Genomics and Biotechnology for Improved Energy from Woody Biomass

John Carlson, Ali Barakat, Teo Best, Scott DiLoreto, Chris Frost

The Schatz Center for Tree Molecular Genetics,
The School of Forest Resources and
The Huck Institutes for the Life Sciences
at
Pennsylvania State University
Why Tree Crops for Biomass Energy?

Research Objectives and Opportunities with Poplar and Other Biomass Energy Trees
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

Spitzley and Keoleian, 2004

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

**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

Willow biomass plantation. Credit: Daniel Peck
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

Cellulose
- Polymer of β-(1,4)-glucan;
- degree of polymerization ~300 to 15,000
- Production: ~35 to 50%

Lignin
- Polymer derived from coniferyl, coumaryl, sinapyl alcohol
- Production: ~15 to 30%

Hemicellulose
- Short-chain branched, substituted polymer of sugars;
- degree of polymerization ~70 to 200
- Production: ~25 to 30%

Fig. 3. Key global biomass resources from agricultural residues, wood, and herbaceous energy crops.

(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