



# *Genomics and Biotechnology for Improved Energy from Woody Biomass*

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# Why Tree Crops for Biomass Energy?

## Research Objectives and Opportunities with Poplar and Other Biomass Energy Trees



Combined harvesting and chipping of a willow plantation in Sweden.

(Photo: Stig Larsson)



# Economic Relevance of BioEnergy Trees:

Uses of solid biomass from trees for energy production:

- Conversion into Fuel Gas
- Conversion into liquid fuels such as ethanol
- Production of Steam and Electricity by direct burning

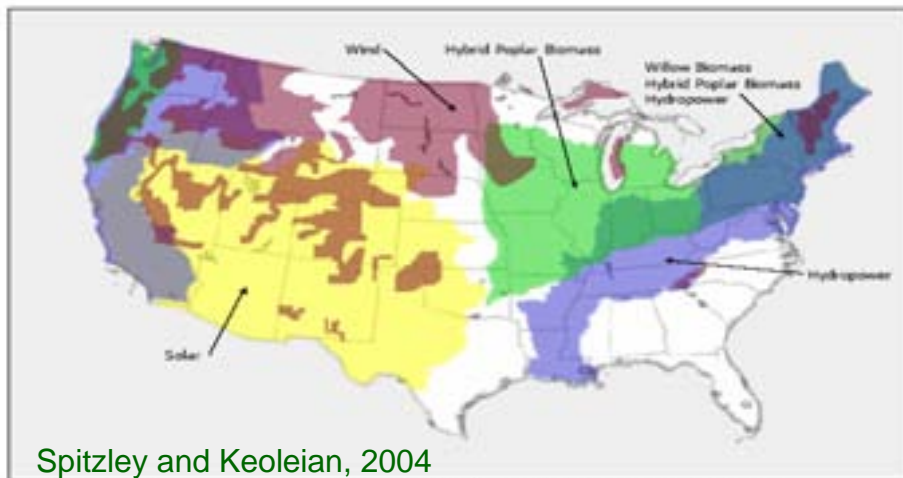


Figure 8. U.S. Capacity for Development of Alternative Electricity Generation



NRG Dunkirk power plant on Lake Erie

# Advantages of Power Generation from Woody Biomass:

- Tree biomass is renewable
- Trees protect soil from erosion
- Tree farms create wildlife habitat
- Dependency on fossil fuels reduced
- Less tree and wood waste put to landfills
- Reclamation of mines and polluted sites
- Acid rain emissions are reduced vs. fossil fuels
- Secondary revenues/uses from steam generation
- Preservation of best cropland for food production
- Opportunities for local tree growers and forest industry

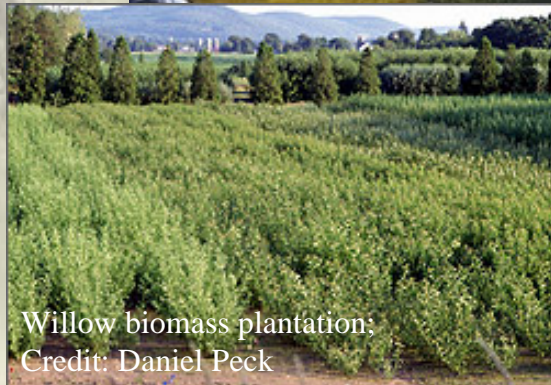
# Potential Biomass Energy Trees

## Biomass trees:

Hybrid Poplar  
Willows  
Yellow-poplar  
Sycamore  
American Chestnut<sup>1</sup>

## Traits for Bioenergy Trees:

Clonal propagation  
Fast height growth  
Rapid volume accumulation  
High density for solid fuels  
Low lignin for liquid fuels  
Suitable for high density plantations



chestnut tissue culture



# Multiple Benefits from Hybrid Poplar

- *High Energy Content* – 334 gallons per acre ethanol (vs. 260 from corn)



- *Riparian Buffers* – Hybrid poplars keep pollutants out of our waterways

- *Phytoremediation* – Hybrid poplars clean the soil and restore strip mines



- *Agroforestry* – Hybrid poplars are for intercropping



- *Carbon Sequestration* – Hybrid poplar reduces greenhouse gases

# Research on Hybrid Poplar for BioFuels



- **Genomics** – *the entire genome sequence is known for poplar*



- **Clonal Selection** – *Genetic variation in poplars is captured by clonal propagation*



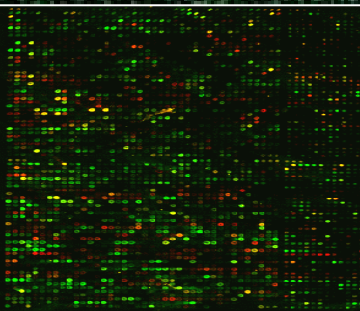
- **Management** – *Production is maximized in short rotation plantations*



- **Biotechnology** – *New genotypes produce more fuel on marginal lands*

# *Hybrid Poplar is both a Model and Practical System for BioEnergy Research*

- **Rapid biomass accumulation (10-20 T/ha)**
- **Genome-enabled research and biotechnology**
- **Efficient transformation for biotech applications**
- **Direct commercial application of results  
via clonal propagation in plantations**





# Areas of Research Related to Biomass

## **Domestication of Trees as Energy Crops**

- Biomass production, carbon sequestration, stress tolerance

## **Modification of lignin content in trees**

- Optimizing ethanol production from lignocellulose

## **Genomics-assisted tree improvement**

- Suppressing flowering; Enhancing vegetative growth

## **Genomics of Asian Longhorned Beetle Gut Microbes**

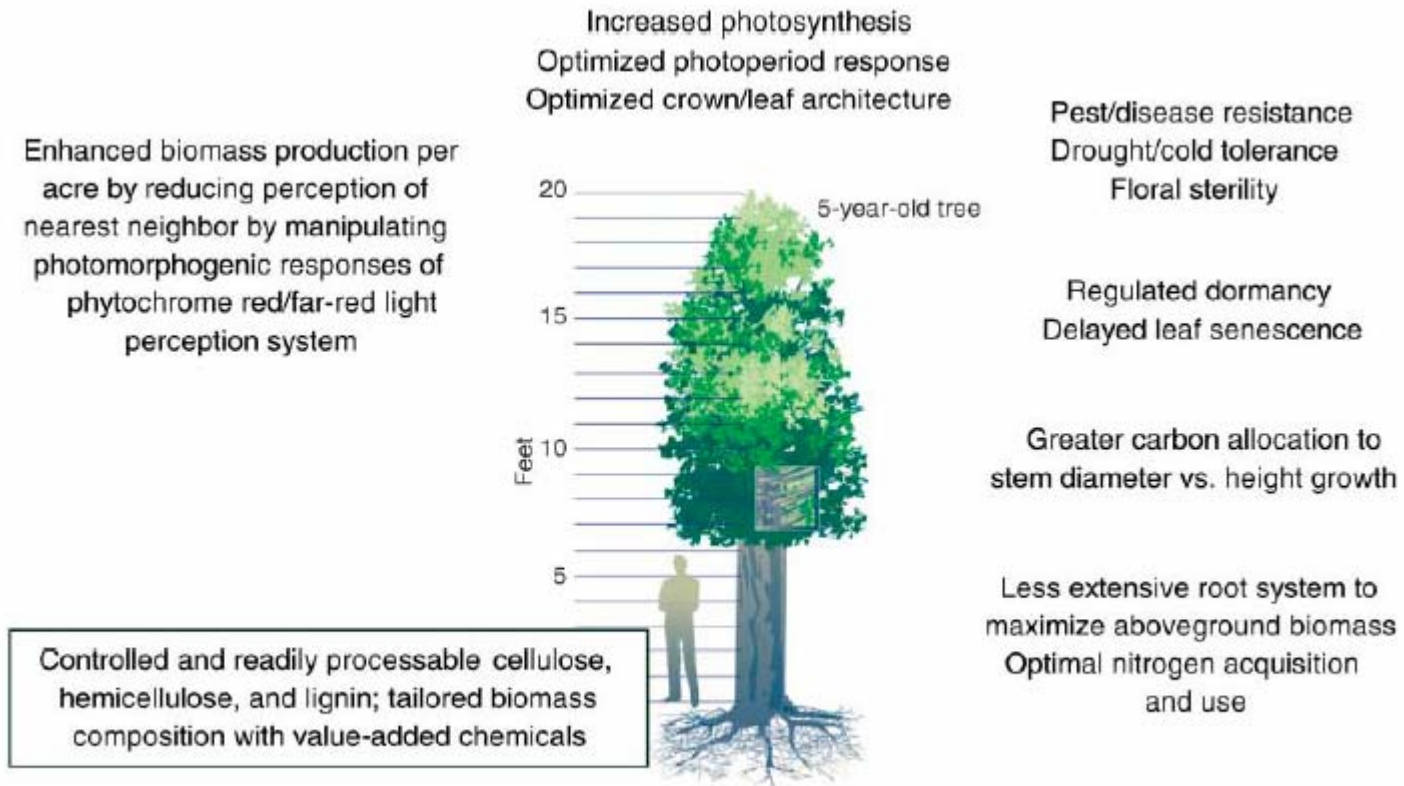
- Discovering genes for conversion of wood to energy



<http://www.aphis.usda.gov/ppq/ep/alb/>



# Domestication of Trees as Energy Crops



**Fig. 2.** Overview of plant traits that can be targeted by accelerated domestication for enhanced plant biomass production and processing.

(Ragauskus et al, Science, 2007, The Path Forward for Biofuels and Biomaterials)

# *Our Projects in Functional Genomics for Domestication of Poplar*

- **Lignin biosynthesis and modification**
- **Gene networks regulating flowering vs. vegetative growth**
- **Volatiles emitted in response to insect herbivory**
- **Response of trees to Ozone stress**



# *Functional Genomics of Flowering in Populus*

Ali Barakat and Scott DiLoreto, with the research groups of Claude dePamphilis, Dawn Luthe, Cetin Yuceer, Amy Brunner, and Grier Page

*The control of flowering is required for research and commercialization of transgenic trees.*

Our approaches:

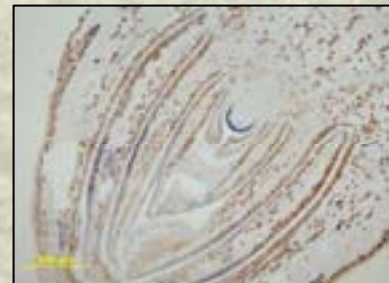
Physiological and genetic manipulations

Functional genomics

Comparative genomics

Evolutionary analysis

Gene expression patterns.



**See Frost et al. Poster in Plant Feedstocks/Products/Materials section**

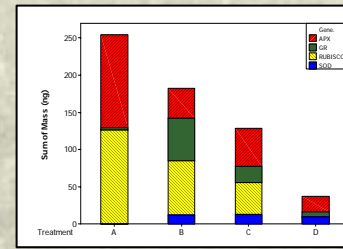
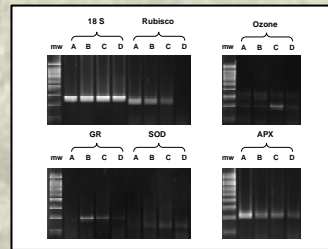
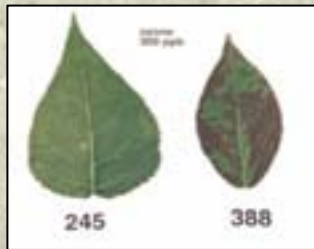
# *The Molecular and Physiological Responses of Poplar to Ozone*

Teo Smart with Don Davis, Dennis Decoteau, Jonathan Lynch

***Ozone causes early senescence and declines in biomass production.***

## Our Objectives:

- Compare ozone sensitive and tolerant hybrid poplar clones for physiological responses to soil conditions and ozone exposures
- Identify gene expression patterns that confer ozone tolerance.



**See Frost et al. Poster in Plant Feedstocks/Products/Materials section**

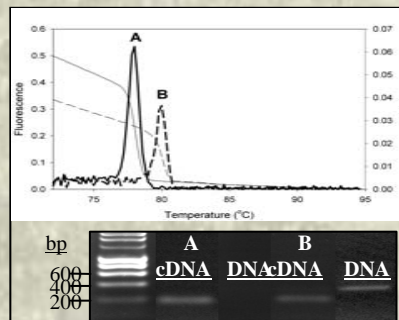
# *Insect Herbivore-Induced Plant Volatiles and Systemic Plant Defenses in Poplar*

Chris Frost, with Mark Mescher, Consuelo De Moraes, Heidi Appel, Jack Schultz, and Haiying Liang

*Herbivores cause significant damage in managed plantations*

Our objectives:

- Measure induced responses to multiple insect herbivores.
- Characterize the priming potential of specific volatiles.
- Determine the ecological effects of volatiles on insect herbivory.
- Study gene expression in leaves exposed to herbivores and airborne volatiles.



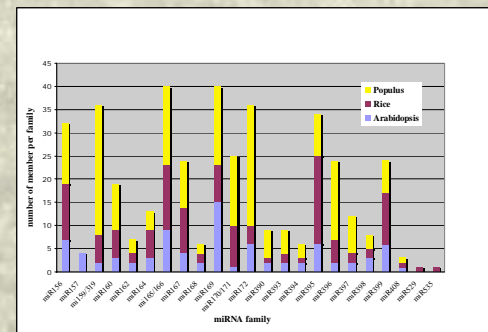
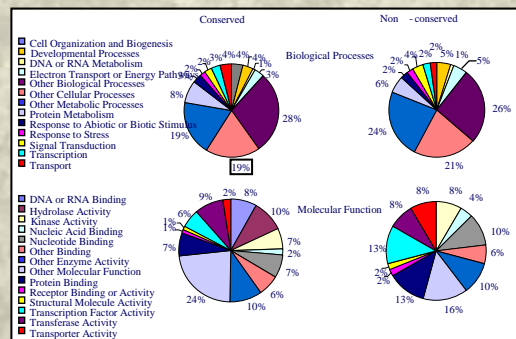
**See Frost et al. Poster in Plant Feedstocks/Products/Materials section**

# Identification of microRNAs from Poplar

Ali Barakat with Claude dePamphilis and Kerr Wall

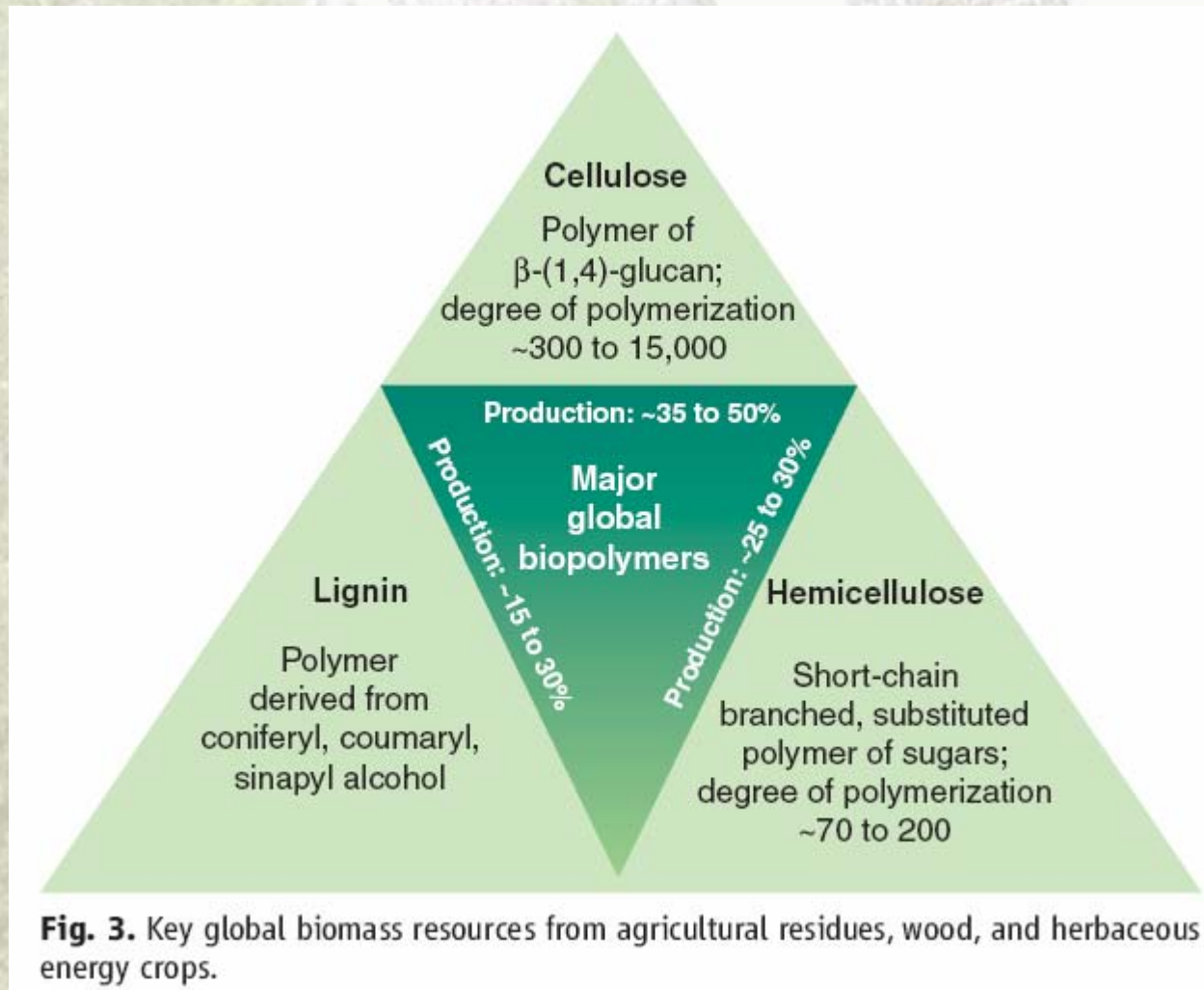
*MicroRNAs are small RNAs that negatively control expression of genes involved in development, stress tolerance, defense, etc.*

- We identified over 160 new miRNA families in *Populus*.
- We compared these with miRNAs in *Arabidopsis* and rice.
- We identified the targets of most of the miRNAs in *Populus*.
- Functional genomics and biotechnology studies underway



See Frost et al. Poster in Plant Feedstocks/Products/Materials section

# Tree Biomass = Lignin + Cellulose + Hemicellulose

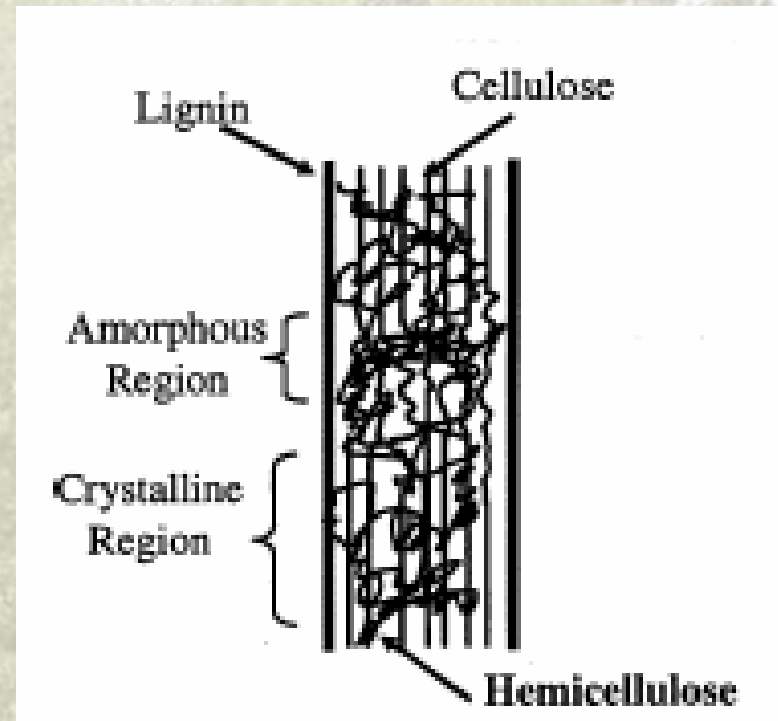


(Ragauskus et al, Science, 2007, The Path Forward for Biofuels and Biomaterials)



# Lignin Degradation and Biomass Utilization

Lignin is the second most abundant compound in the biosphere. It is structurally important in trees. However lignin interferes with pulp and paper production and biomass utilization.



# ***Lignin Modification via Expression of a Tyrosine Rich Cell Wall Peptide in Hybrid Poplar***

**Ming Tien, Haiying Liang, Nicole Brown, and John Carlson**

Our Hypothesis: Free radical coupling between lignol subunits and TYR will result in a lignin structure that can be partially hydrolyzed with proteases. This would permit more efficient extraction of lignin and enzymatic conversion of wood to ethanol.

Approach: Transform Poplar hybrids with a PAL-promoter/CBG-leader/TYR-gene construct and determine effects on the trees and on conversion to ethanol.

**See Tien et al. Poster in Plant Feedstocks/Products/Materials section**

# Metagenomics of the Microbial Community in Asian Longhorned Beetle (*Anoplophora glabripennis*).

Scott Geib, Scott DiLoreto, John Carlson, Maria del Mar Jimenez-Gasco, Ming Tien, and Kelli Hoover

**Goal:** Identify the microbial genes that produce the enzymes for wood degradation in the ALB gut.

## Our Approach:

- DOE (JGI) is sequencing the ALB gut microbial metagenome.
- We will identify expressed genes and their functions.



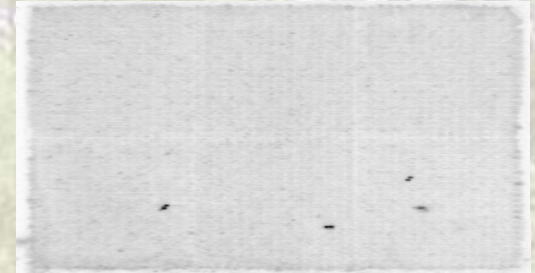
See Geib et al. Poster in Plant Feedstocks/Products/Materials section

# Genomic Resources for other potential biomass trees



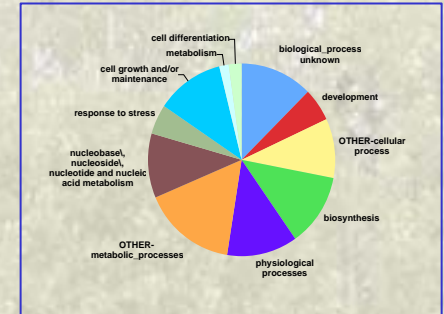
## Liriodendron tulipifera (yellow-poplar)

EST Database  
BAC Library  
Microsatellites  
Transformants  
Mapping Populations



## American Chestnut Breeding

Marker-assisted selection  
Map-based gene cloning



## The Fagaceae Genome Project

EST Databases  
BAC libraries  
Physical maps  
Genetic Maps  
Microarrays



Genome Sequencer FLX System