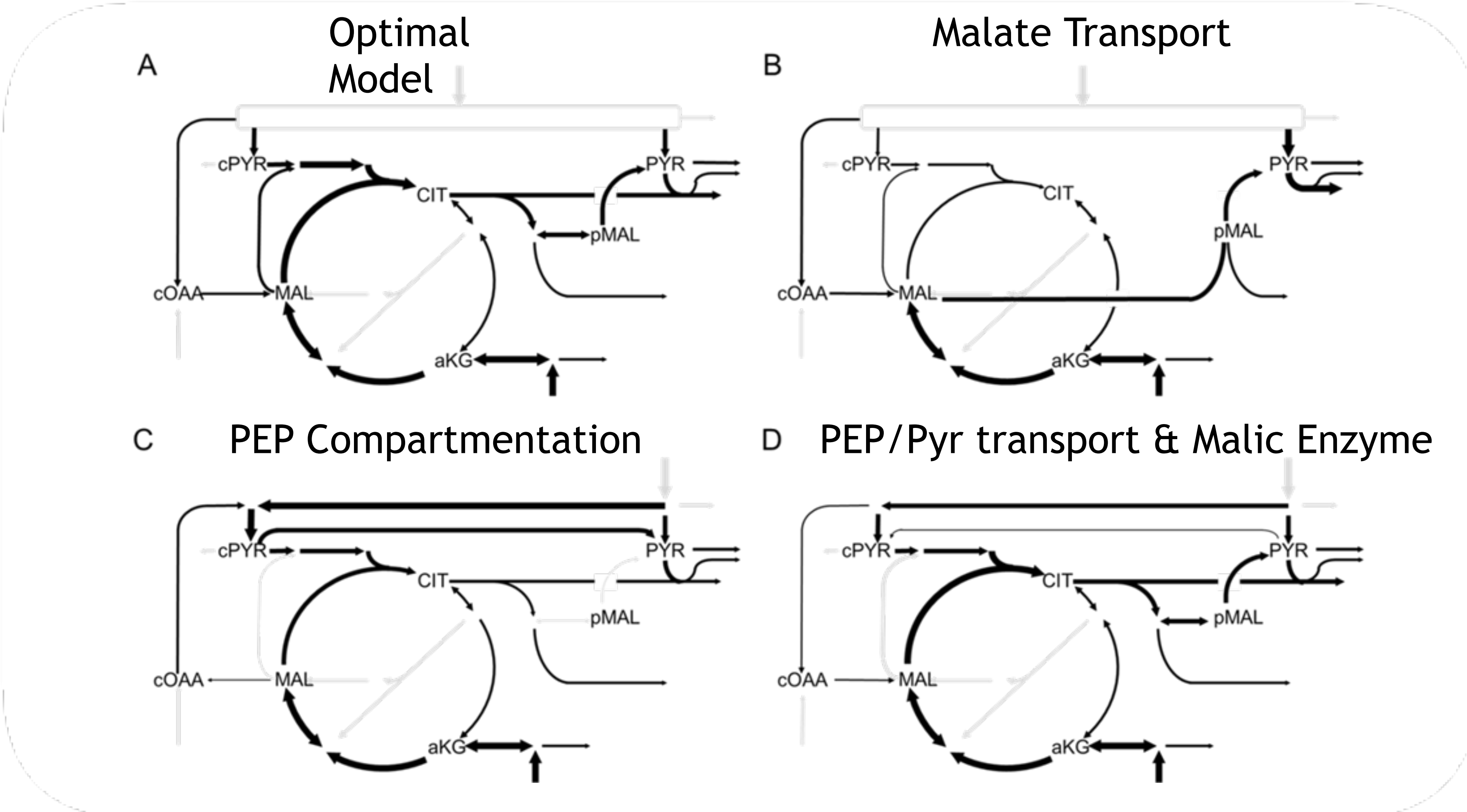
PEPCK is not required for labeling descriptions



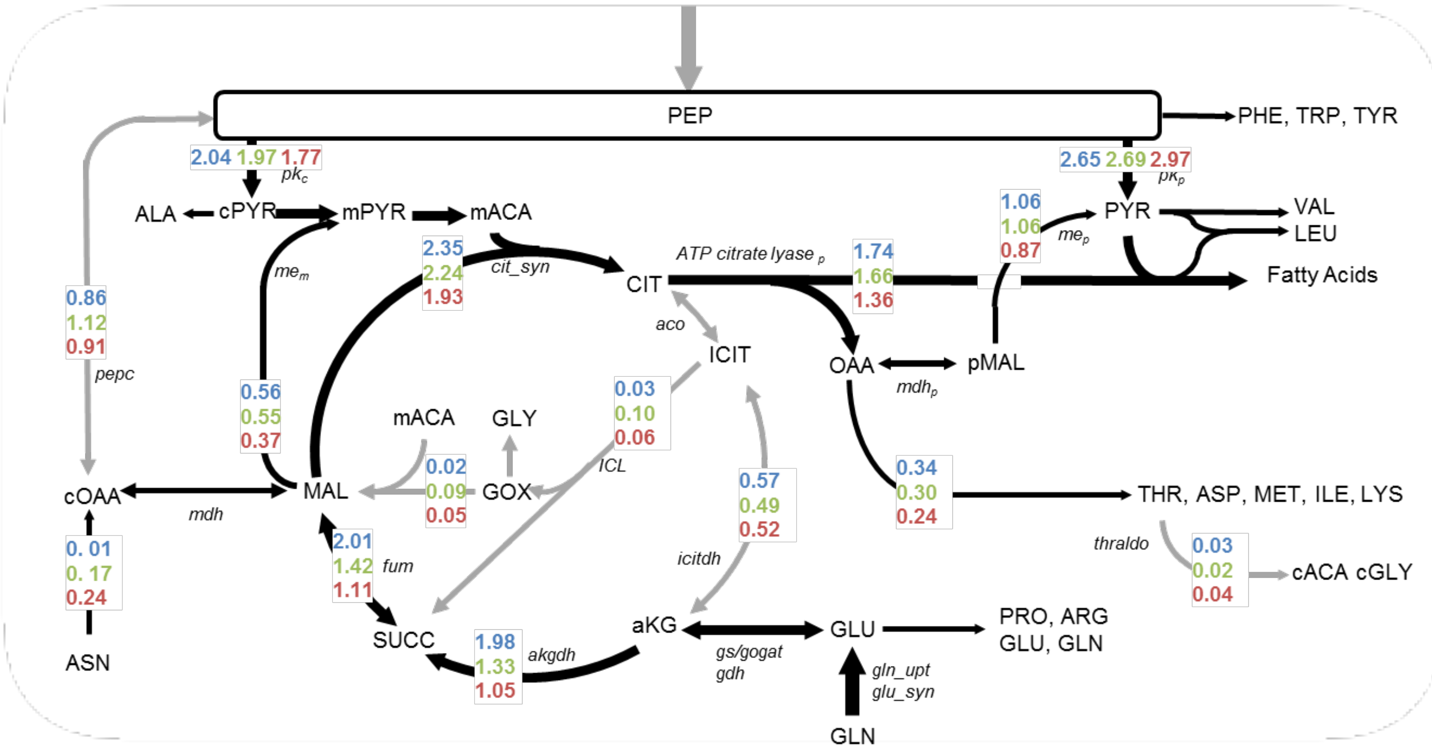
•PEPCK activity frequently unknown

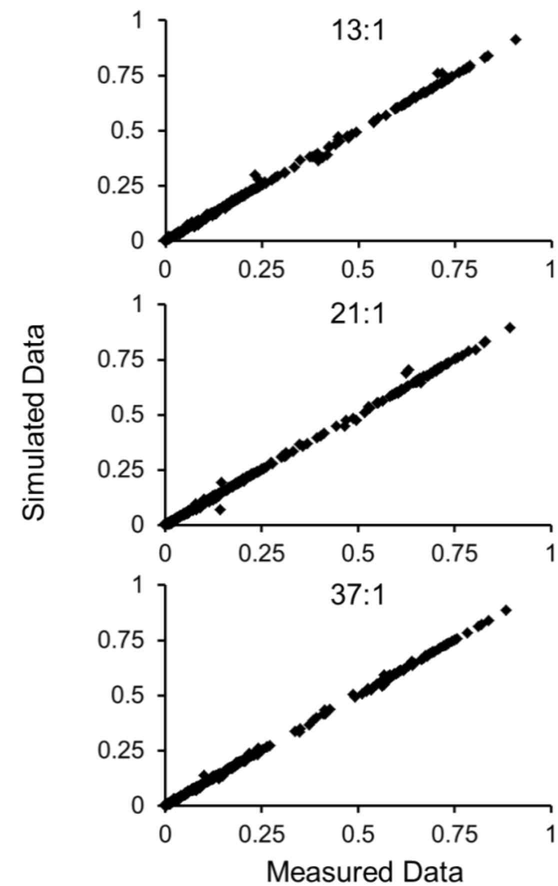
•Modeling results: PEPCK activity is not necessary for fit

Models Considered



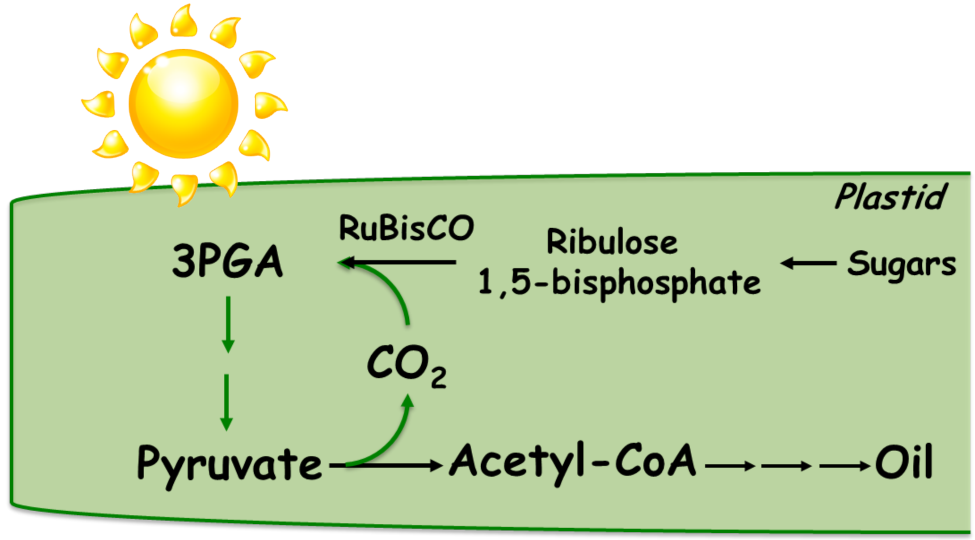
Fluxes vary for C:N ratios



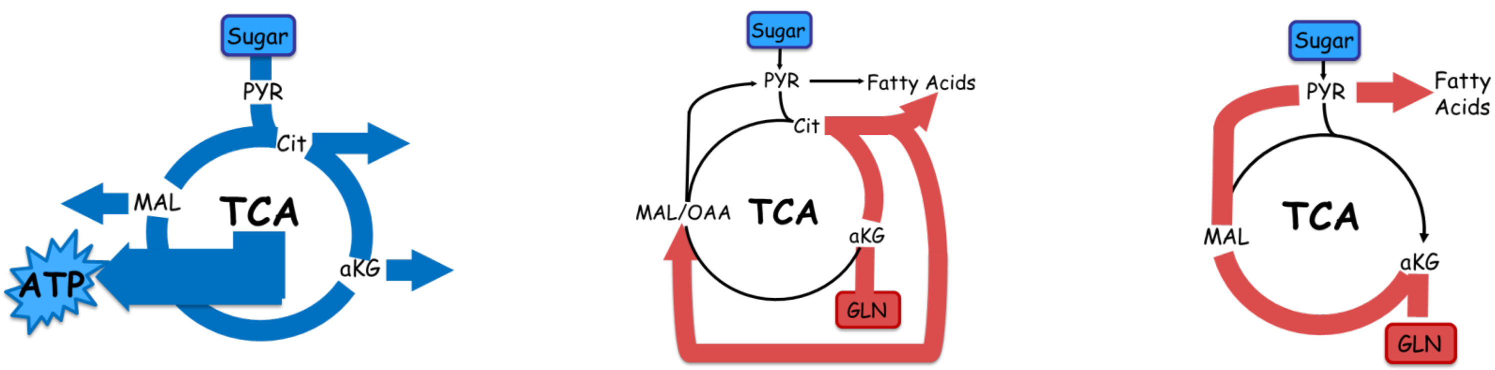


Metabolism in Green Oilseeds

- <u>Hypothesized Roles for Light:</u>
  - Prevent hypoxia
  - Produce NADPH, ATP
  - Improve carbon use efficiency



- <u>Altered Tricarboxylic Acid Cycles:</u>
  - Cyclic vs. non-cyclic
  - Citrate export for cytosolic acetyl-CoA
  - Amino acids supplied maternally



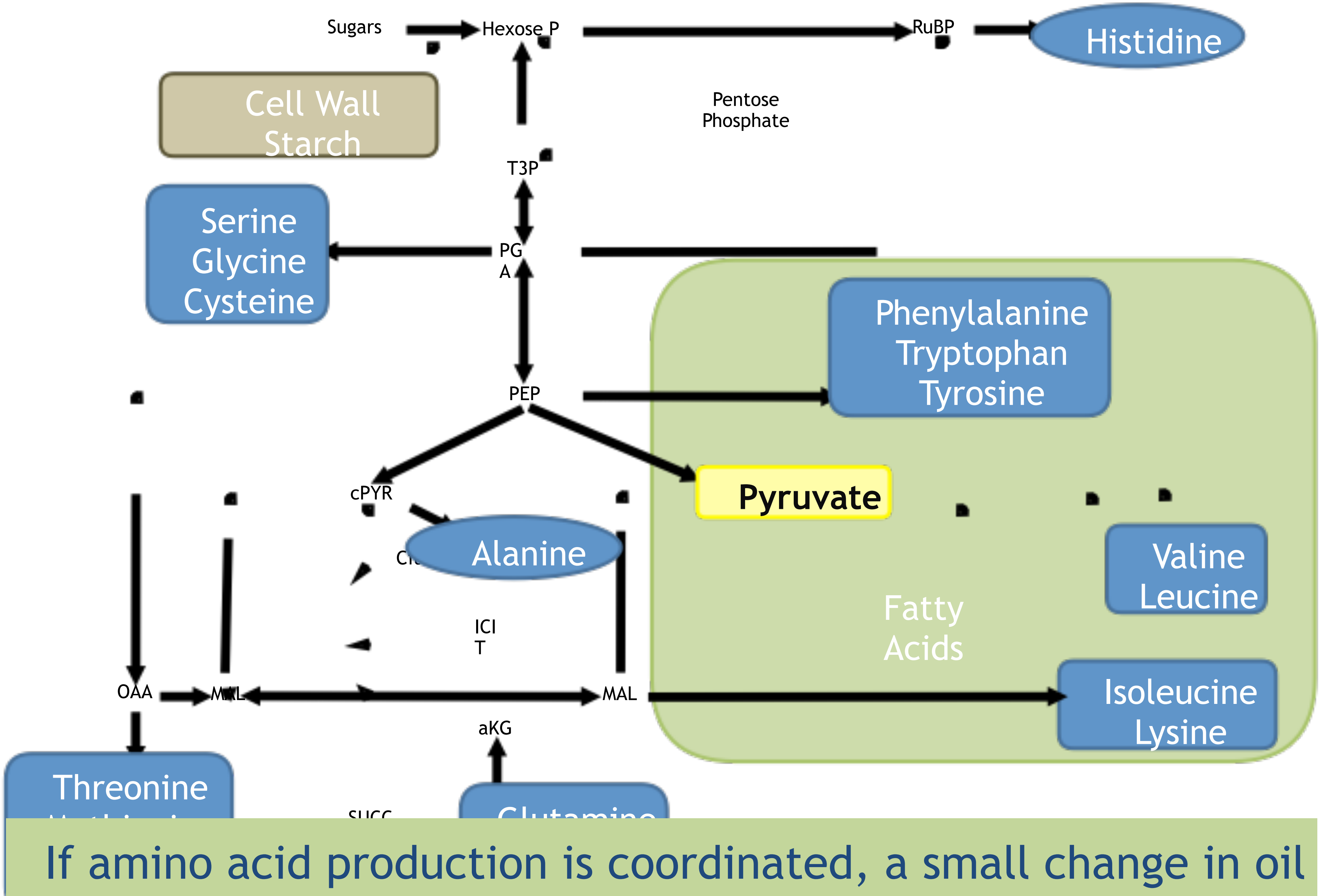
14C Labeling Experiments (Balancing Carbon)

C:N Ratio	Biomass growth (mg day^{-1} embryo^{-1})
13	5.0 ± 1.3
21	4.8 ± 0.7
37	5.1 ± 0.4

Protein (DPM) ↓	Oil (DPM) ↑
27809 ± 2684	9279 ± 513
24324 ± 4217	10523 ± 717
20860 ± 1070	14163 ± 1273

- Oil production changes inversely with protein on a carbon basis
- Competition for carbon

Pyruvate Carbon Used for Oil or Protein



If amino acid production is coordinated, a small change in oil will have more dramatic impact on protein levels

Summary

- Oligosaccharides have been successfully diverted to sucrose
- To move sucrose to protein or oil will require explorations in central metabolism
- Can the insoluble fraction within a seed be reduced?
- Protein and oil are inversely tied, possibly through the node of pyruvate

Acknowledgements

doug.allen@ars.usda.gov

<u>Open Positions:</u>
Danforth Post Doc
USDA Biol Scientist

USDA
United States Department Of Agriculture
Agricultural Research Service

DONALD DANFORTH
PLANT SCIENCE CENTER
DISCOVERY | COMMUNITY | IMPACT

Lab Members/Alumni:

Matt Juergens
Partha Ghosh
??
??

Alumni:

Stephen Plassmeyer
Wenyu Yang
Jia Li
Fangfang Ma
Thuy Truong
James Gierse
Christine Diepenbrock

Collaborators:

Jan Jaworski (DDPSC)
Phil Bates (USM)
Kent Chapman (UNT)
Young-Jin Lee (ISU)
Sam Wang (DDPSC)
John Ohlrogge (MSU)
Jamey Young (Vanderbilt)
Tom Brutnell (DDPSC)
Mel Oliver (USDA)
Jan Miernyk (USDA)
Brad Evans (DDPSC)
Yinjie Tang (WUSTL)
Pramod Wangikar (IIT)

NSF

U.S. DEPARTMENT OF ENERGY

arpa·e
Advanced Research Projects Agency • ENERGY