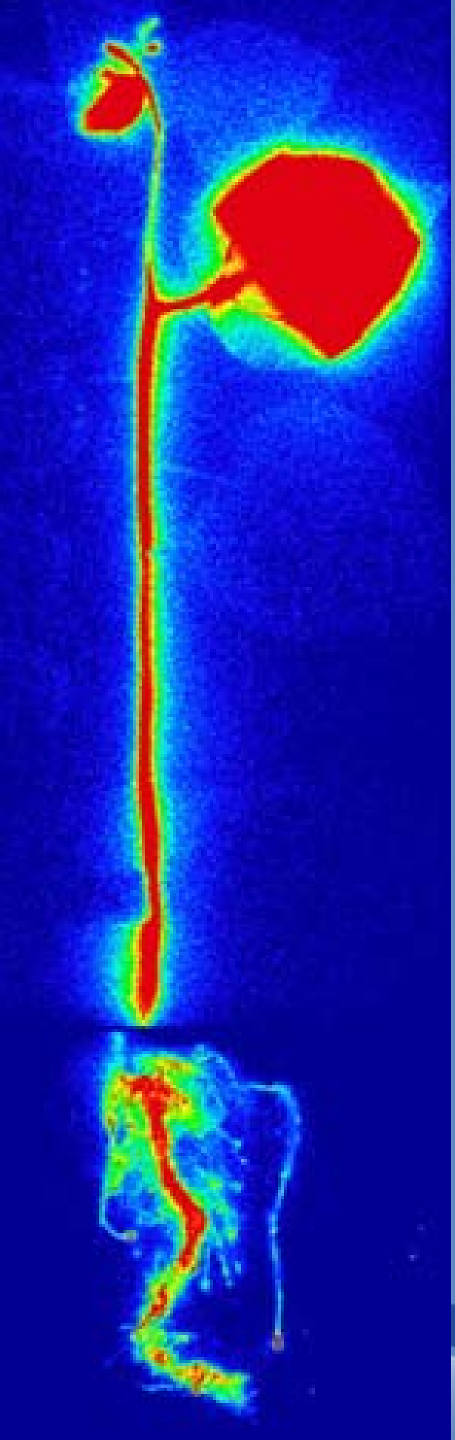


PET Plants: Imaging Natural Processes for Renewable Energy from Plants

Benjamin A. Babst

Goldhaber Postdoctoral Fellow

Medical Department

Plant Imaging

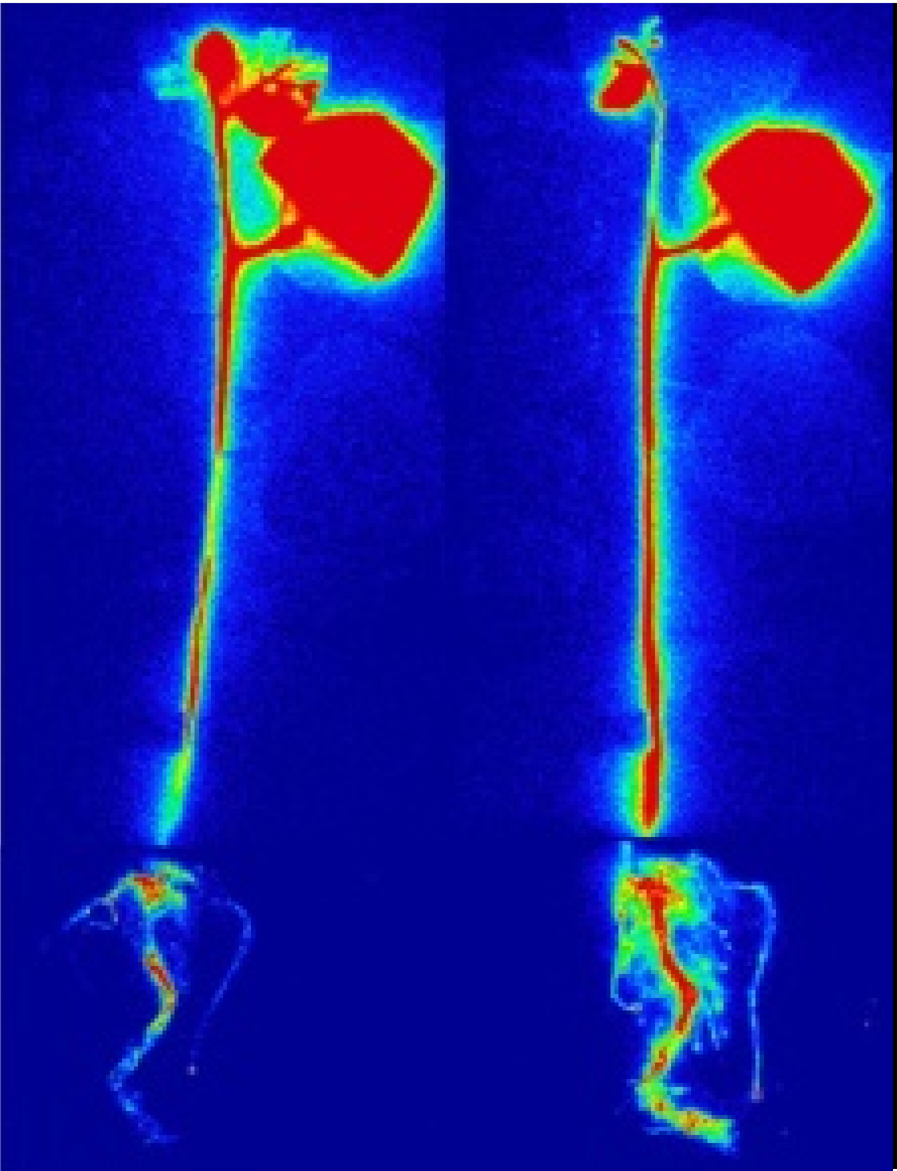
BROOKHAVEN NATIONAL LABORATORY

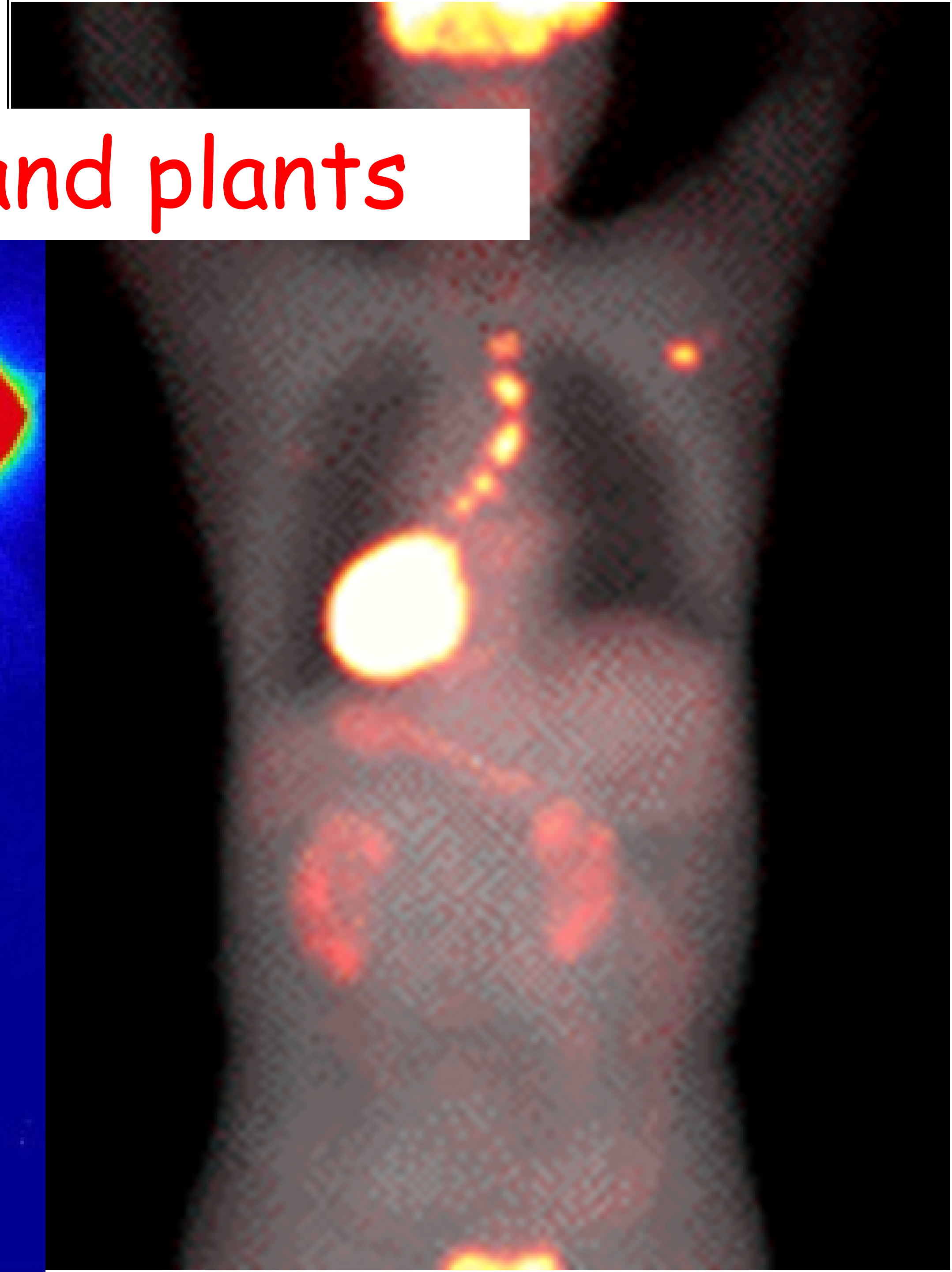
a passion for discovery

Office of Science

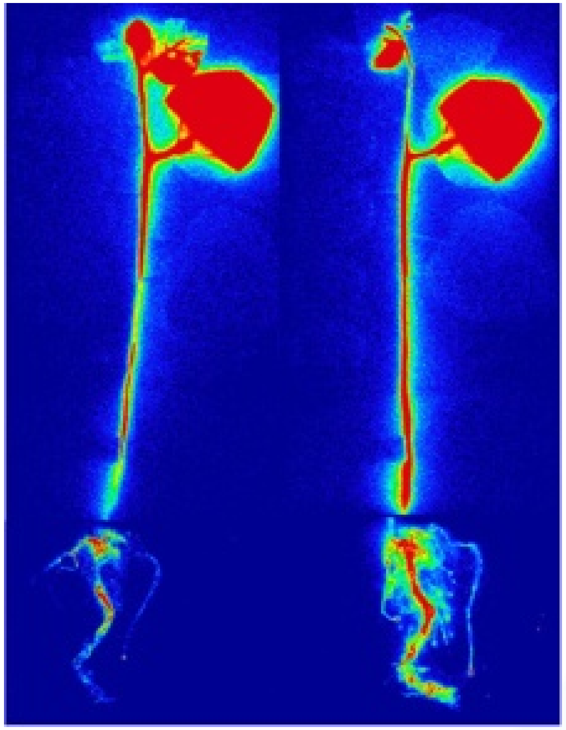
U.S. DEPARTMENT OF ENERGY

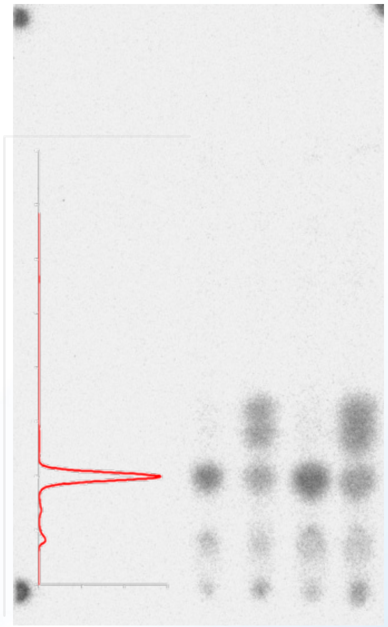
PET imaging for medicine and plants





Brookhaven's Unique Capabilities

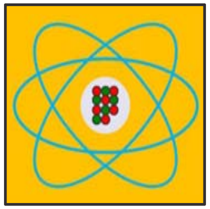




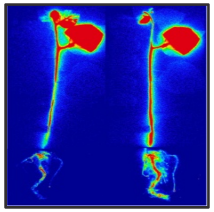
Movement, distribution, and metabolism of molecules in plants.

Outline

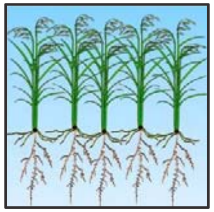
Bioenergy to mitigate energy crisis



PET tools & imaging



Plant resource movement



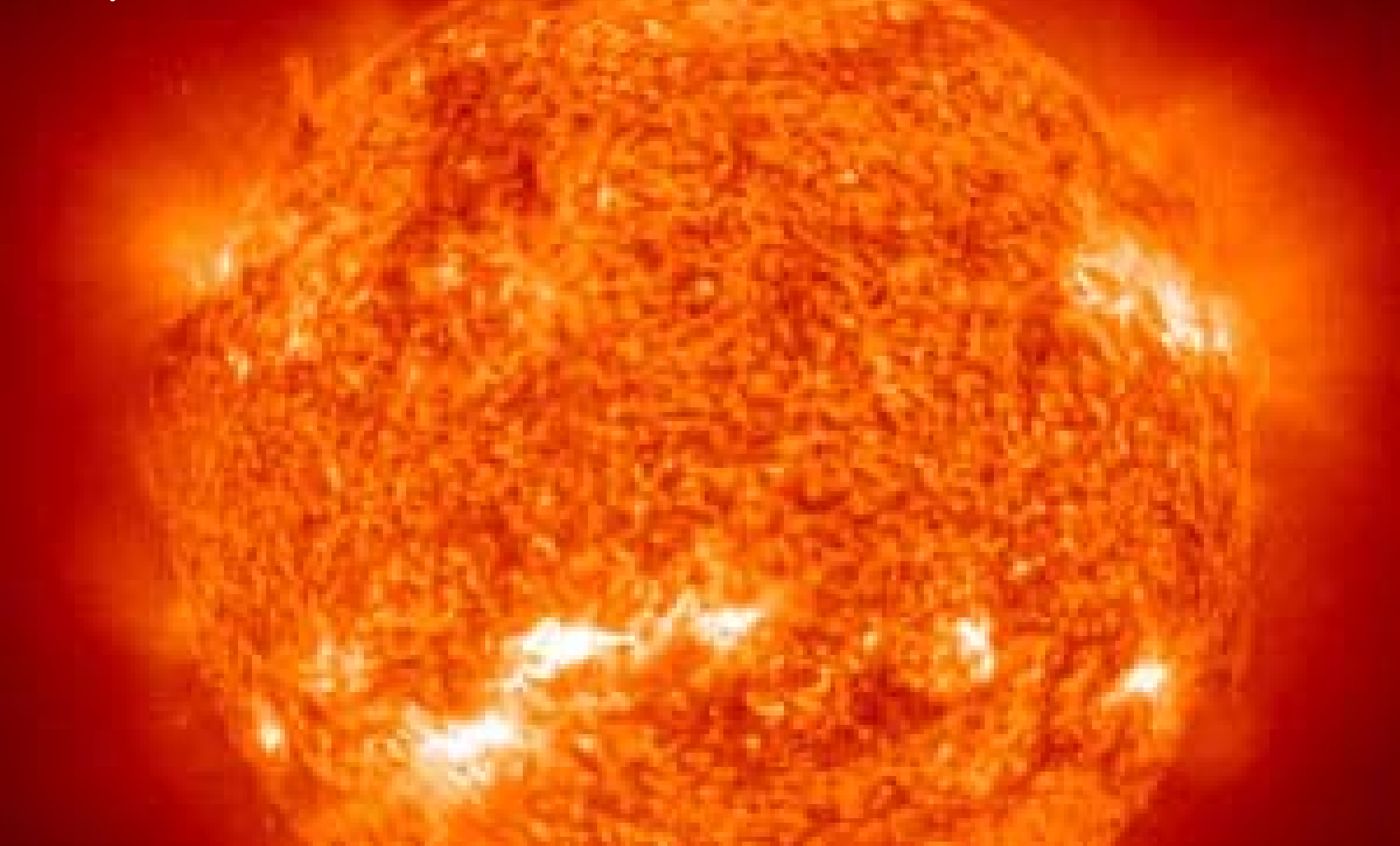
Plant signaling

Importance of Energy

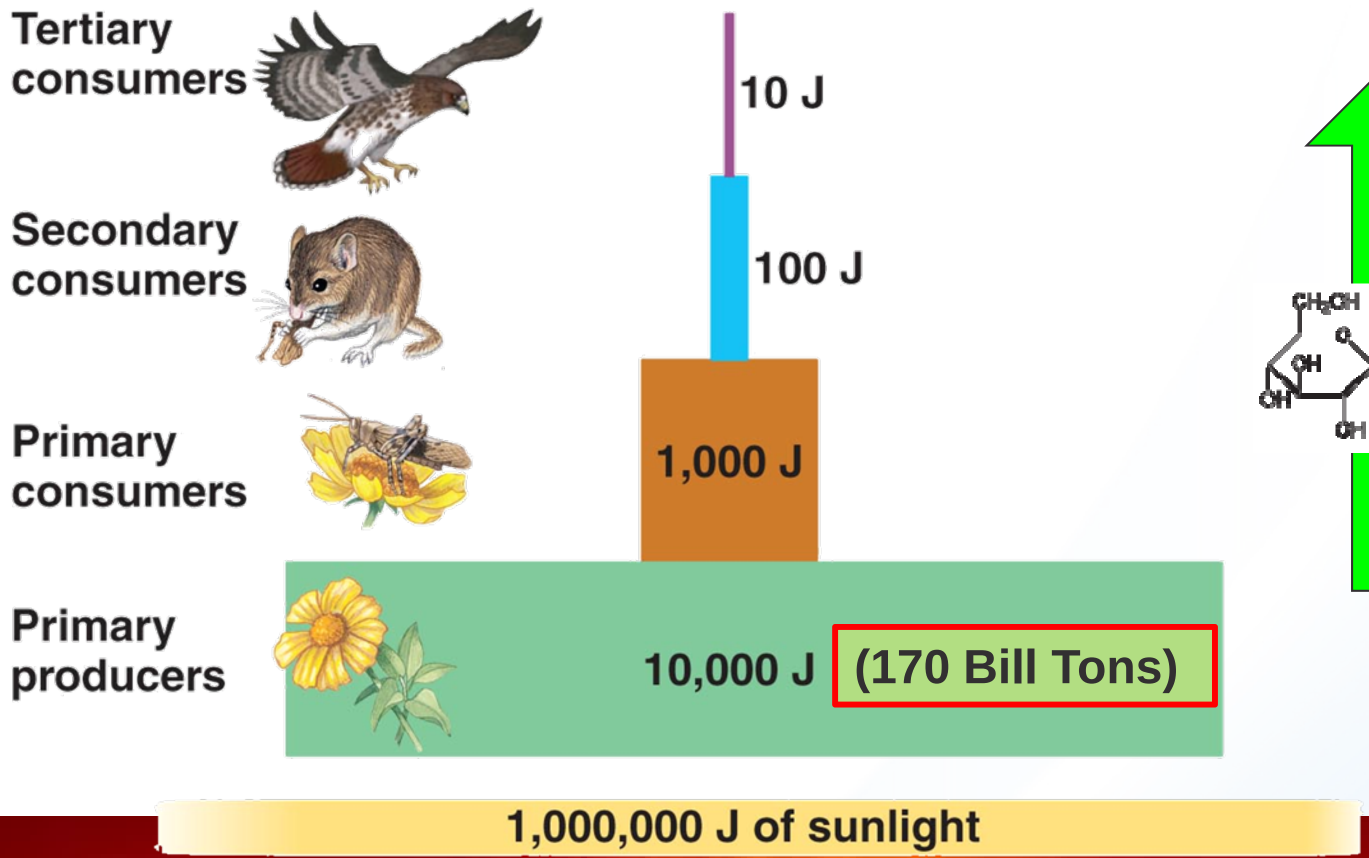
Economy

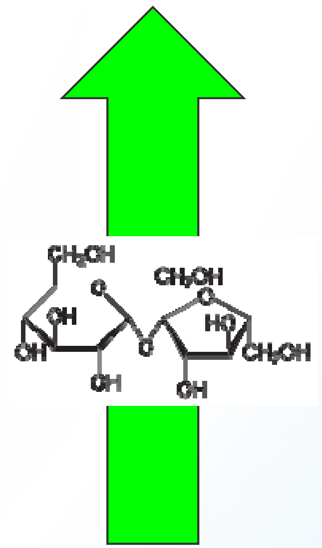
All energy comes from the sun.

^ Almost

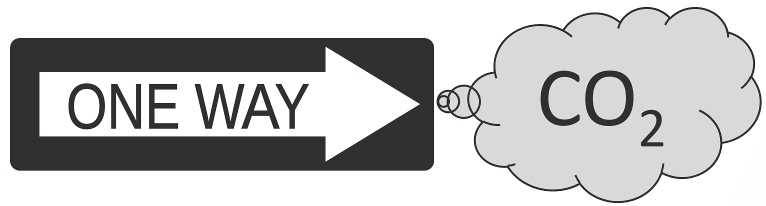


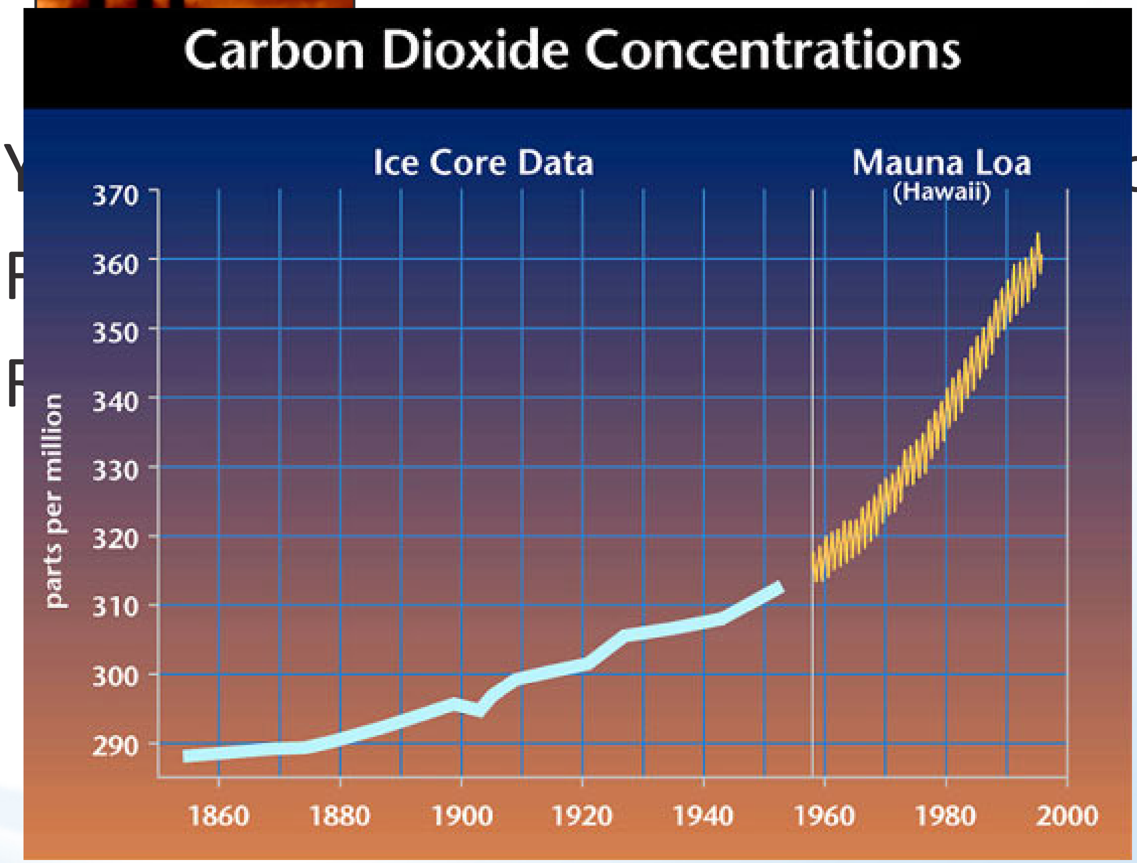
Energy in Ecosystems





Fossil Fuel Dilemma





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- F
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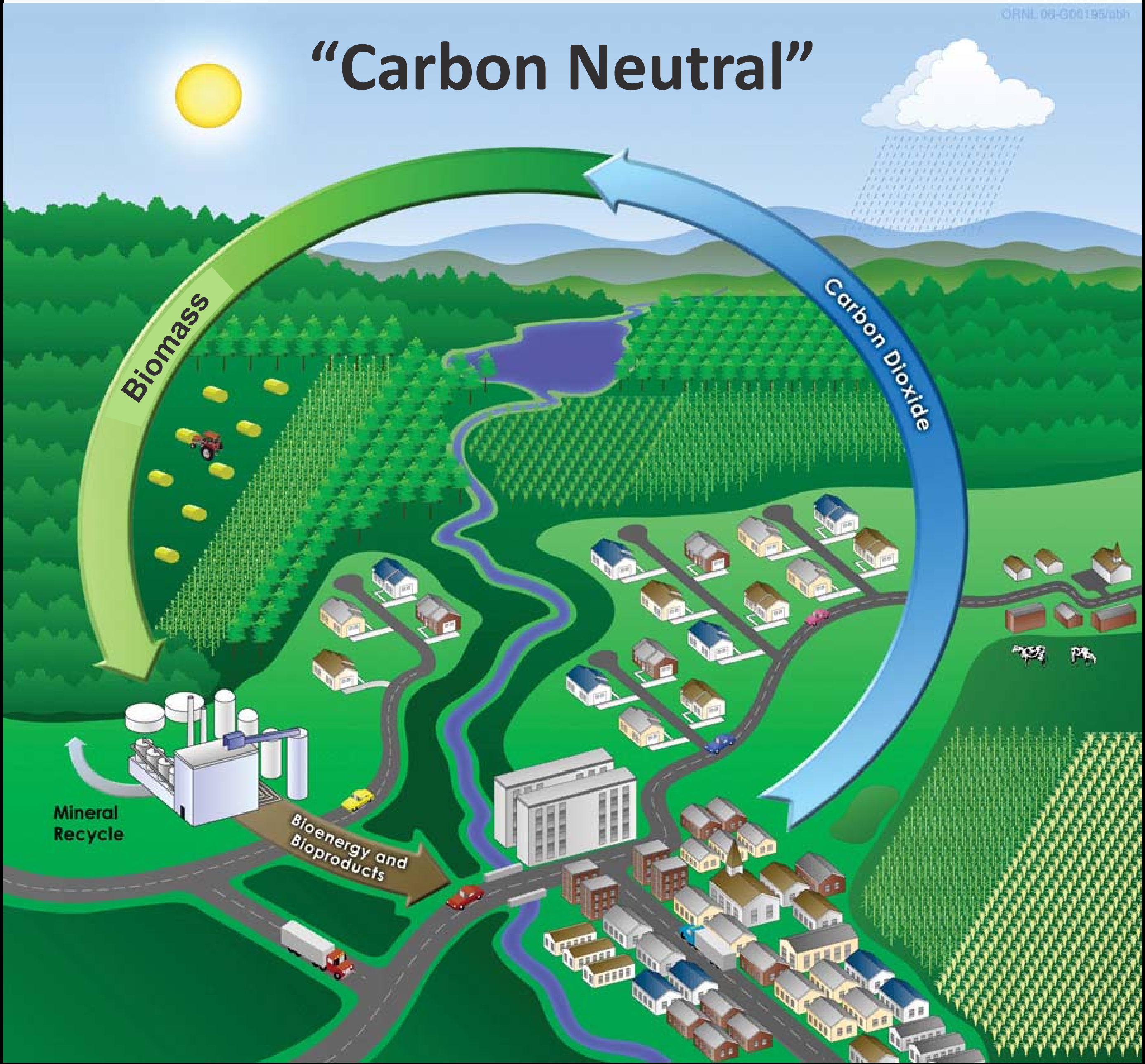
Bioenergy


"Carbon Neutral"
Biomass
Carbon Dioxide
Mineral Recycle
Bioenergy and Bioproducts
ORNL 06-G00195/abh

What is biomass?

- **Edible biomass**
  - sugars (sugar cane)
  - starches (corn)

- **Inedible biomass:** fibrous or woody parts (cellulosic or lignocellulosic biomass)

What is biomass?

- **Edible biomass**
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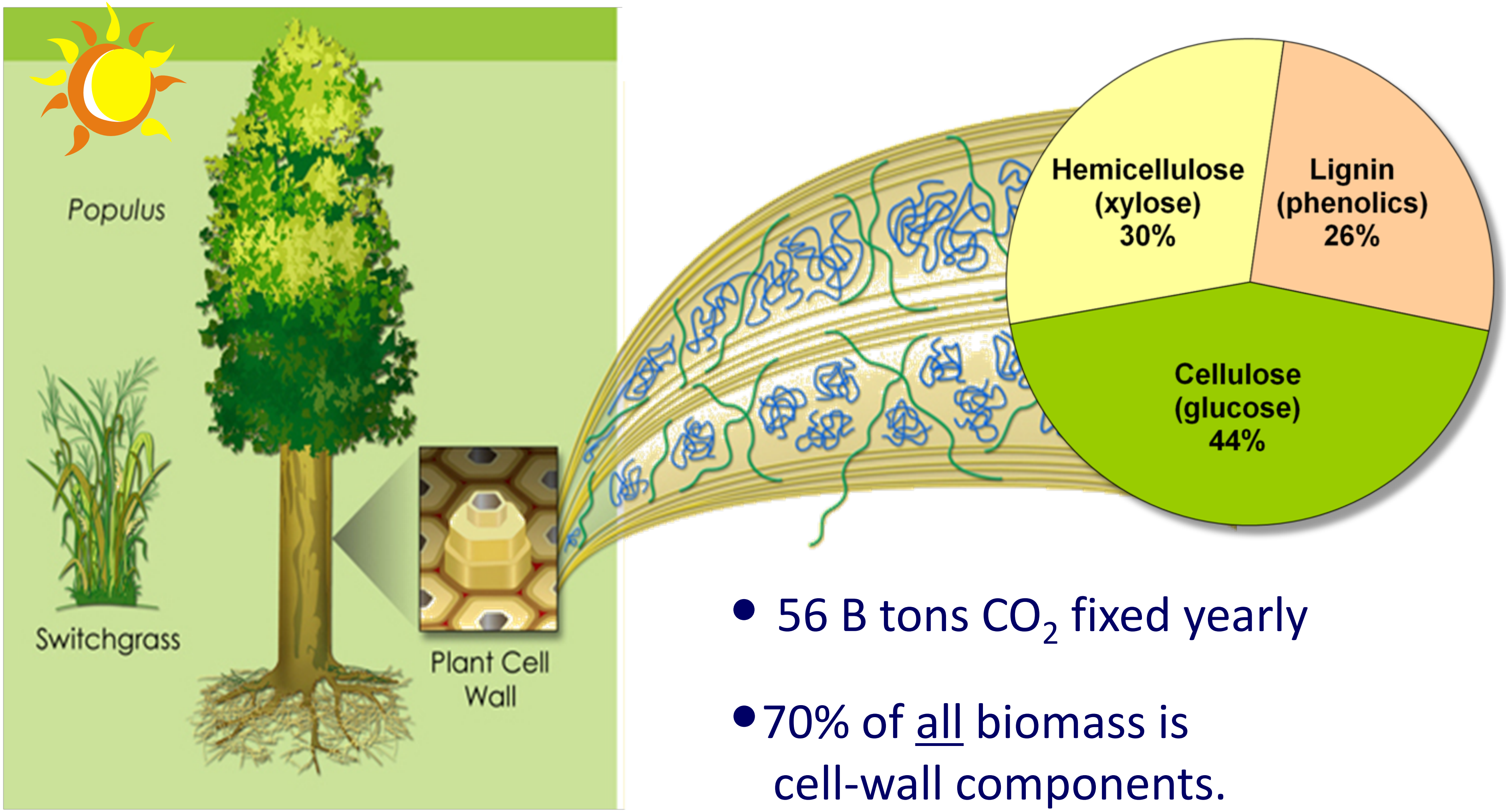
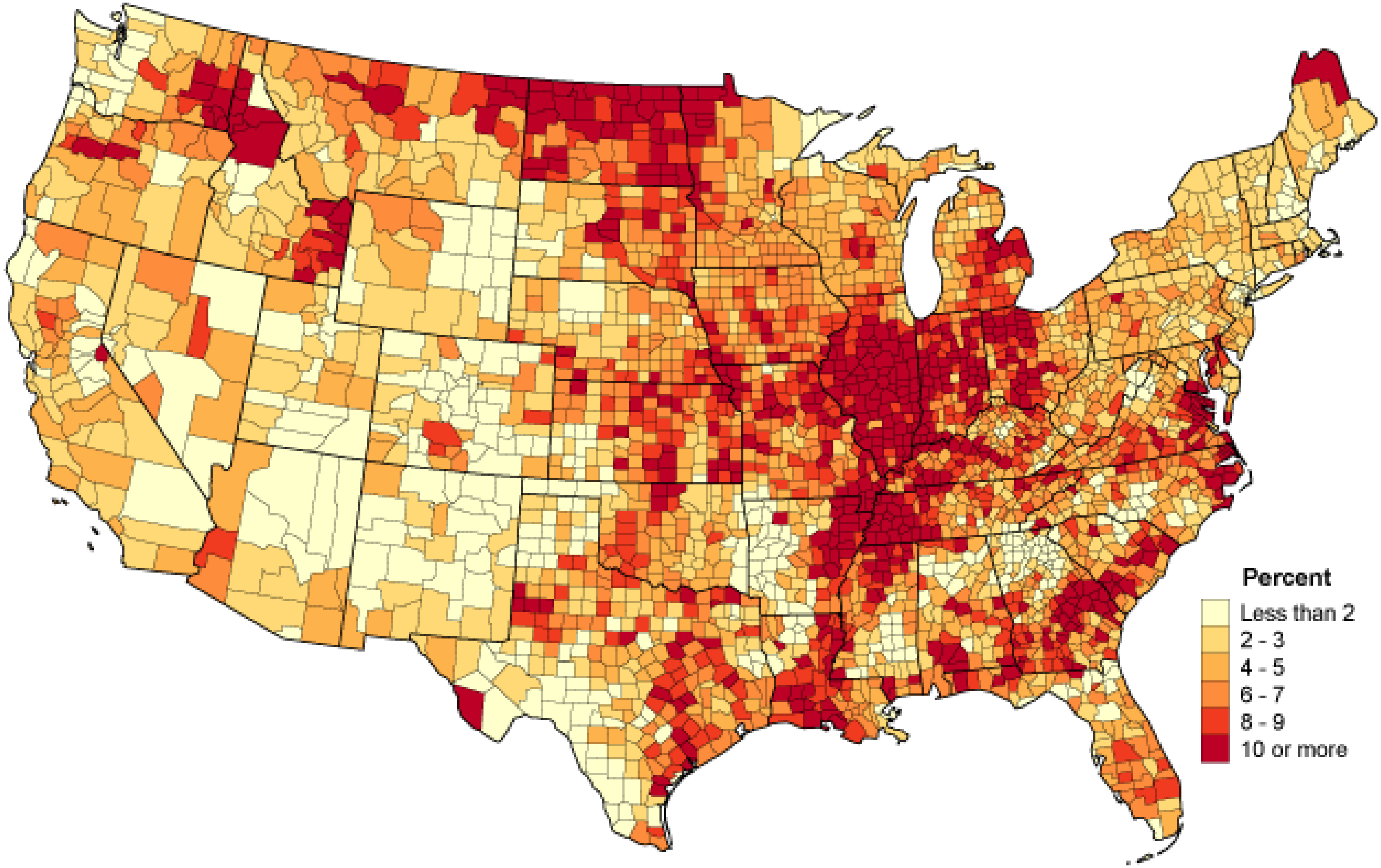
Inedible biomass – *how*?



- 56 B tons CO_2 fixed yearly
- 70% of <u>all</u> biomass is cell-wall components.

Displace Prime Farm Lands?



"…there is potential for using… **marginal lands** for producing biomass feedstock." – USDA-DOE Billion Ton Update

Big picture challenges: Technology

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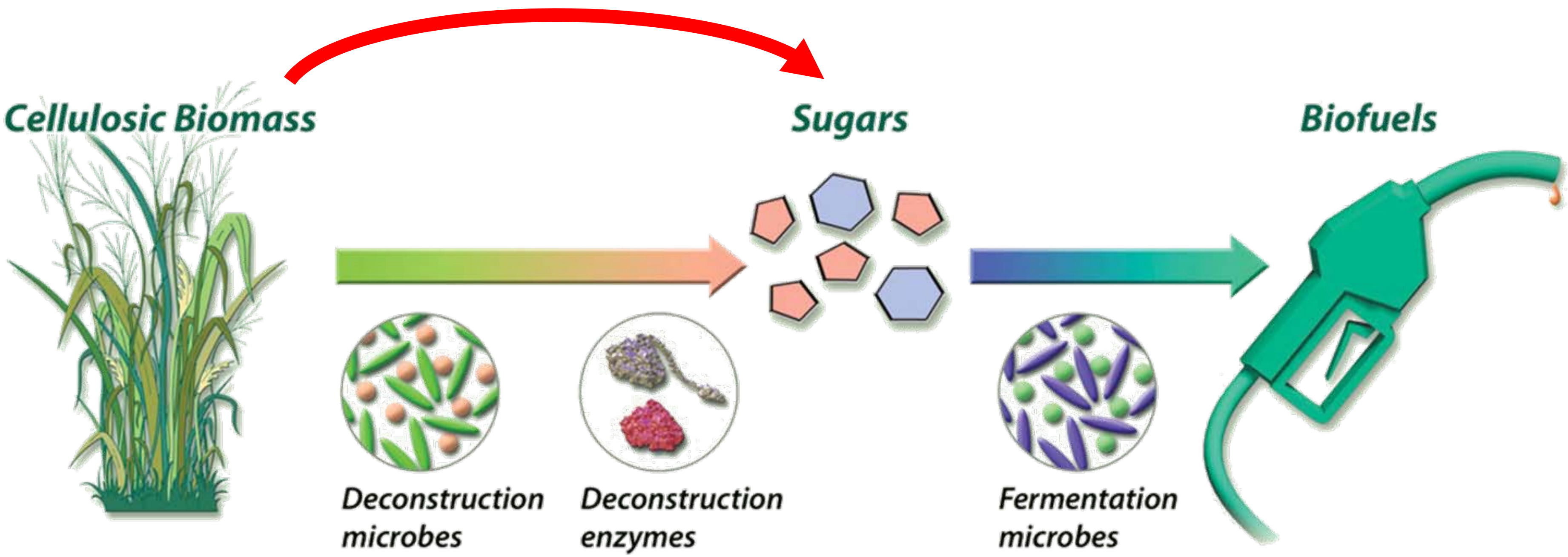
Big picture challenges: Technology

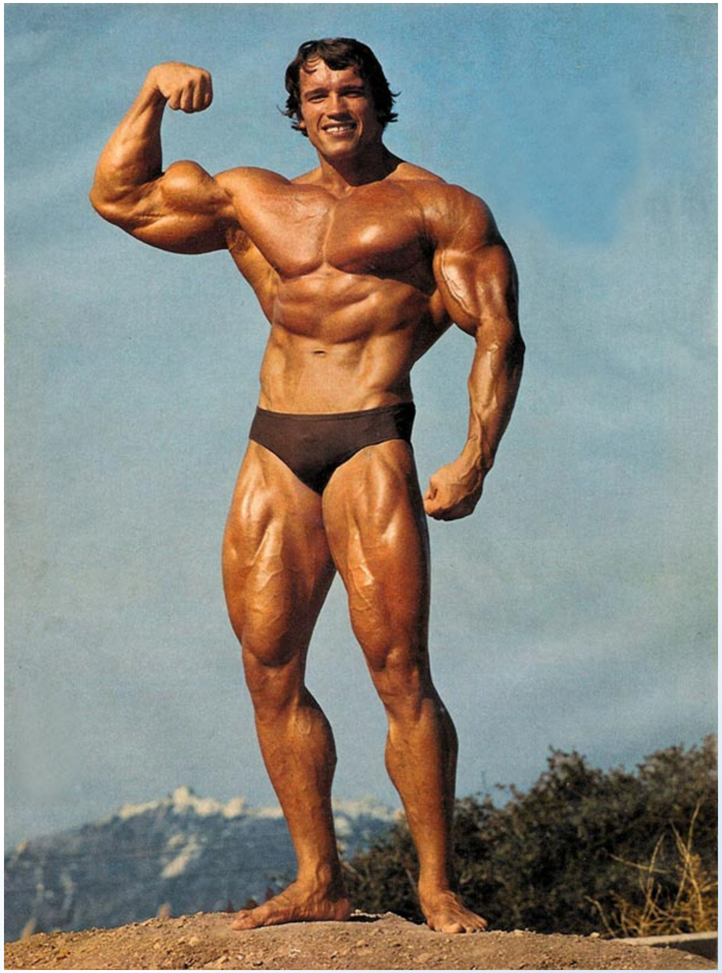


*** Develop crops dedicated to biofuel production

Improve conversion of cell wall to sugars

Optimize conversion of sugar to biofuels

Get resources in the right place, AND make the right stuff



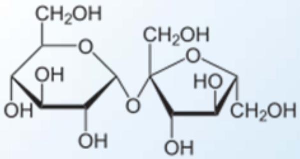
Maximize Grain vs. Biomass?

Green Revolution

Bioenergy Revolution

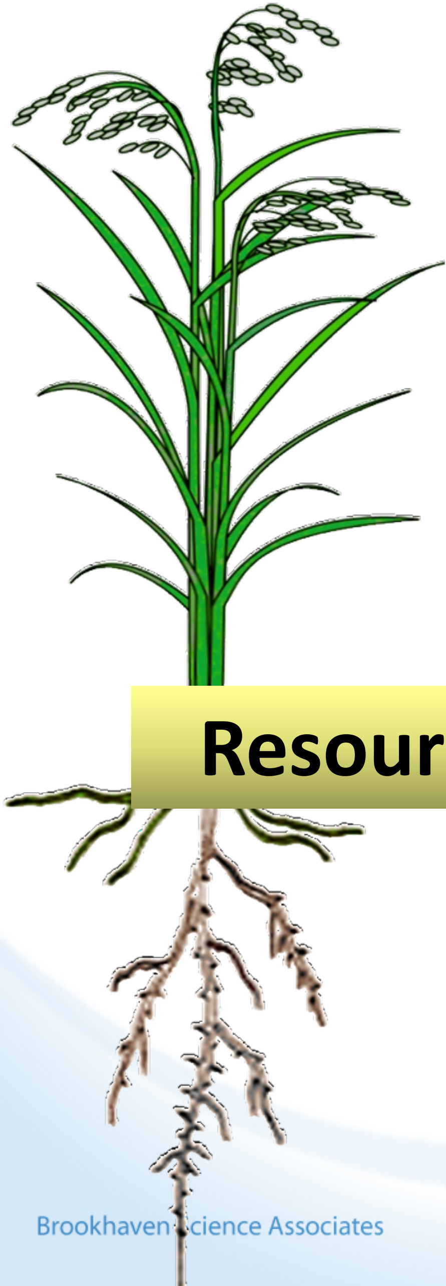
Plant Improvement for Feedstock

- **Fast growth**
- **Stress tolerant**
  - Nutrients
  - Water
  - Salt
- **Pest resistant**
- **Composition for conversion**
  - High cellulose, carbohydrates
  - Low lignin
  - Lipids



Challenge: How to improve plant composition, growth, and robustness?

Grasses as model plants

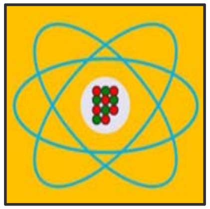


- Fast growth
- Stress tolerance (marginal lands)
- Pest resistant
- Transgenic and mutant lines available
- Composition – high sugar varieties

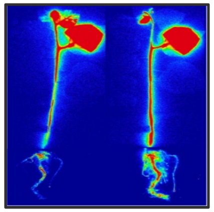
Resource Allocation & Chemical Partitioning

Outline

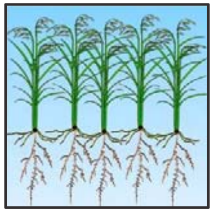
Bioenergy



PET tools & imaging



Plant resource movement



Plant signaling

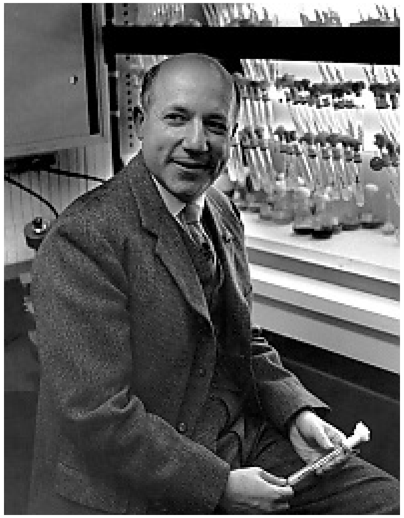
Radio-isotopes

	* ^{11}C	^{12}C	^{13}C	* ^{14}C
Electrons	6	6	6	6
Protons	6	6	6	6
Neutrons	5	6	7	8
	unstable	stable	stable	unstable

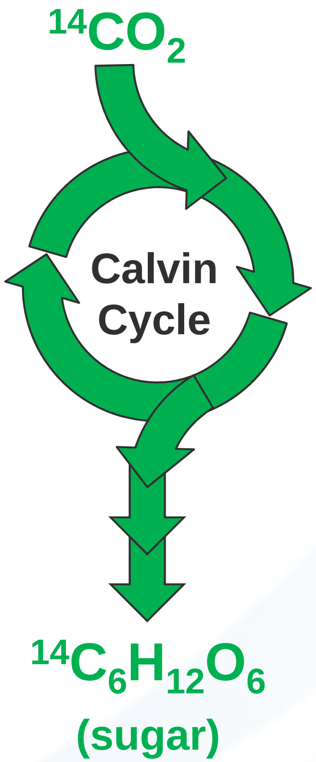
- Same number of protons & electrons
- Chemically identical
- Different number of neutrons

Radiotracers in plant biology, historically

Carbon-14



Melvin Calvin—
1961 Nobel Prize in Chemistry



Martin Gibbs —
BNL 1949-1957

Higher Sensitivity & Non-invasive

carbon-11 | carbon-14

$^{11}CO_2$ | $^{14}CO_2$

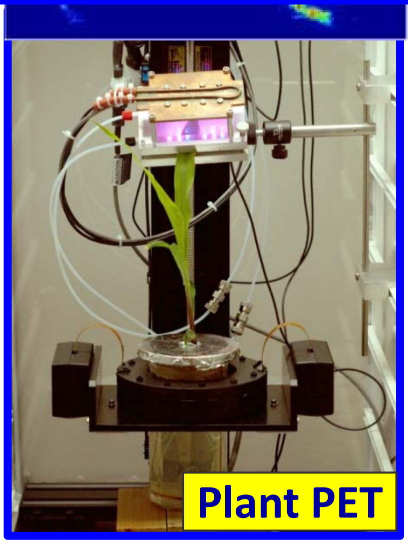
10^{-15} g | 10^{-6} g

1 μCi

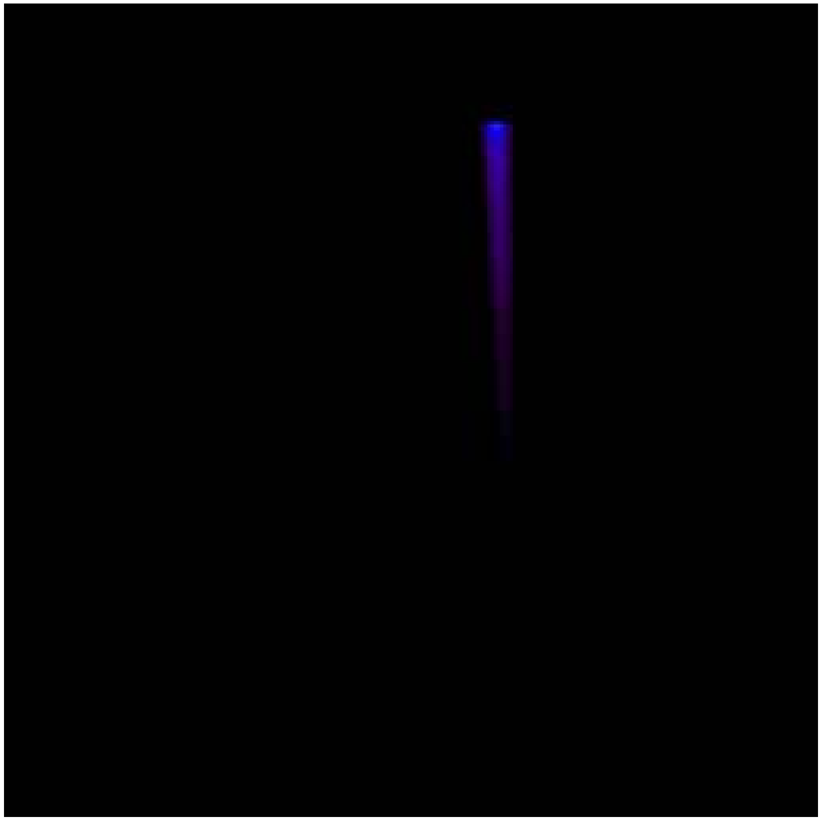
PET Scientific Tools

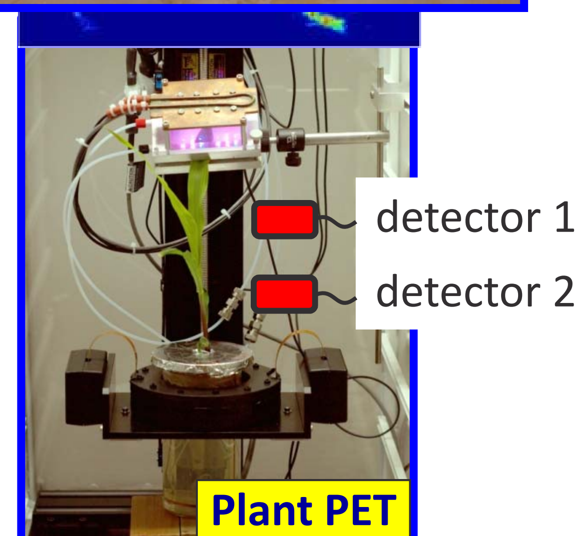
Isotope	Half-life
carbon-11	20.4 min
nitrogen-13	10 min
oxygen-15	2 min
fluorine-18	110 min

Plant Science

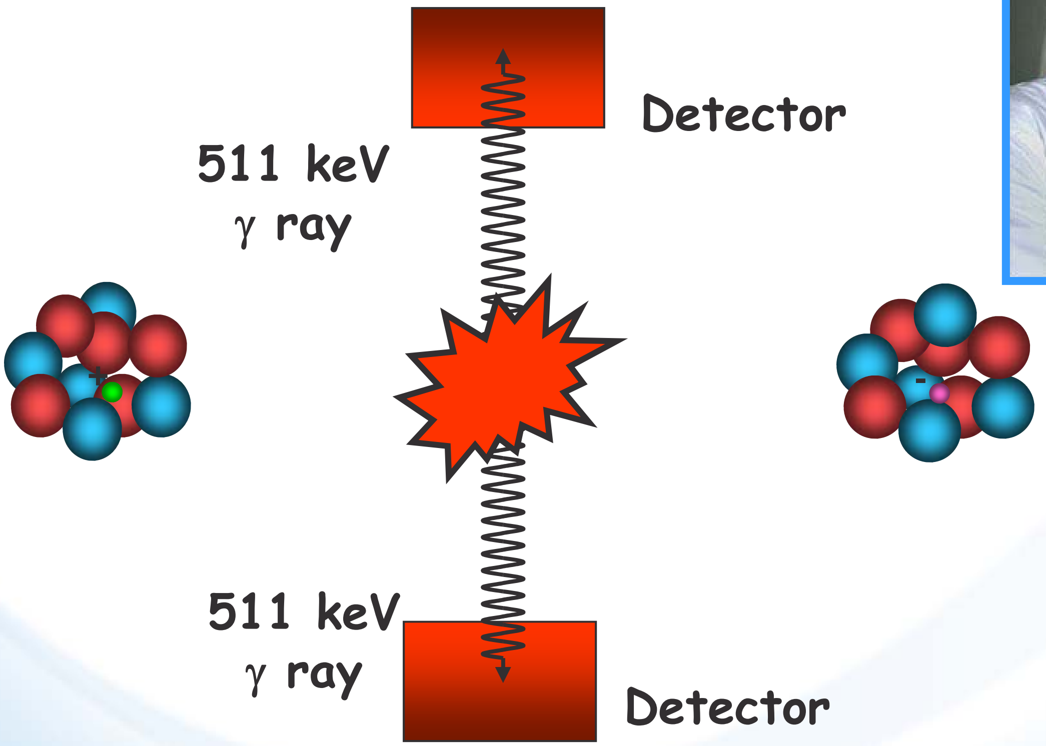


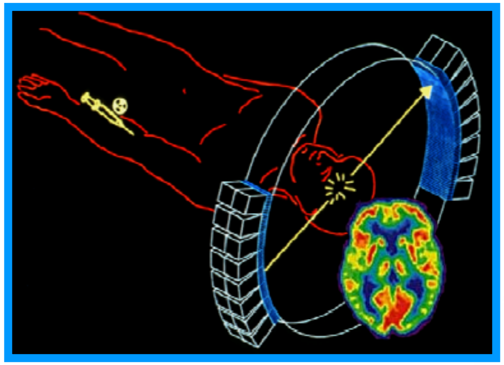
PET Scientific Tools

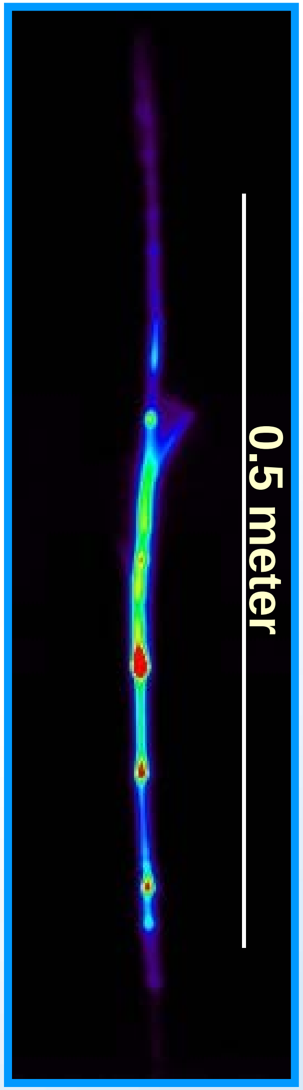




Positron Emmission Tomography (PET)

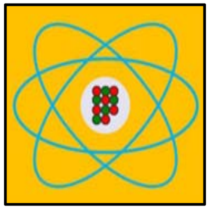




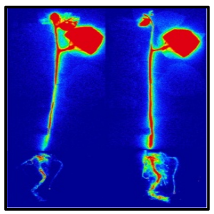


Outline

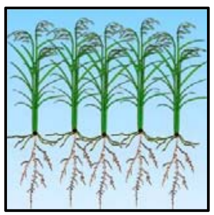
Bioenergy



PET tools & imaging

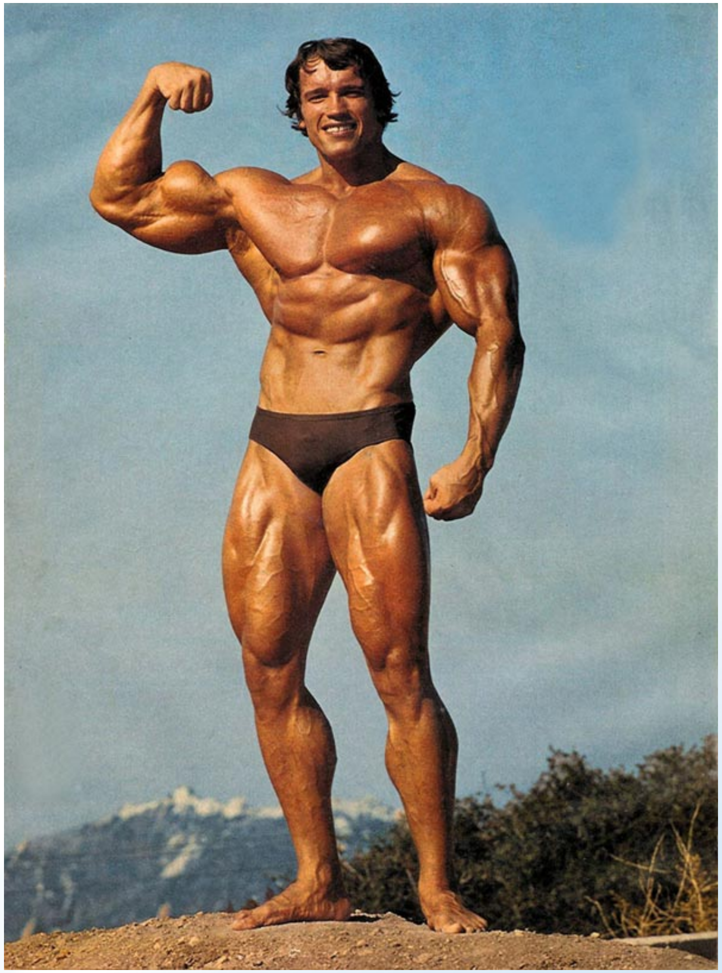


Plant resource movement

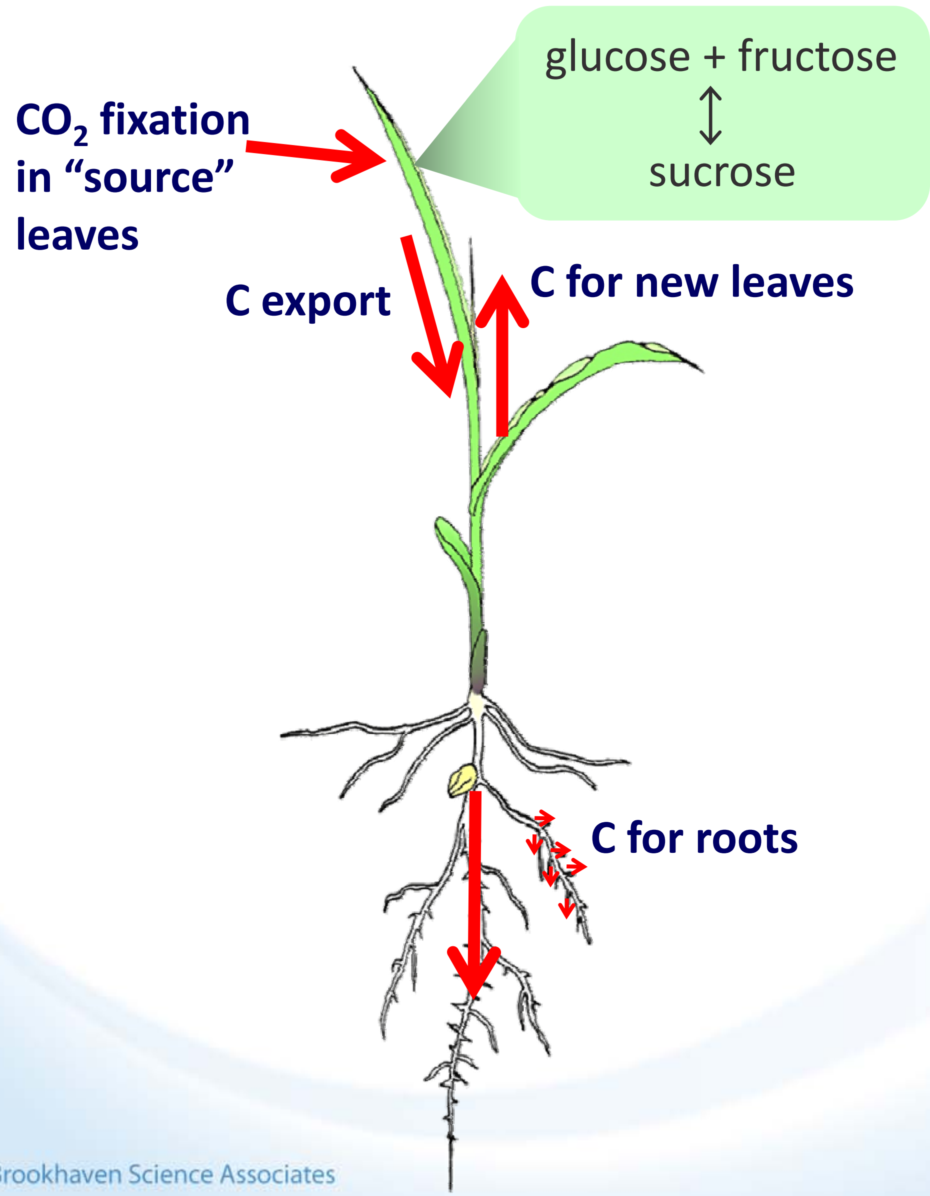


Plant signaling

Get resources in the right place, AND make the right stuff

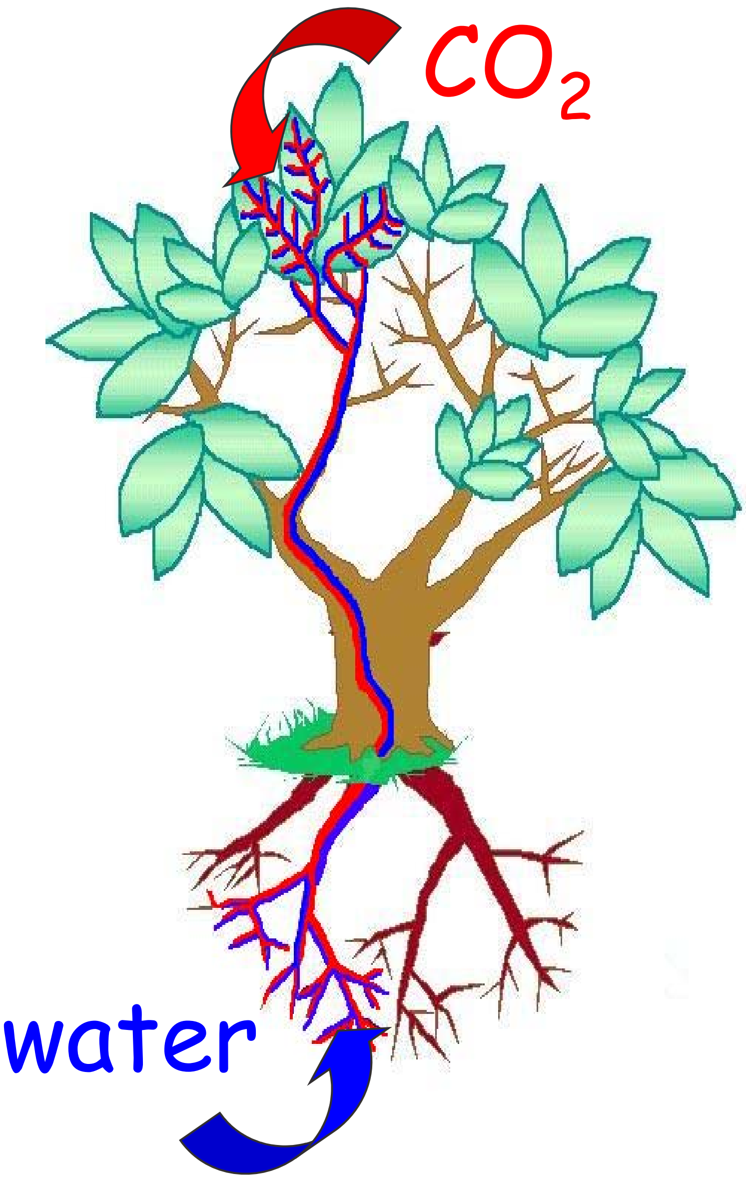


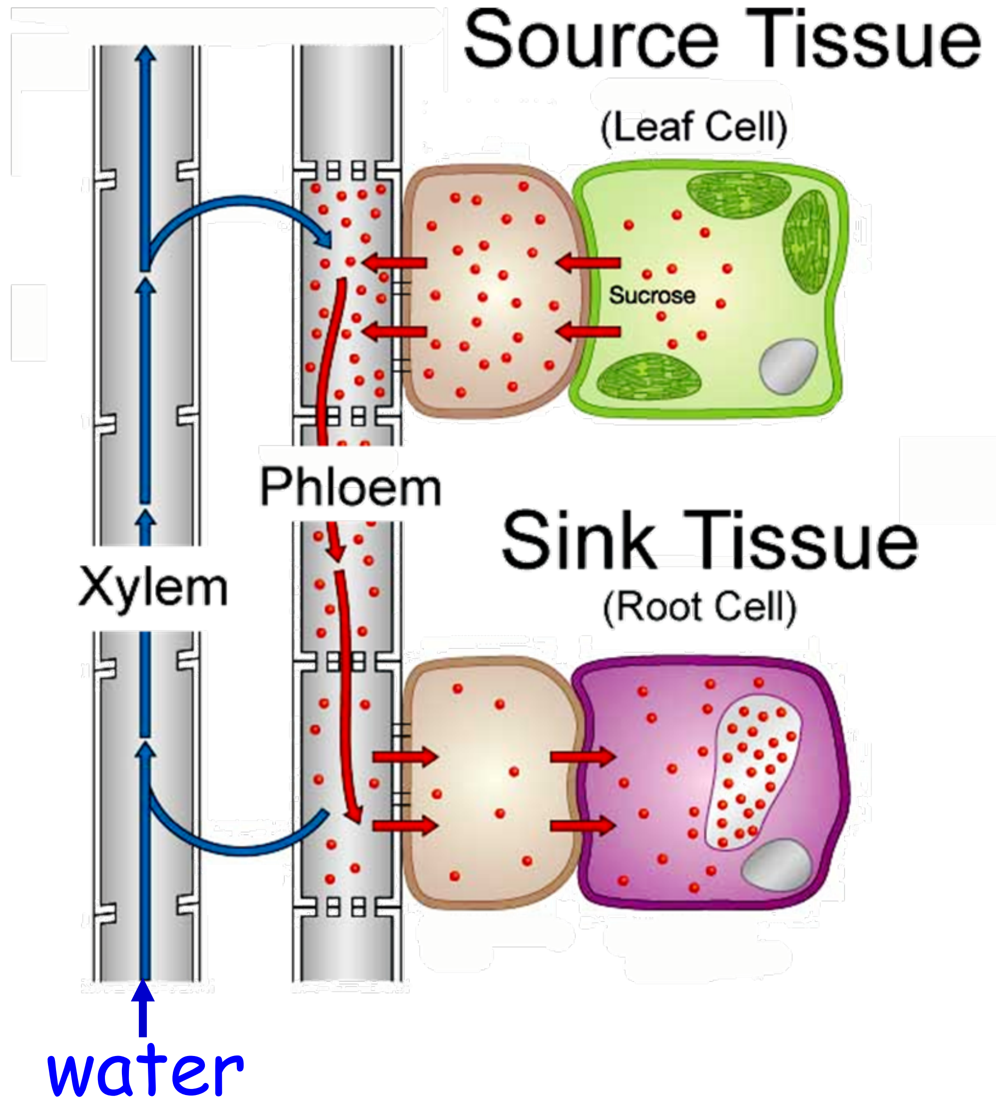
Carbohydrate Transport



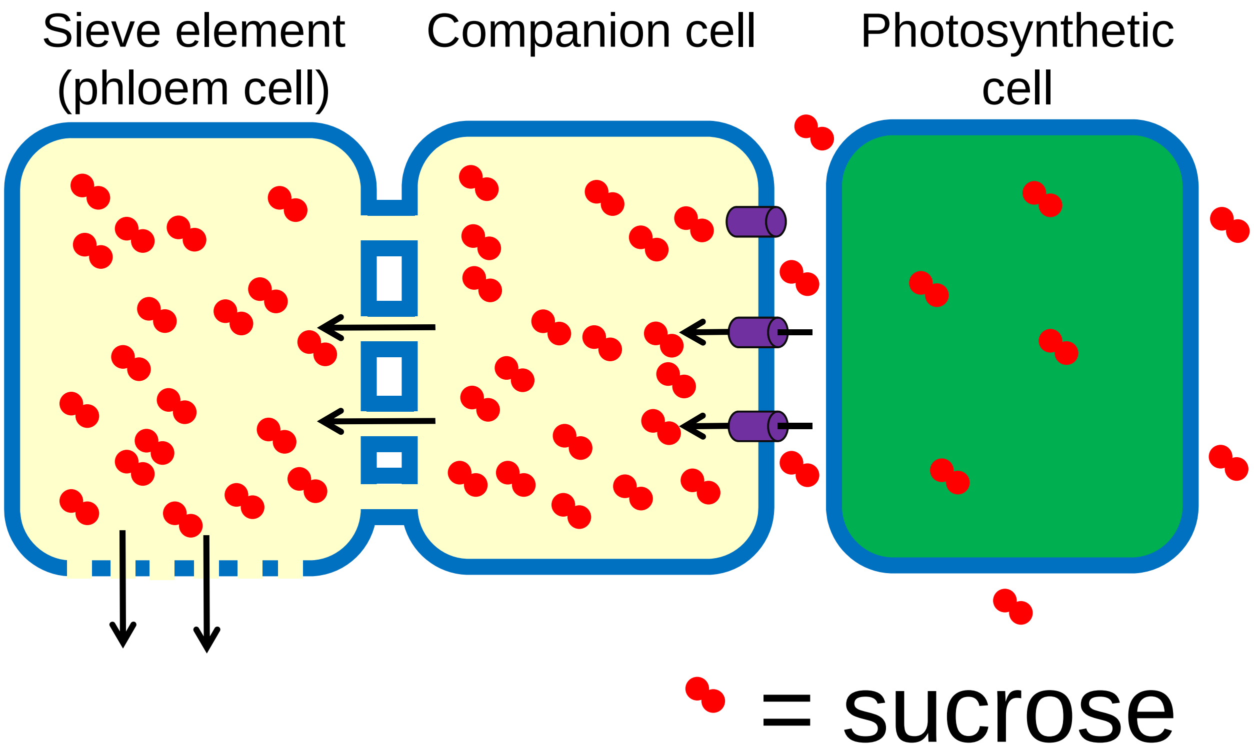
- **Growth of “sinks”**
  - Stem
  - New leaves
  - Roots
- **Cellulosic biomass**
- **Bioproduct yield**
- **C *drives* transport**

Mass-Flow Model: Ernst Münch 1930





Sugar loading into Phloem

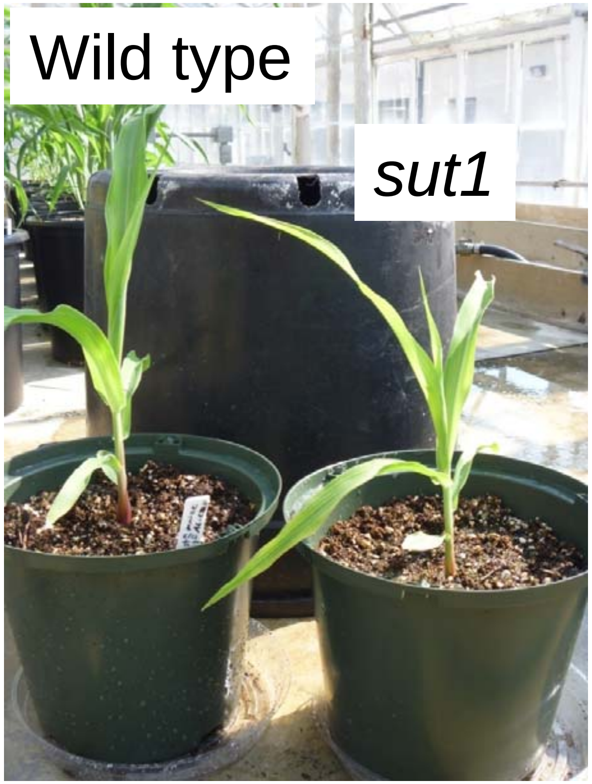


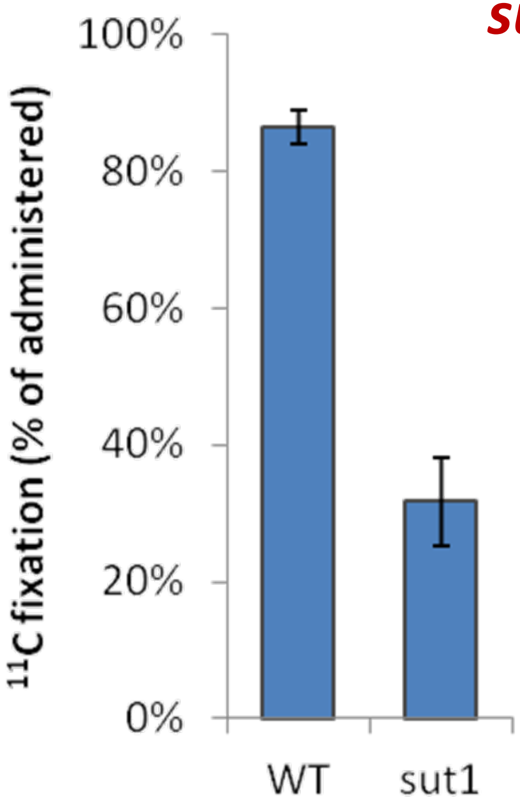
Sucrose transporter (SUT)

- Genetic model: *Zea mays*
- *Zea mays*: 7 sut genes

Sucrose transporter 1 mutant (*sut1*):

Carbohydrate export from leaves



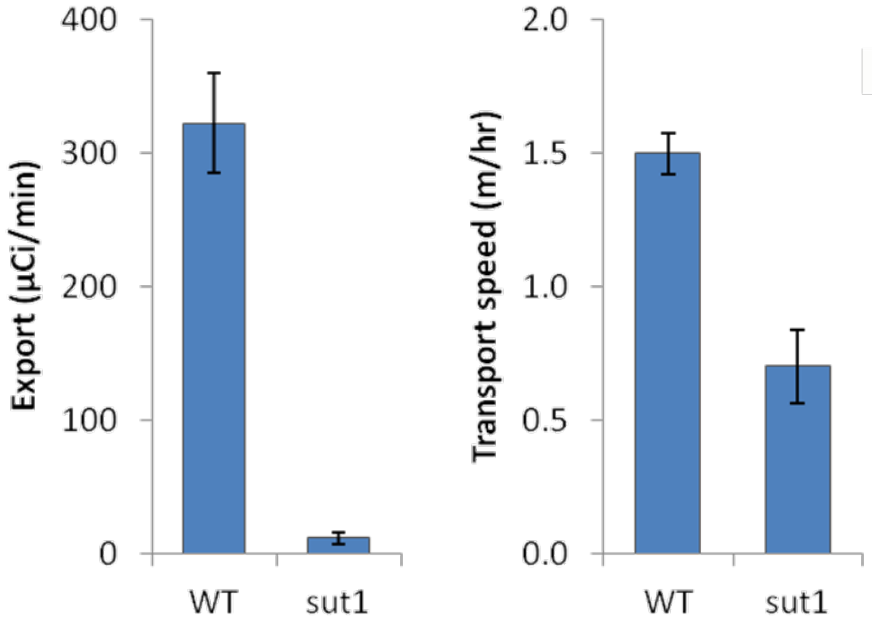


sut1 mutant phenotypes:

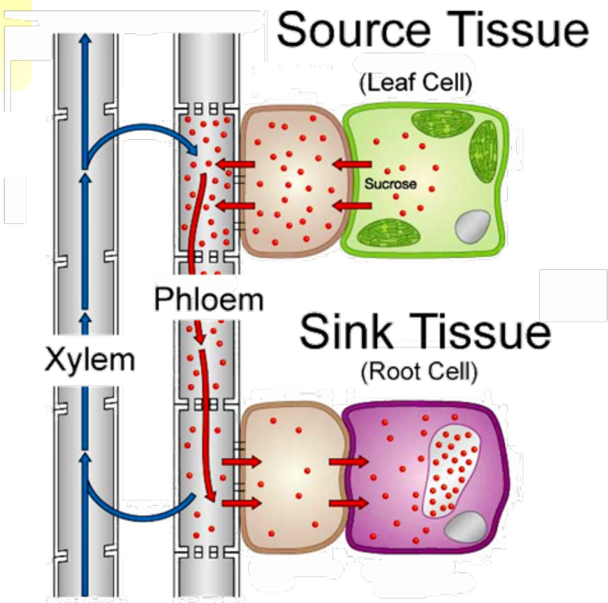
- -Reduced growth
- -Reduced leaf number
- -Sterile
- -Reduced photosynthesis
- -High Leaf carbohydrates

^{11}C-Sugar Export

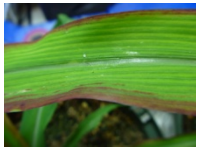
Was Münch wrong?
What maintains sap flow?



Münch hypothesis

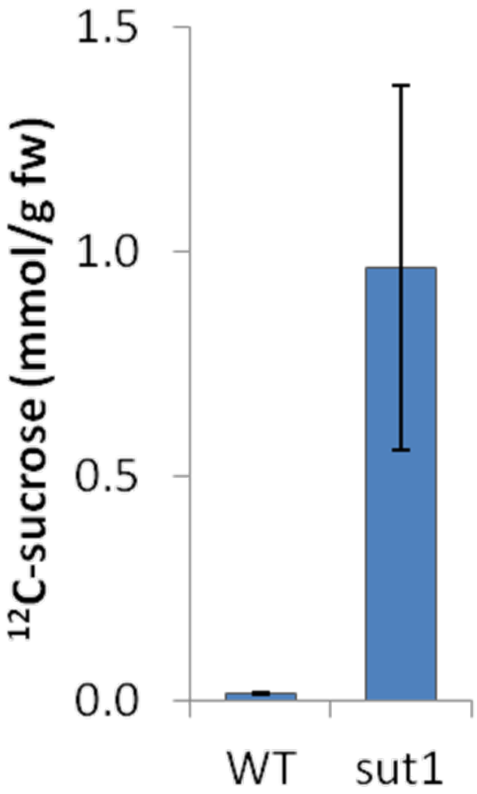


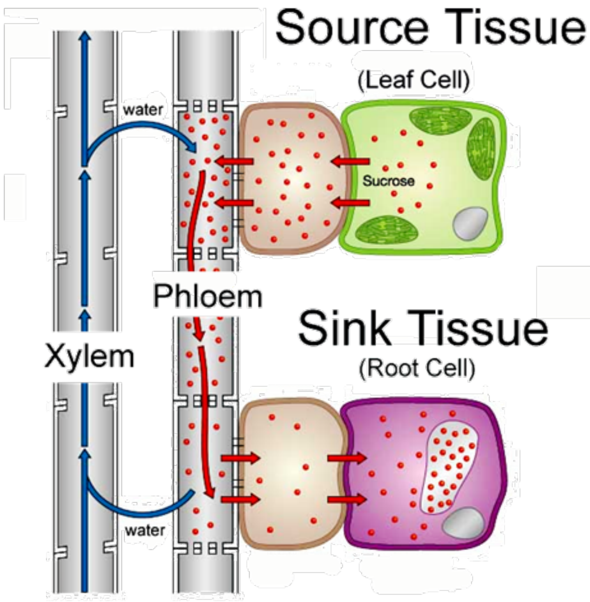
Drastically reduced export… moderately reduced sap flow speed

sut1 leaf carbohydrates

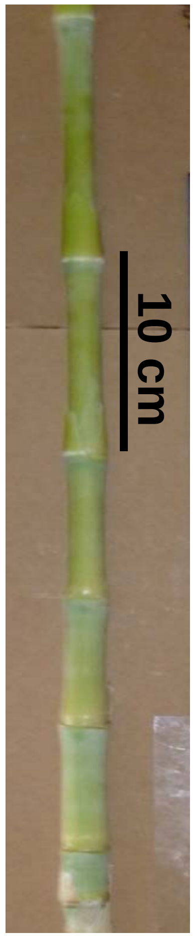
Sugars accumulate in response to the reduced transport out of mutant (*sut1*) leaves.





Sugary Stems

Sugar in Sweet Sorghum Stems

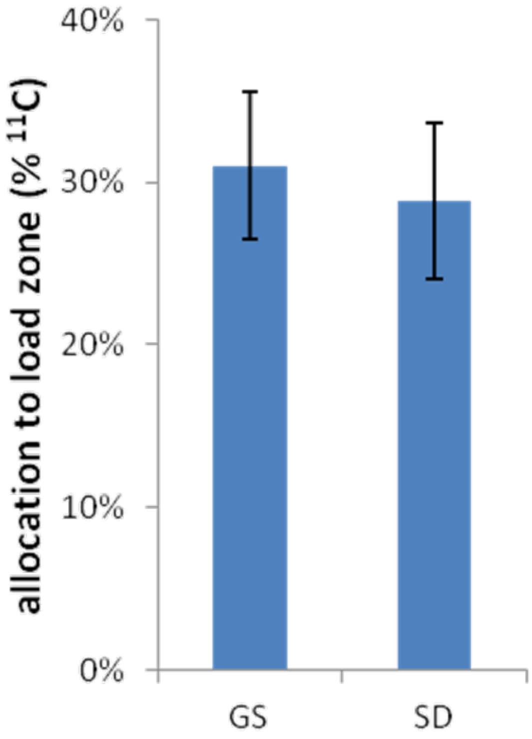


- **Abundant sugars → directly fermented**
- High biomass
- Lower nutrient/water requirements
- Drought tolerance (stay green)
- Adaptable to temperate climates
- Genetic resources

Sugar in Sweet Sorghum Stems

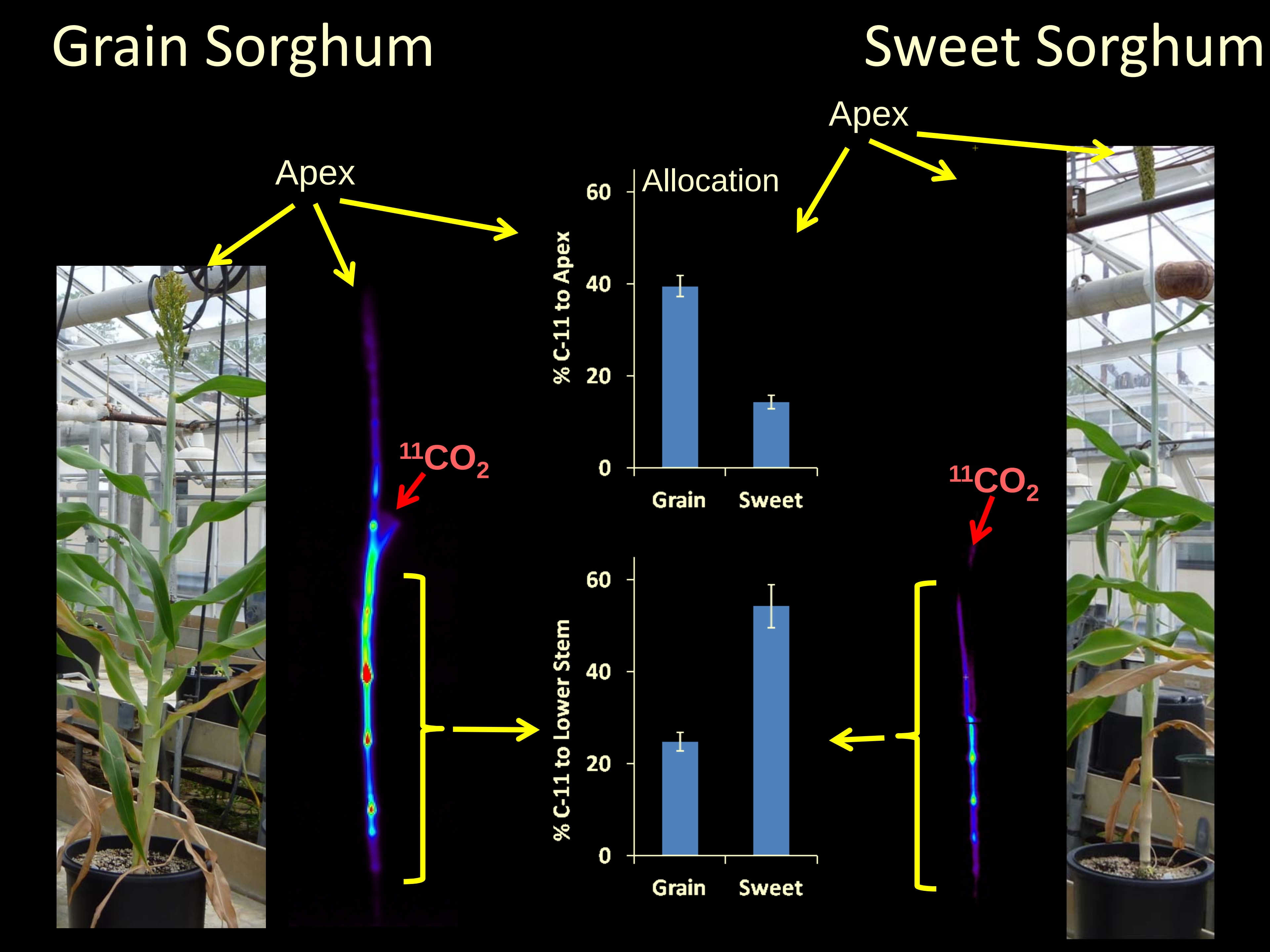
Study model for sugar accumulation

Leaf export similar to grain sorghum.

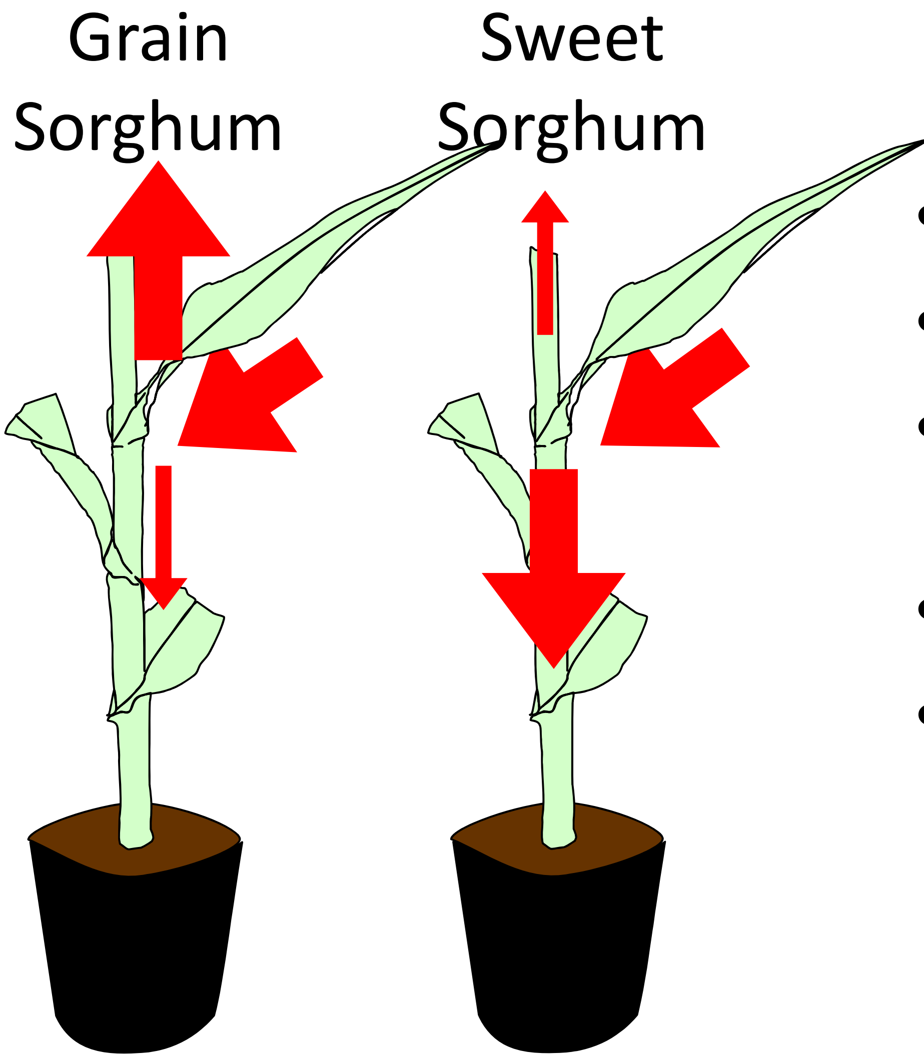


Abhijit Karve
BNL, 2012

Supported by:
USDA-DOE Plant Feedstock Genomics for Bioenergy

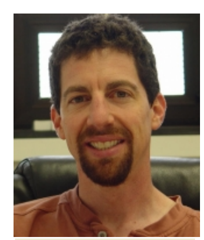

Grain Sorghum
Sweet Sorghum
Apex
Apex
Allocation
% C-11 to Apex
60
40
20
0
Grain
Sweet
$^{11}CO_2$
$^{11}CO_2$
% C-11 to Lower Stem
60
40
20
0
Grain
Sweet

Sugar in Sweet Sorghum Stems



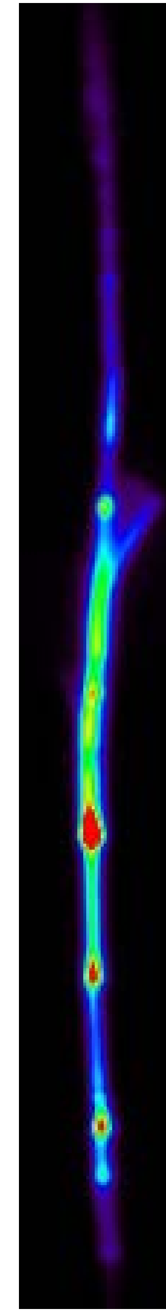
How controlled?

- Weaker competition
- Stronger sink strength
- Sucrose transporters
  - Sut mutant isolation
- Nodes in control?
- Other mechanisms?
  - Genetic mapping



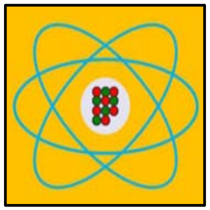
Mizzou University of Missouri

University of Nebraska Lincoln

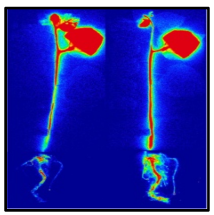


Outline

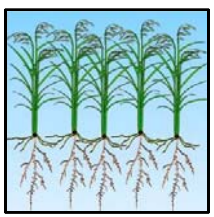
Bioenergy



PET tools & imaging

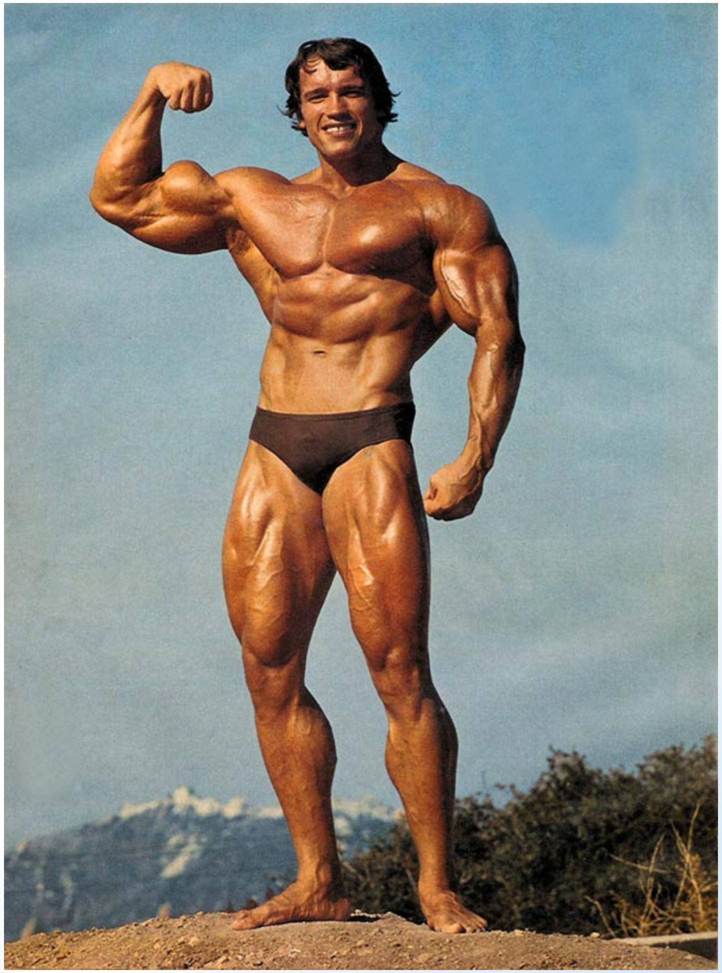


Plant resource movement



Plant signaling

Get resources in the right place, AND make the right stuff



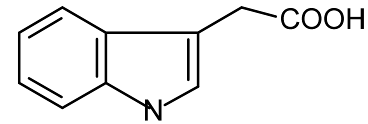
Phytohormones in Stem Growth

Green Revolution

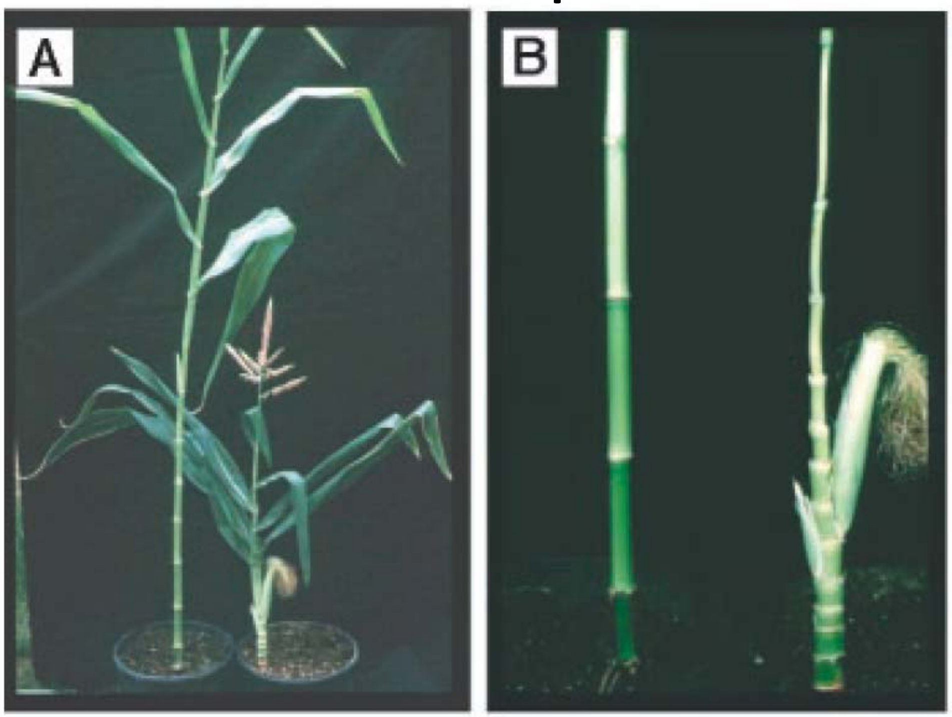
Bioenergy Revolution

Gibberellin
Brassinosteroid
Auxin

Auxin in Stem Growth



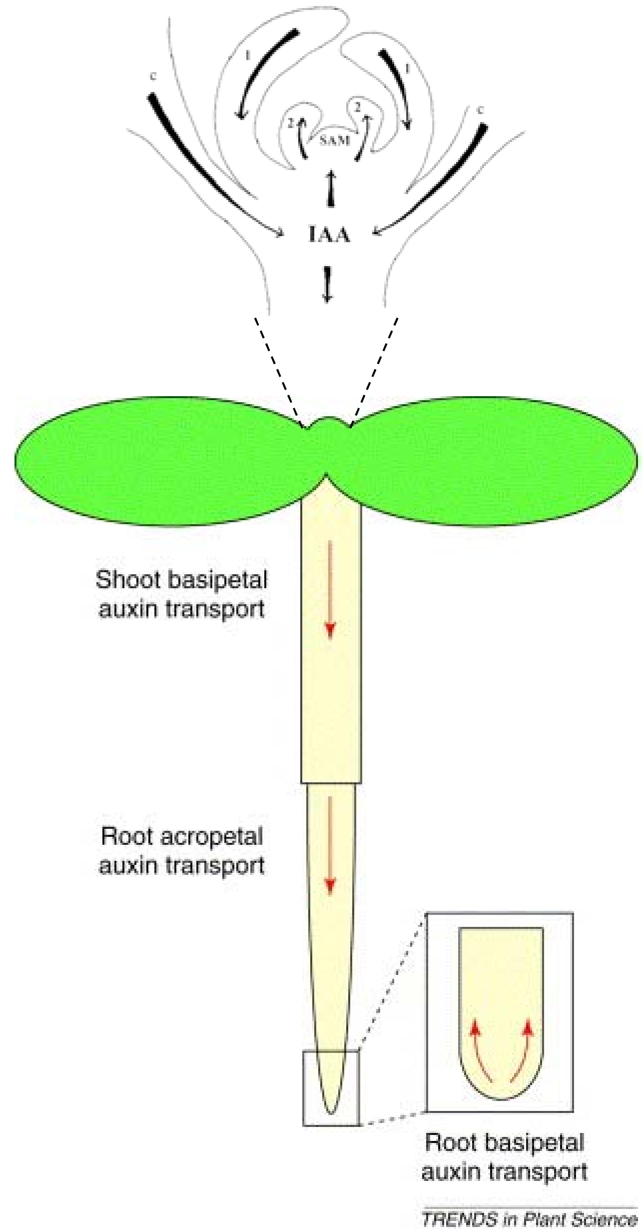
br2: auxin transport mutant



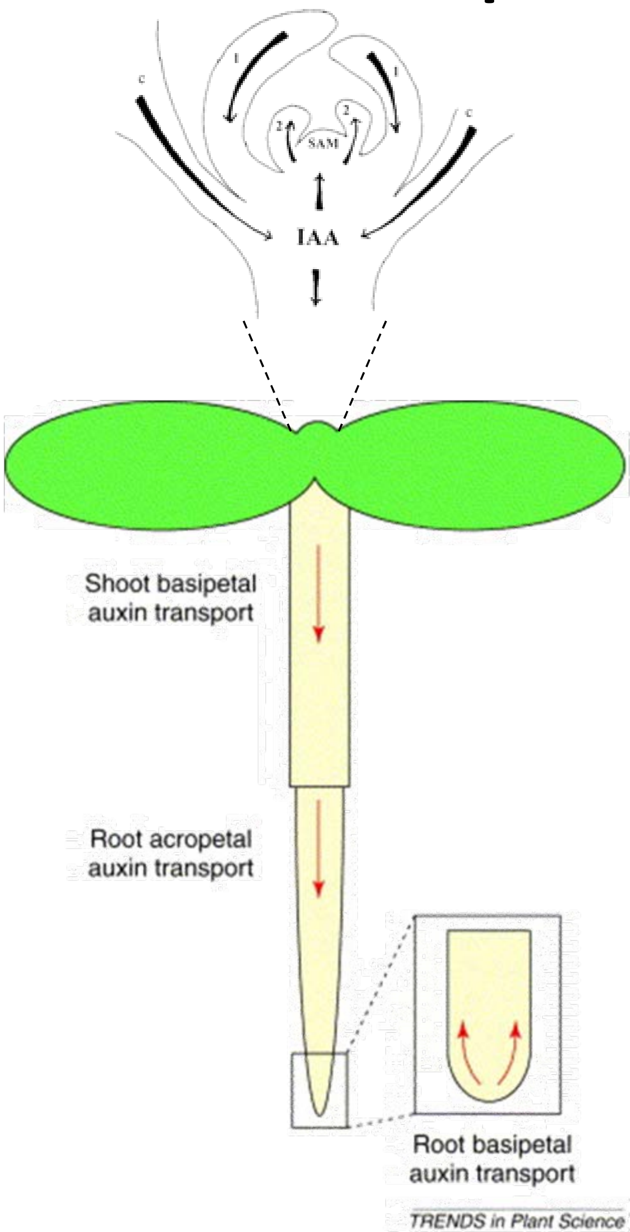
WT *br2*

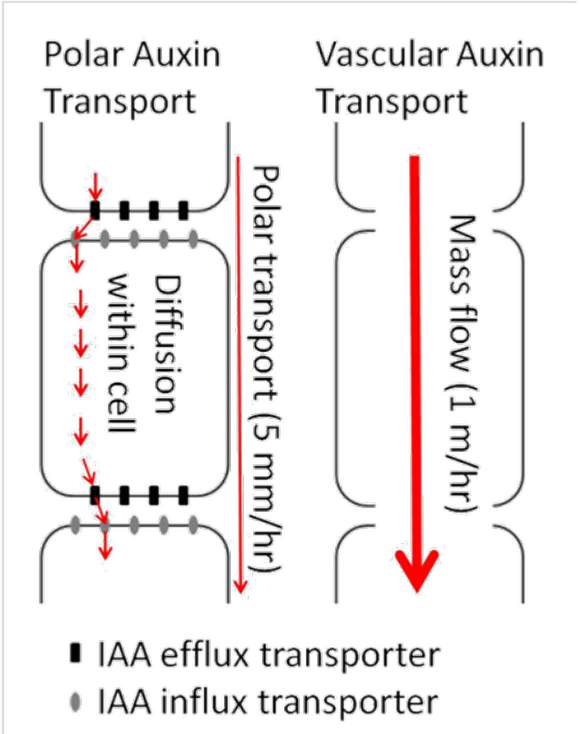
WT *br2*

Multani et al. (2003) Science

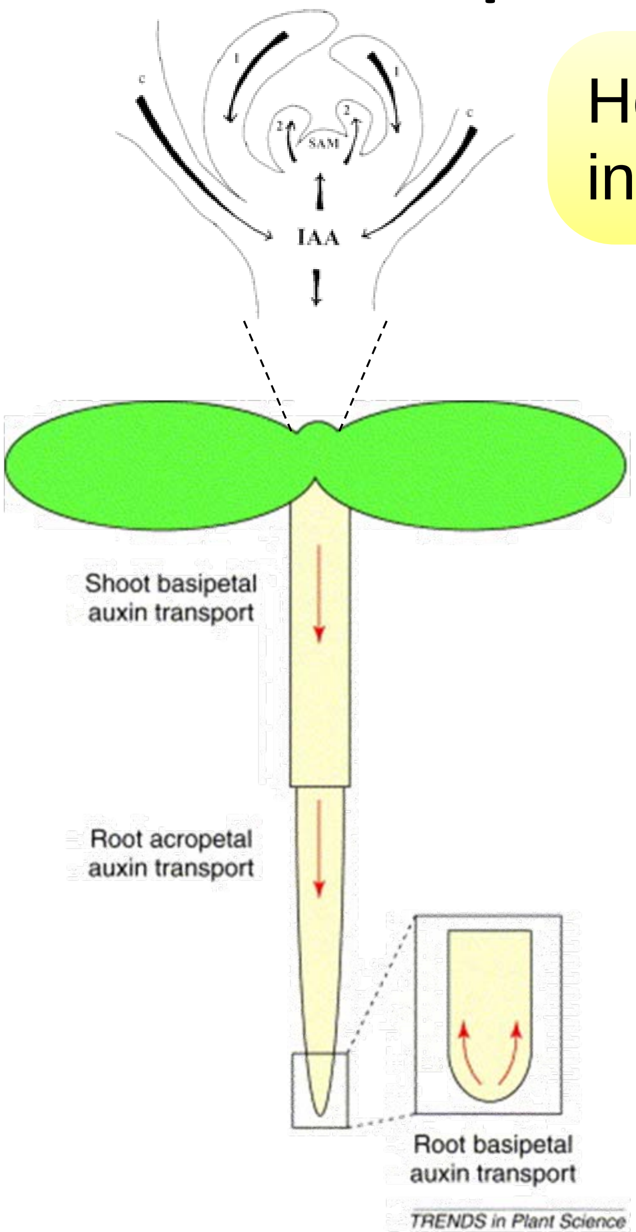


Auxin Transport in Stem Growth

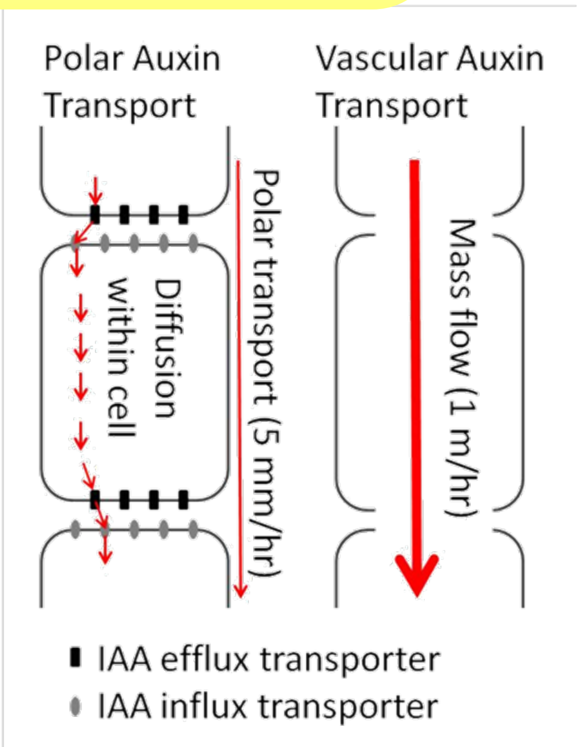




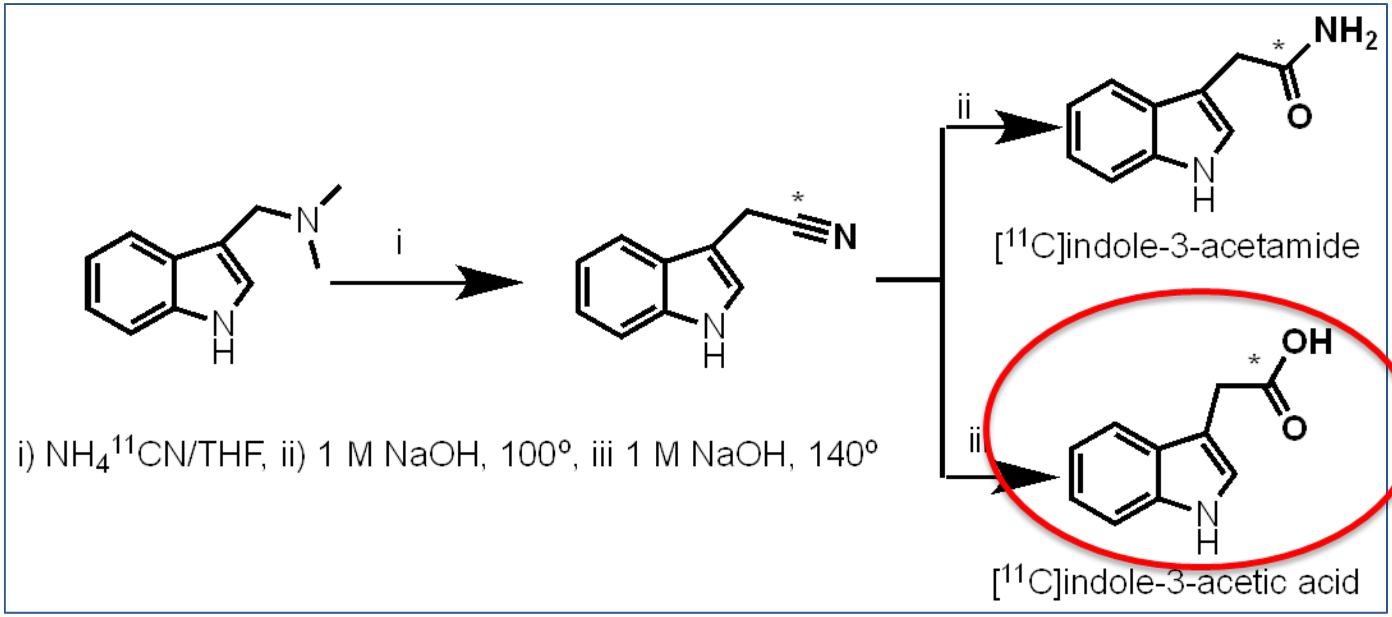
Auxin Transport in Stem Growth



How do grass nodes influence auxin transport?



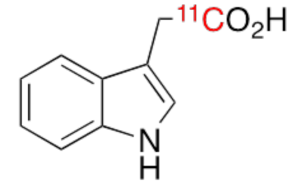
Synthesis of [11C]Indole-3-acetic acid (Auxin) from gramine (Reid et al., 2011)



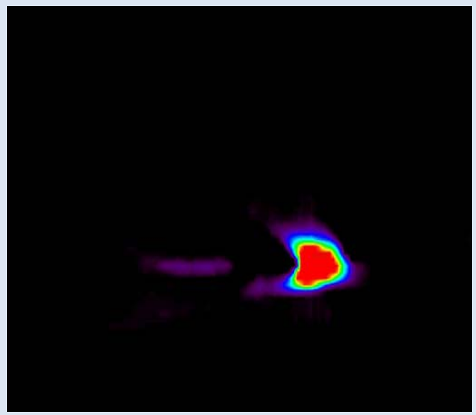
Alicia Reid

- This rapid, high yield radiosynthesis yields [11C]indole-3-acetic acid and 2 other labeled auxins from one general method.
- [11C]indole acetonitrile is formed in very high yield and is a key intermediate for other labeled auxin intermediates for auxin biosynthesis studies in grasses.

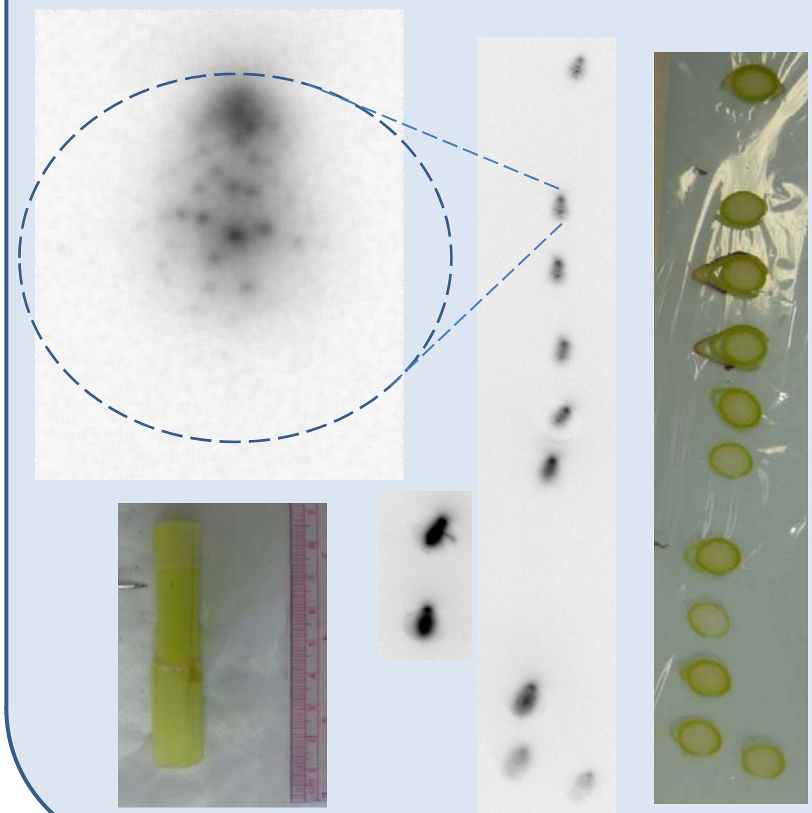
Imaging Auxin Transport
New Data:



microPET



autoradiography



1. Upward transport of auxin in stem.
2. Transport associated with vascular bundles (autoradiography).

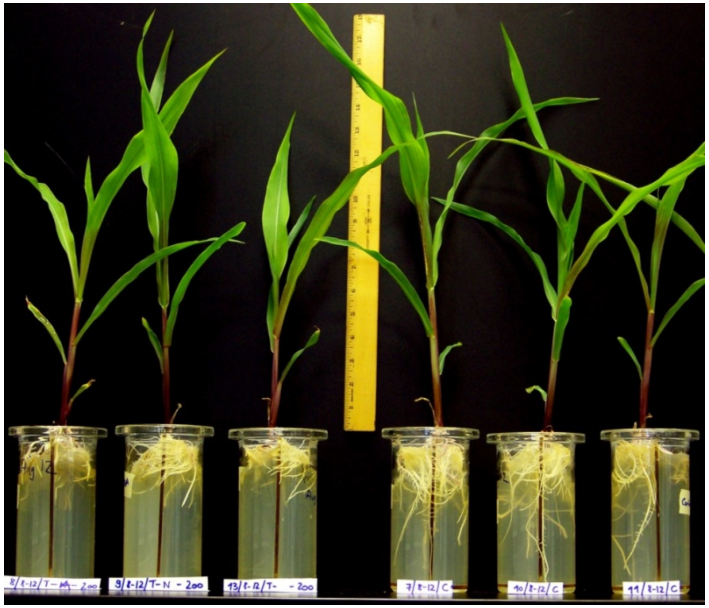
Getting at the Root of the Problem…

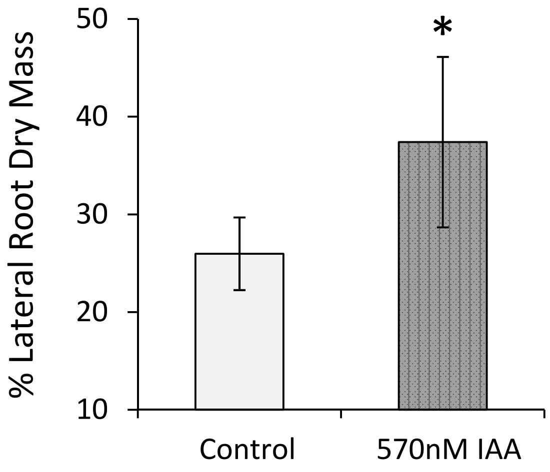
- **USDA & DOE:**

Understanding root system architecture is critical to developing more robust plants for bioenergy that are capable of growing on marginal soils.

- **What plant signals regulate root architecture?**

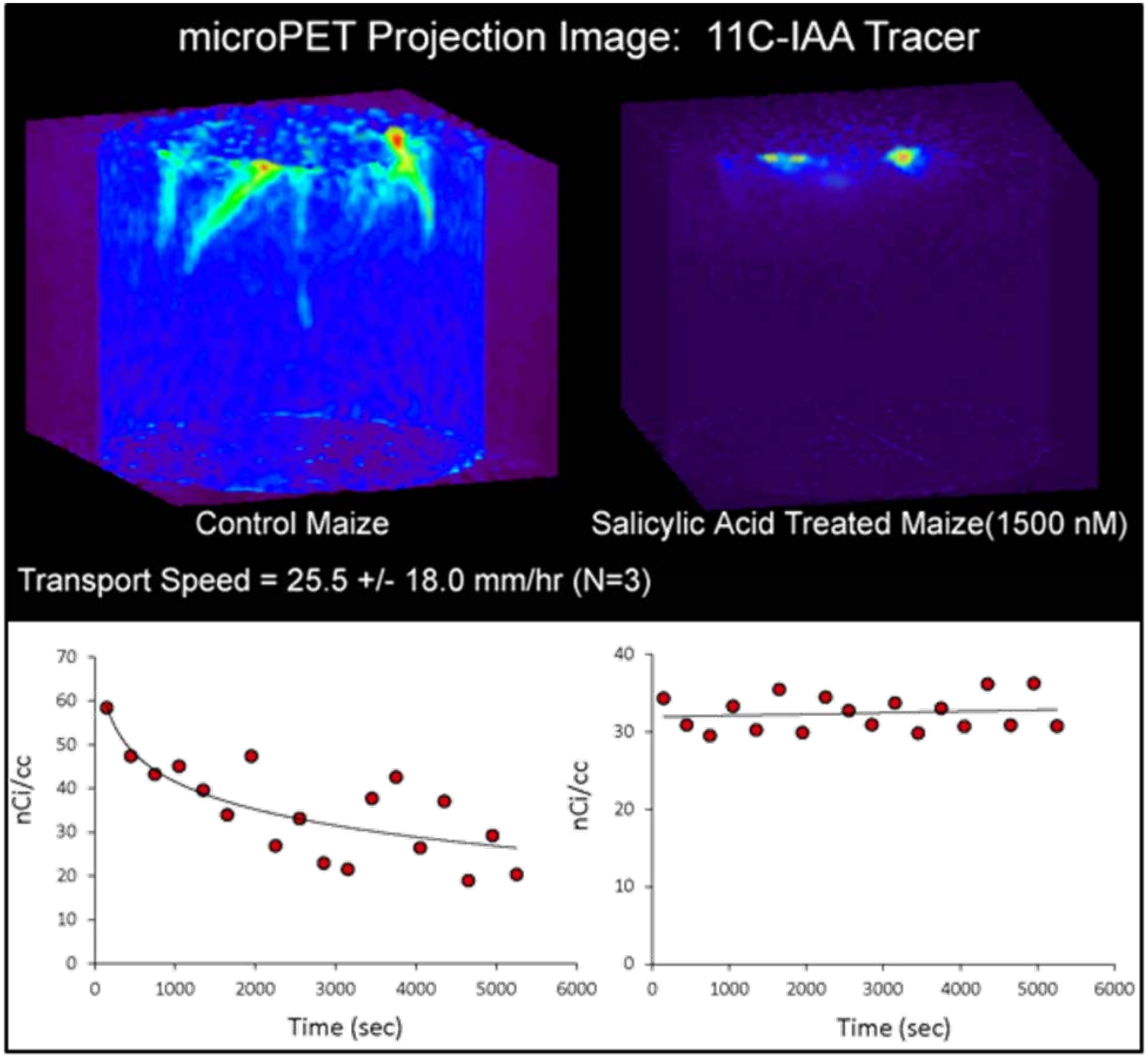
Treatment of roots using auxin will result in shorter roots, but higher lateral root density.

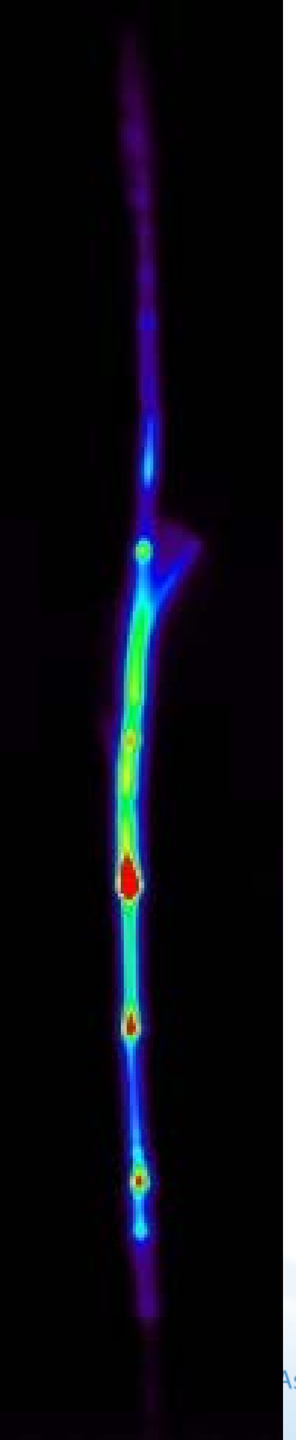




Radiographic images of ^{11}C-photosynthate distribution

Salicylic Acid Inhibits Auxin Transport





Summary

- Transport important for plant quality and yield
- Sink import drives sugar accumulation
- Roles of plant hormones
  - Stem elongation
  - Root architecture
- How do plant hormones interact
- Key role for $^{11}CO_2$ and PET imaging

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