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GENETICS INSTITUTE

Commercial Production of Terpene Biofuels in Pine

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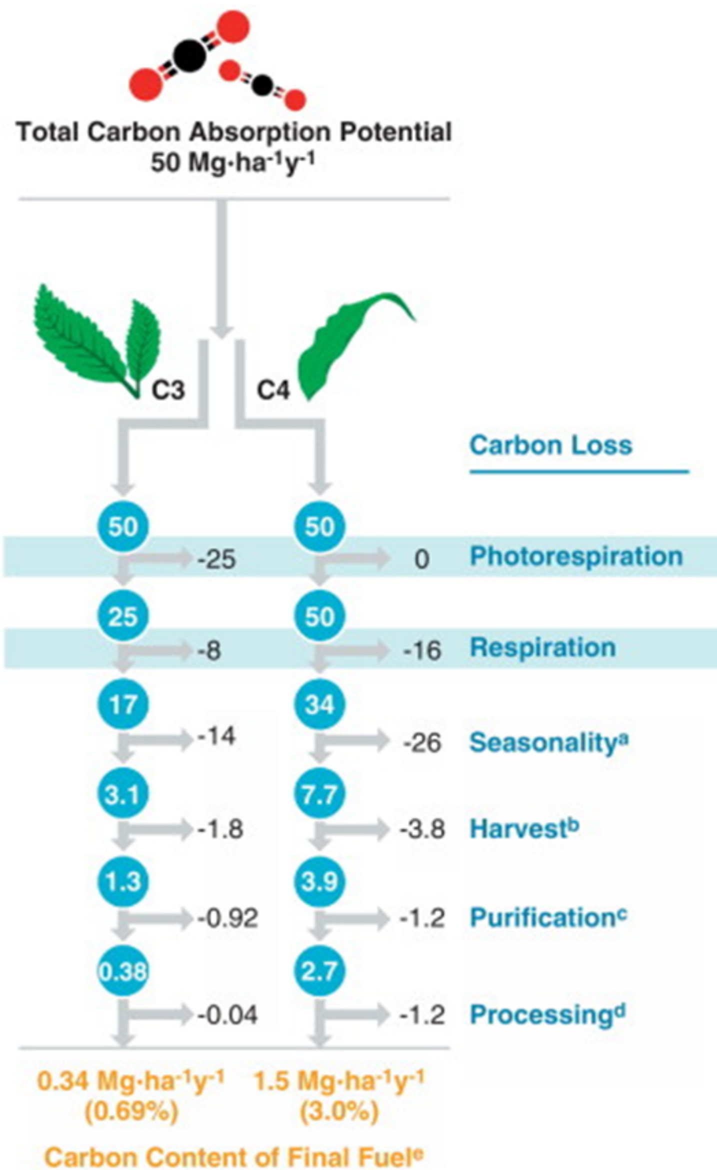
Biofuels: A Carbon Challenge



Borak et al. 2013 Cur Opin Biotech 24 369-375

1st GENERATION BIOFUELS

Extraction

- Sugar
  - Ferment to EtOH
  - Sugar
- Starch
  - Amylase + ferment to EtOH
  - Oil, animal feed
- Oil
  - Transesterification to biodiesel
  - Glycerin

Come from domesticated plants breed & selected for concentration & yield of edible food molecules

2nd GENERATION BIOFUELS

Deconstruction

- Lignocellulose
  - Sugar Platform
    - Size reduction + degradation + fermentation
    - Power, lignin
  - Gas Platform
    - Anaerobic digestion to biogas
    - Gasification + catalytic synthesis to liquid fuel
    - Power
  - Liquid Platform
    - Cracking / pyrolysis + upgrading

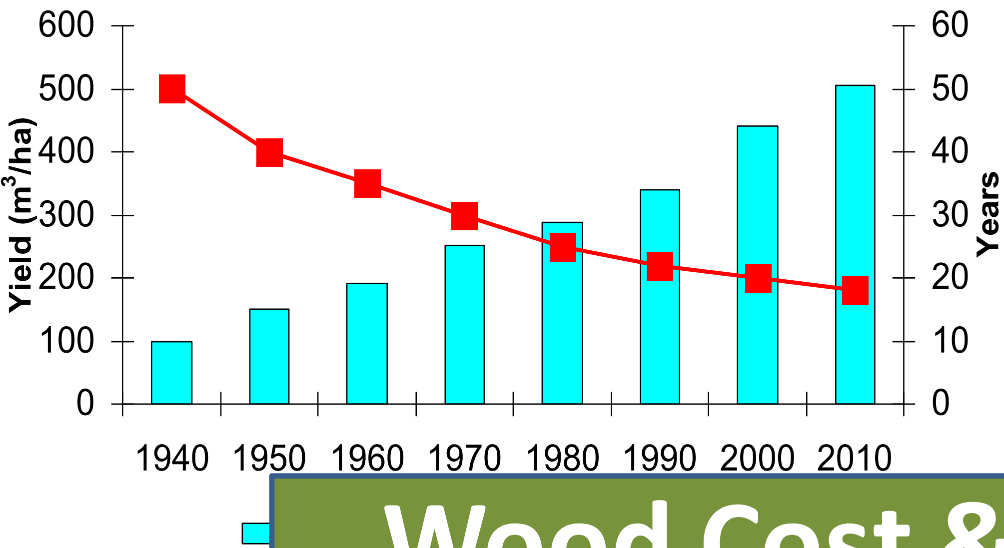
Non-edible parts of food plants & undomesticated grasses & trees which have high heterogeneity & low chemical uniformity

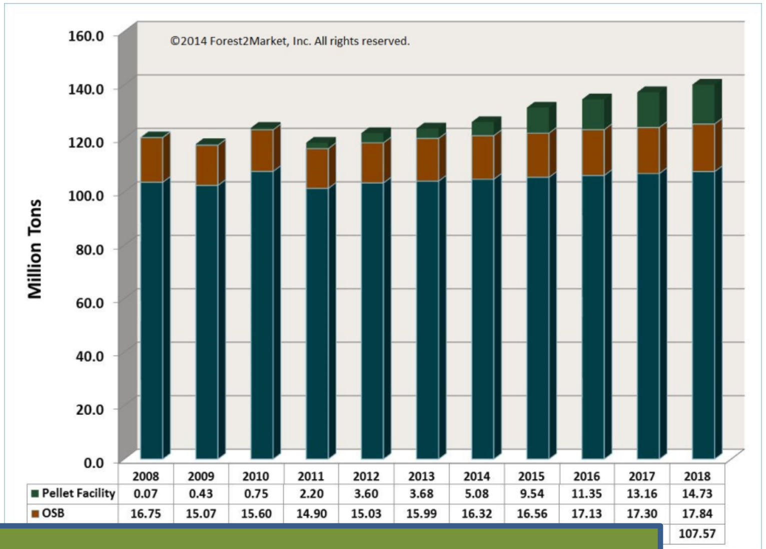
	Maize		Soybean		Sugarcane		Switchgrass	
	MgC h^{-1} y^{-1}	Yield	MgC h^{-1} y^{-1}	Yield	MgC h^{-1} y^{-1}	Yield	MgC h^{-1} y^{-1}	Yield
Captured	7.7	15.0%	3.1	6.3%	24	48.0%	12	24.0%
Harvested	3.9	7.8%	1.3	2.5%	16	32.0%	7.4	15.0%
Purified	2.7	5.4%	0.38	0.8%	7.7	15.0%	3.4	6.8%
Processed	1.5	3.0%	0.34	0.7%	4	8.0%	1.1	2.2%
Final energy content	52 (Ethanol)		50 (FAME)		52 (Ethanol)		52 (Ethanol)	
Overall fuel yield (GJ h^{-1} y^{-1})	78		17		207		57	

Planted Southern Pines: The Renewable Biomaterial, Chemicals & Bioenergy ☆STAR☆

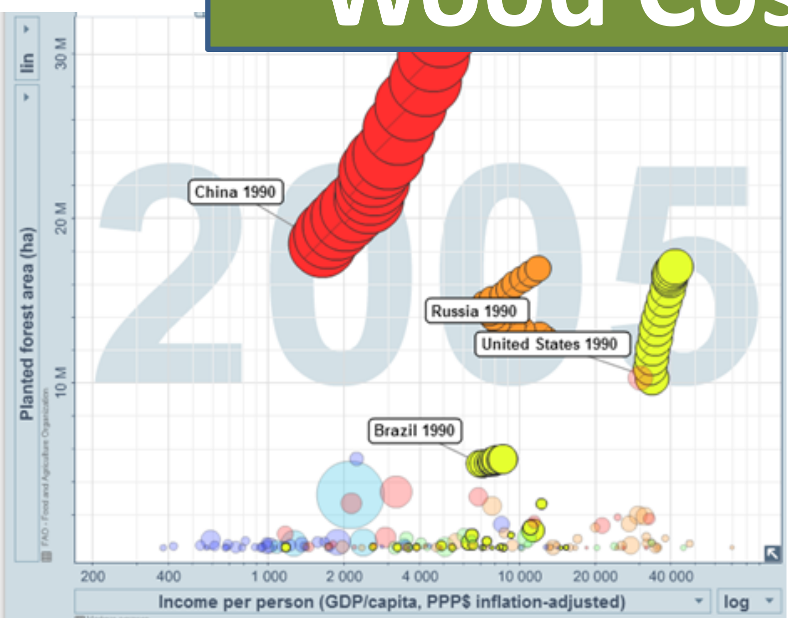
- Meets all sustainability metrics
  - Supply exceeds demand
  - Economically viable for multiple products
    - Top 1-3 industries in most SE states
  - Positive net energy & negative CO_2
- Largest biomass supply chain in world
- Largest source of long fiber pulp
- Largest source of saw timber
- Expansion of wood pellets
- Biofuels??

Impact of Silviculture & Tree Improvement on Harvest Volume, Rotation Length & Markets





Wood Cost & Abundance



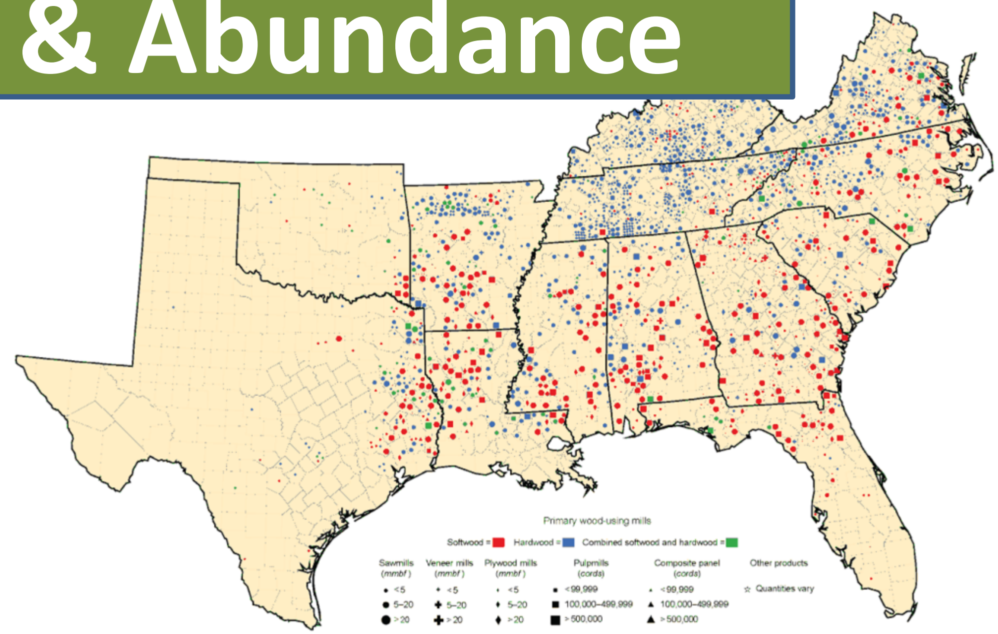
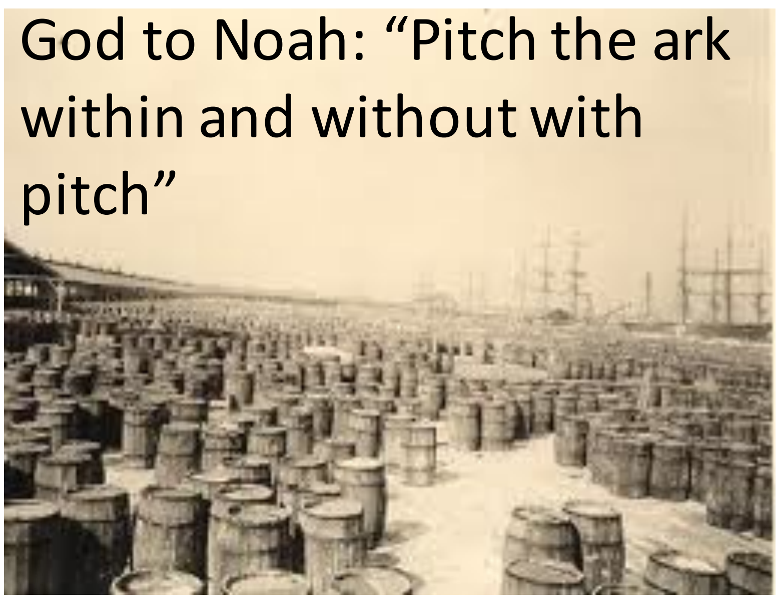


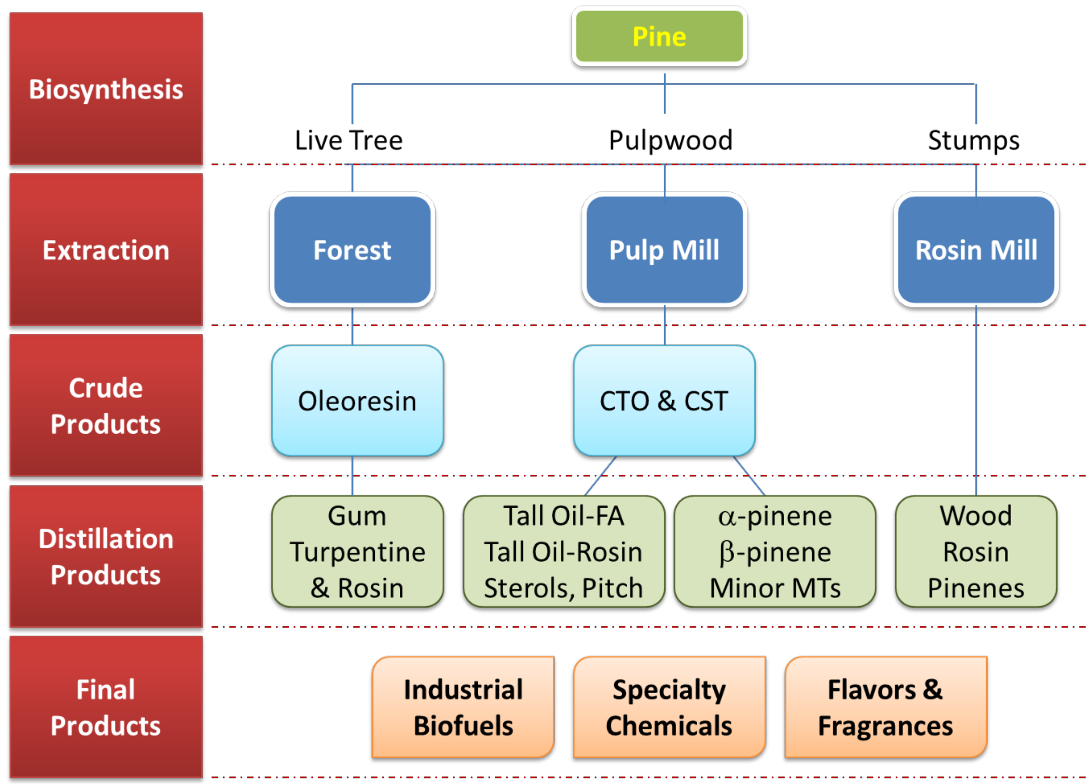
Figure 6—Primary wood-using mills in the South by State, 2007.

Pine Chemicals: First Industrial Chemicals & Current Industry Supply

~ Regional production

	Oleoresin	CTO	CST
Asia	**92%**	**-**	**-**
S. America	**8%**	**6%**	**2%**
Europe	**-**	**40%**	**35%**
N. America	**-**	**50%**	**60%**

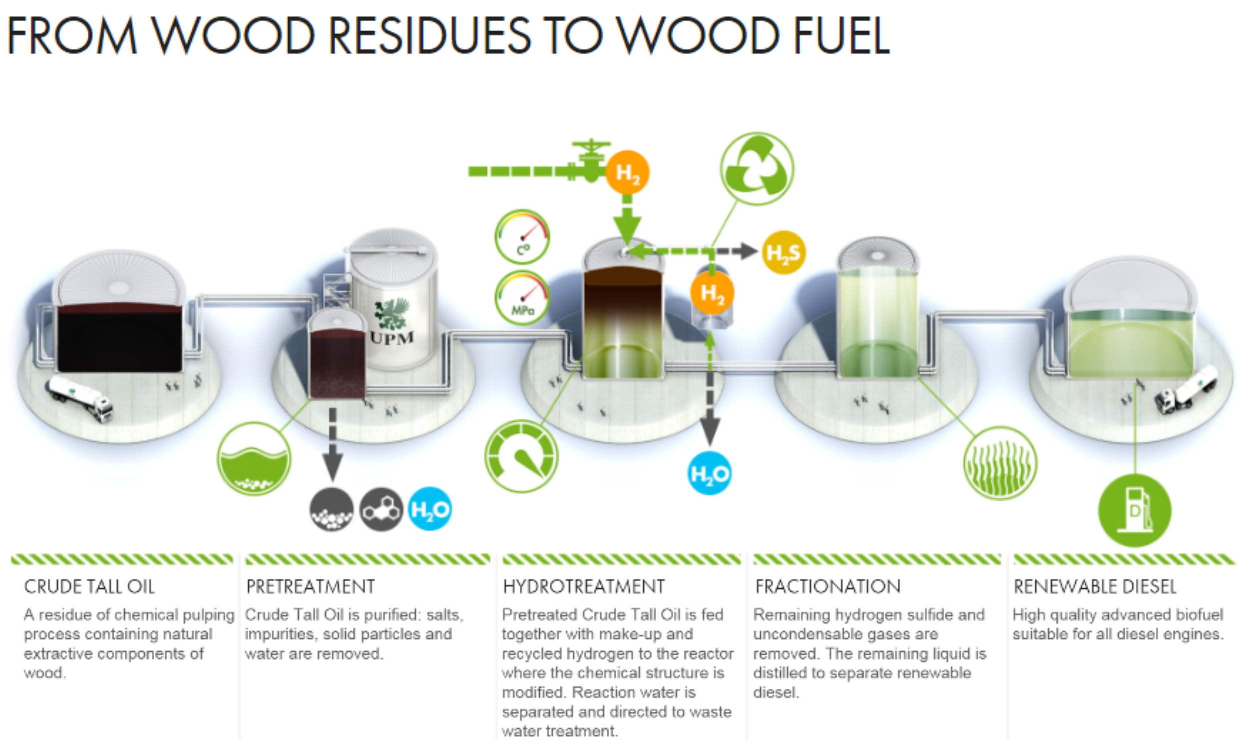




Conifer terpenes as feedstock for liquid **biofuel**

- Terpenes have **a high energy density**
- May be **blended with fossil fuels**
- **Minimal fertilizer** and **irrigation** requirements
- Large renewable chemicals industry competitive with petroleum based feedstocks

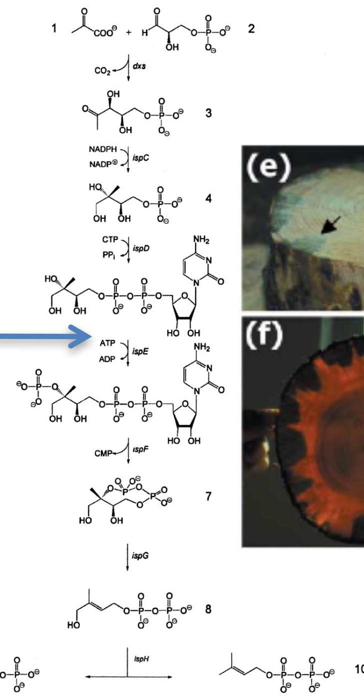
Sunpine tall oil refinery

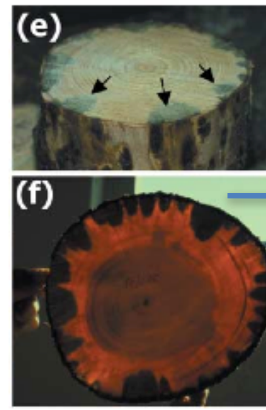


Stem terpene defenses against bark beetles

Constitutive

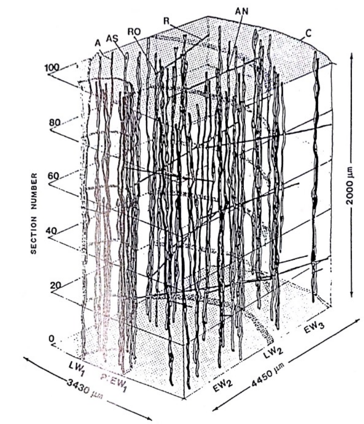
Bark beetle penetrates stem
Physical barrier:
Constitutive oleoresin flow
Chemical defense: terpenes toxic to bark beetles & pathogenic fungi

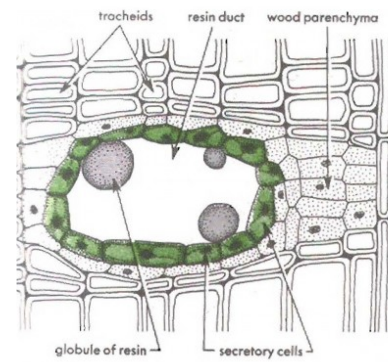




Wounding and fungi induce terpene synthesis & new resin canals in wood

Induced





Resin canal epithelial cells

Immediately → **0-7 days** → **2-4 weeks**

Jasmonate and ethylene signaling

Dual Strategy to Increase Wood Terpenes in Pine

Breeding

- Slash pine high gum selections increase oleoresin tapping yields 1.5-2.0 fold
- Extensive genetic analysis of constitutive traits
- Accelerated breeding with genomic selection models in loblolly pine

Genetic Engineering

- Three synergistic strategies
  - Increase resin canal #/volume
  - Increase carbon flux through DXP/MEP pathway
  - Increase enzyme efficiency

Three Levels of Engineering

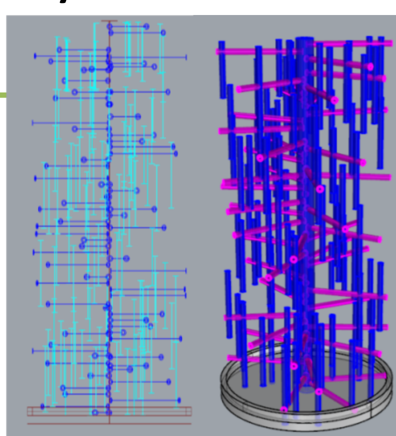
Development

Goals

- Increase # of cells synthesizing terpenes
- Increase storage

Approaches

- Discover regulators of resinosis
  - Inducers of new resin canal formation
  - Inducers of terpene synthesis



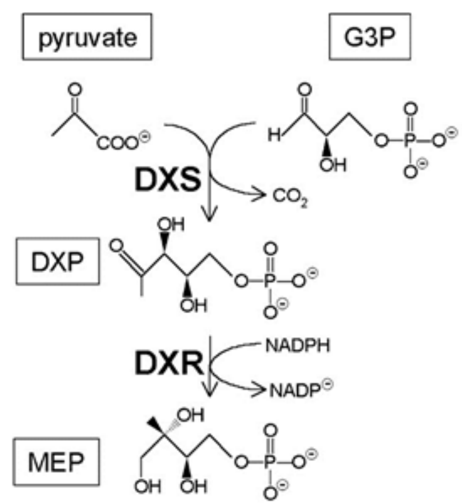
Pathway

Goals

- Increase flux
- Increase efficiency of carbon conversion

Approaches

- Enzyme shunts that reduce carbon loss
- Overexpression of rate limiting enzymes



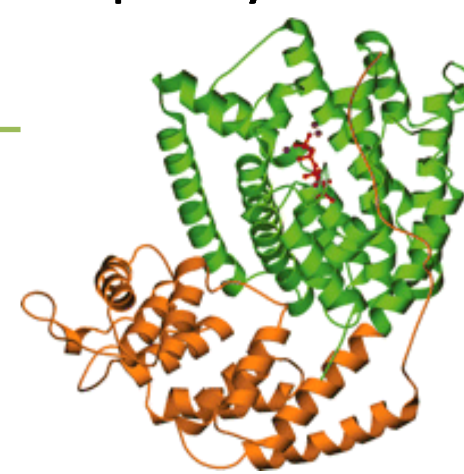
Enzyme

Goals

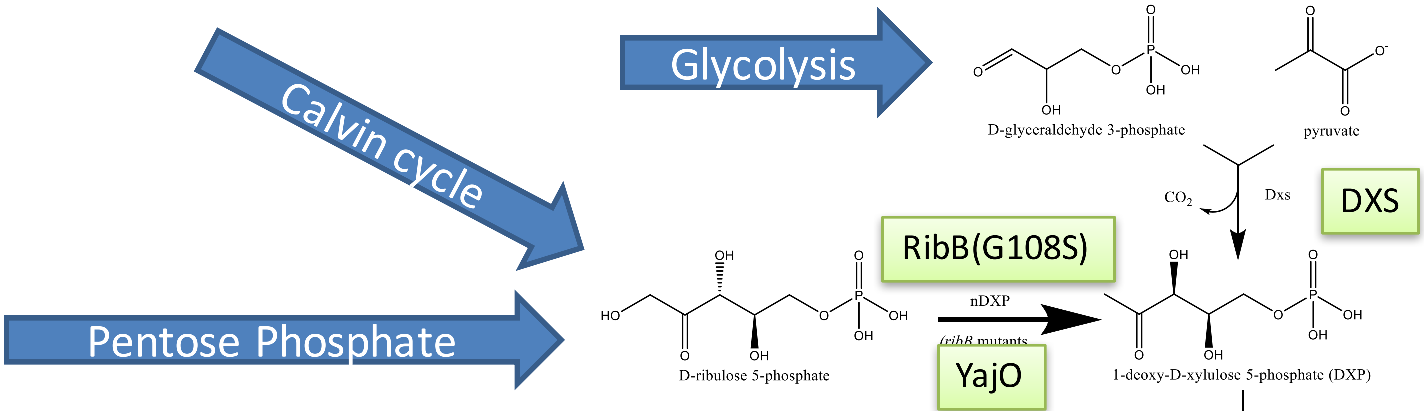
- Alter terpene composition
- Increase efficiency

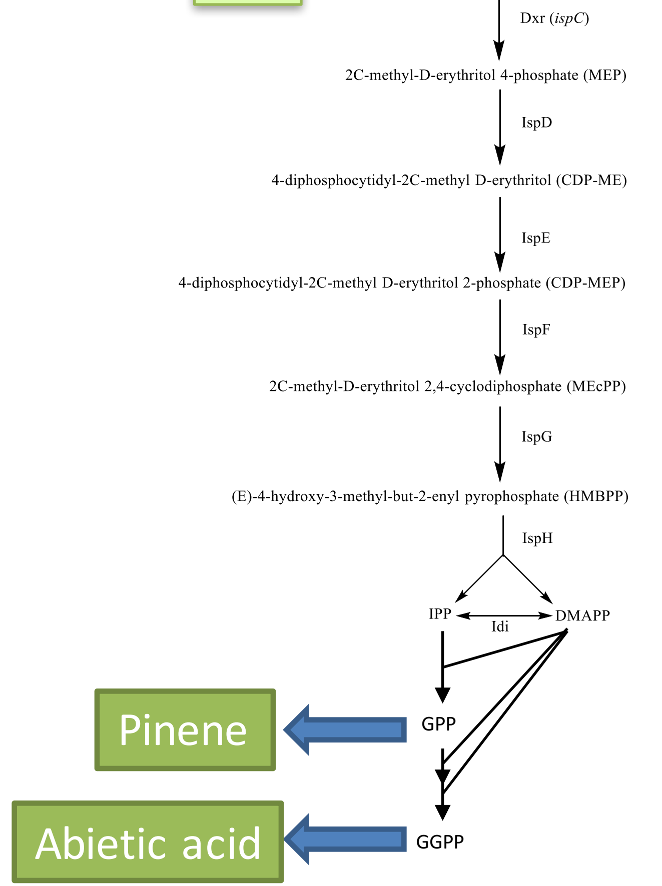
Approaches

- Produce bisabolene in wood
- Improve prenyl transferase and terpene synthases



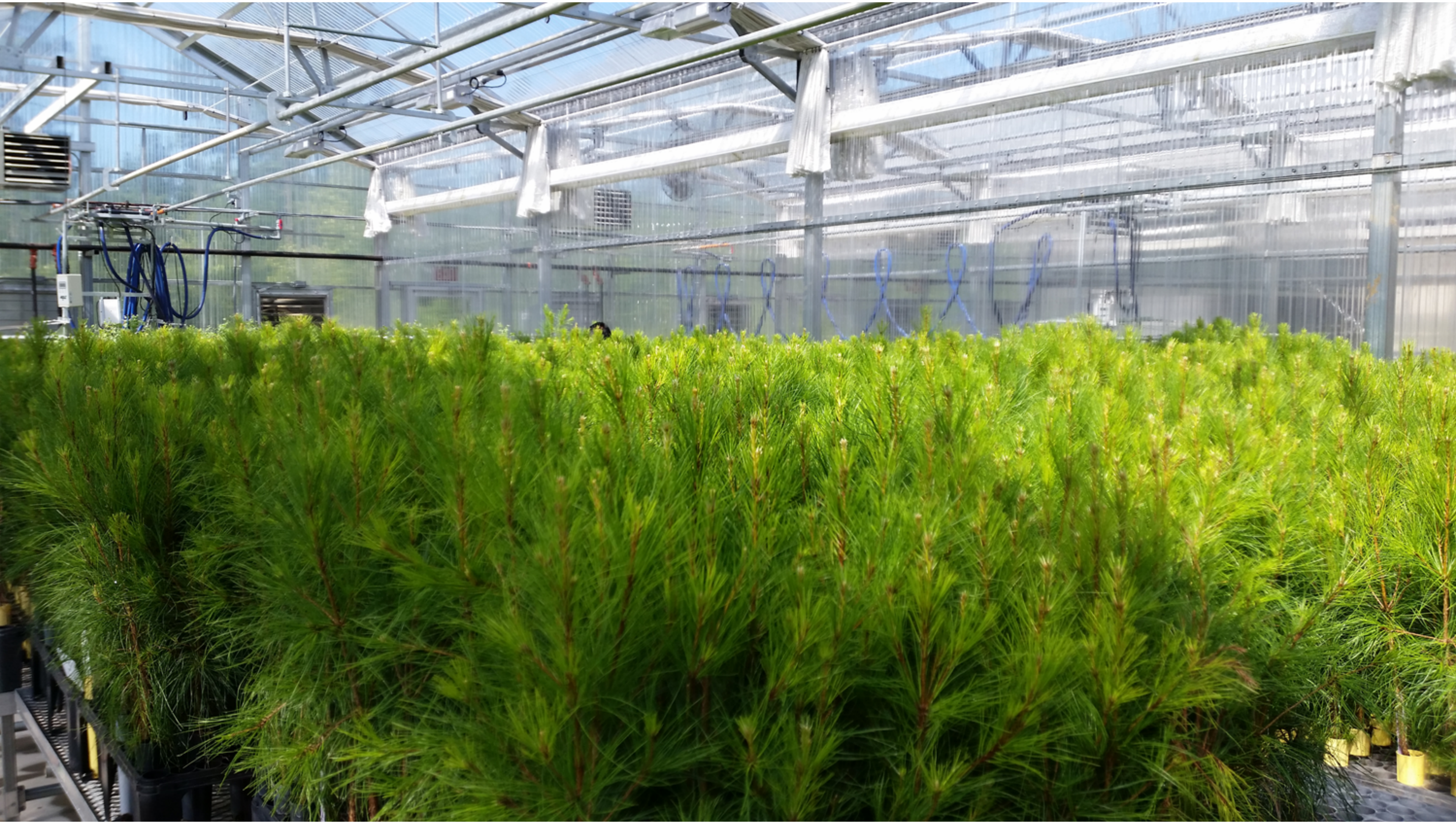
Global Analysis of Round 1 DXP-engineered plants





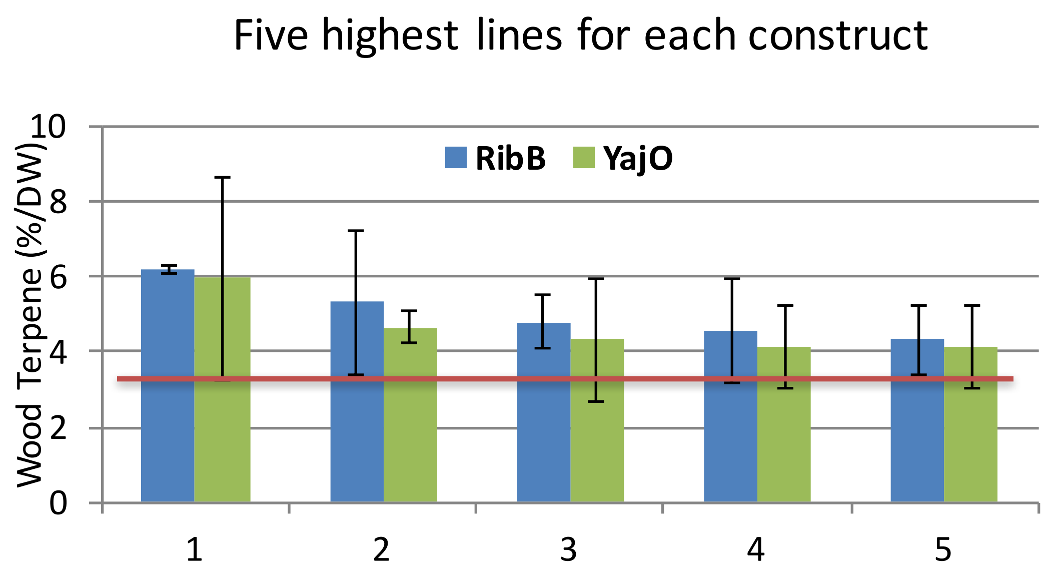
How does DXP pathway engineering affect global metabolism?

Greenhouse of 1.5Y Loblolly Pine Genetically Transformed Seedlings



Overall Mean Terpene Content from All Lines

Gene	# lines	N	C10 Mean (%/DW)	C15 Mean (%/DW)	C20 Mean (%/DW)	Total Mean (%/DW)	Total STD
RibB	28	81	0.99	0.00007	2.46	3.44	0.68
YajO	15	44	0.84	0.0008	2.36	3.20	0.96
DXS	4	12	0.77	0.0	2.38	3.10	0.77
Control	9	24	0.83	0.0	1.82	2.65	0.55



Summary of Pathway Engineering

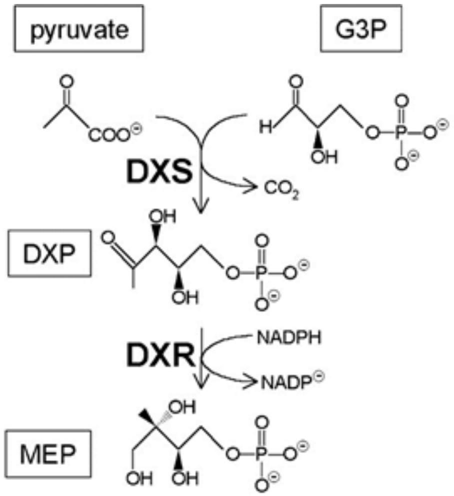
Pathway

Goals

- Increase flux
- Increase efficiency of carbon conversion

Approaches

- Enzyme shunts that reduce carbon loss
- Overexpression of rate limiting enzymes



- RibB & YajO (nDXP) utilize ribulose-5-phosphate which is more efficient than DXS
- RibB is not known to be regulated by feedback inhibition like DXS
- RibB increases young seedling total terpenes in wood by > 2 fold

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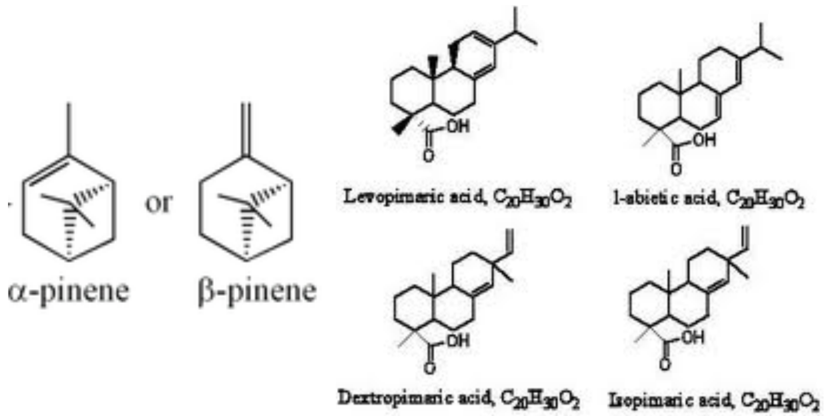
Innovation in Transportation

Pine Terpenes

- Naturally synthesize a diversity of terpenes as defense compounds



- Terpenes accumulate in wood naturally to >20%
  - Constitutive synthesis
  - Inducible synthesis

- Broad genetic diversity
- USFS selected slash pine trees that produce 2-3x greater amounts of resin upon tapping
- Terpene resin flow and α to β pinene ratios are under moderate to strong genetic control