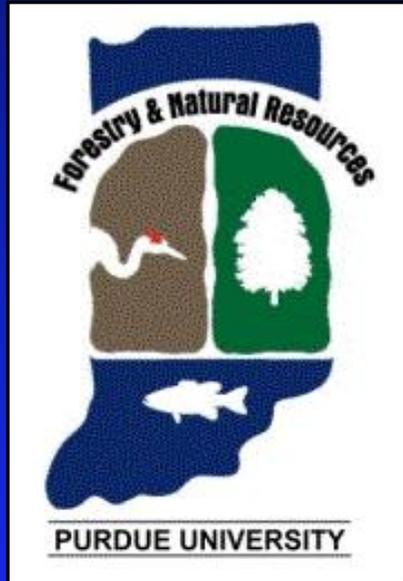


Poplar as an Energy Crop: A View from Indiana



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PURDUE
UNIVERSITY

GAME PLAN

- ◆ **Renewable Portfolio Standards**
 - **Electrical generators**
- ◆ **Aviation fuels**
 - **Civilian needs (MASBI)**
 - **Department of Defense**
- ◆ **Economic modeling**
 - **Predict profitability for growers**
 - **Yield component**
- ◆ ***Corngrass1* work**
 - **Flowering control**
 - **Other desirable traits**
- ◆ **Regulatory climate**
 - **Impediments to investment, implementation**

RENEWABLE PORTFOLIO STANDARDS

- ◆ **Percent of electricity generated from renewable sources**
 - **30 states, different targets and deadlines**
 - **Last session of Indiana General Assembly**
- ◆ **Hoosier Energy (HE) anticipating need**
 - **Demand for electricity growing by 25 MW/year**
 - **Explored various options, decided on woody biomass**
- ◆ **HE is Peabody's biggest customer in Indiana**
 - **4.5 million tons of coal each year**
 - **Continue providing HE with feedstock**
- ◆ **Peabody has abundant land, reclaims 10,000's of ac/yr**
- ◆ **Need to determine amount of land needed to sustainably supply a power plant with wood chips (on reclaimed sites)**

AVIATION FUEL

◆ Department of Defense

- **Goal of Navy and Air Force: Obtain 50% of their domestic fuel from biomass by 2020.**
- **This amounts to 745 million gallons annually by 2020**

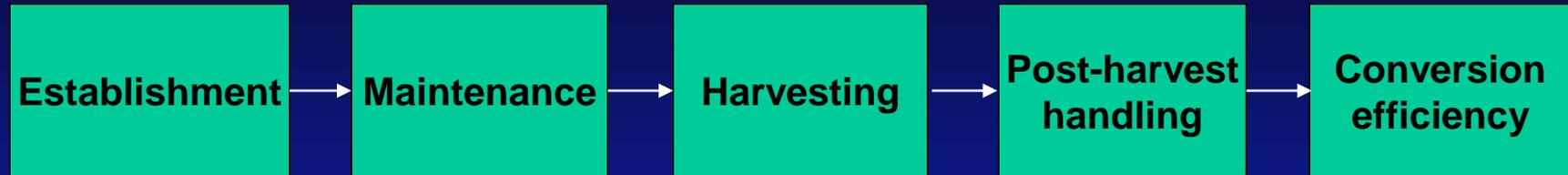
◆ Civilian needs

- **Commercial aviation consumes nearly 9 times more fuel than the U.S. military, and is also highly motivated to support the development of aviation biofuels**

◆ Midwestern Aviation Sustainable Biofuels Initiative

- **MASBI goal: By next May, publish a white paper that will serve as a roadmap demonstrating how it will be possible to develop an economically viable and sustainable aviation biofuels industry in the Midwest (“Seed to Sky”)**

ECONOMIC MODELING



- Obtain planting stock (seed, etc.)
- Sowing seed, inserting sticks

- Obtaining, applying pesticides
- Irrigation (?)

- Biomass production
- Machinery

- Chipping
- Transporting
- Drying
- Storing

- Liquid fuel production
- Direct combustion

◆ Need to predict profitability

- Convince landowners to plant a dedicated energy crop
- Account for all costs and earnings along the supply chain

◆ Working with Wally Tyner

- Department of Agricultural Economics, Purdue
- “Wood to Wheels”

SPECIES SUITABILITY AND YIELD TRIALS

Purdue Agricultural Centers (PACs)

- ◆ Pinney PAC (east of Valparaiso) and SWPAC (north of Vincennes)
- ◆ 69 poplar genotypes
- ◆ 4 management regimes
- ◆ Contrasting soil types
- ◆ Different climatic zones
- ◆ Established May 2011
- ◆ Measurements:
 - Survival
 - Yield (total biomass)
 - Pest (disease and insect susceptibility)
- ◆ Sponsored by Hoosier Energy



DESIRED TRAITS FOR BIOENERGY CROPS

- ◆ **Vegetatively propagated**
- ◆ **Rapid growth**
- ◆ **High conversion efficiency**
 - **Lignin content, composition**
- ◆ **Reproductive sterility**
 - **Diversion of carbohydrate away from vegetative growth**
 - **Transgene confinement**
- ◆ **Ease of harvesting**

microRNAs (miRNAs)

- ◆ **miRNAs control developmental timing in animals such as *C. elegans* and *Drosophila*, and in plants such as *Arabidopsis thaliana*, *Zea mays*, *Antirrhinum majus*, and *Petunia hybrida***
 - **Often have stage- or tissue-specific expression**
 - **Post-transcriptional control**
 - ✓ Hairpin precursor formed as a result of inverted repeat; it gets cleaved and unwound
 - ✓ Hybridizes to mRNA for degradation of target gene
 - **Control can also be at the level of translation, depending on the degree of complementarity of the binding site to the miRNA**

CORNGRASS1 (Cg1)

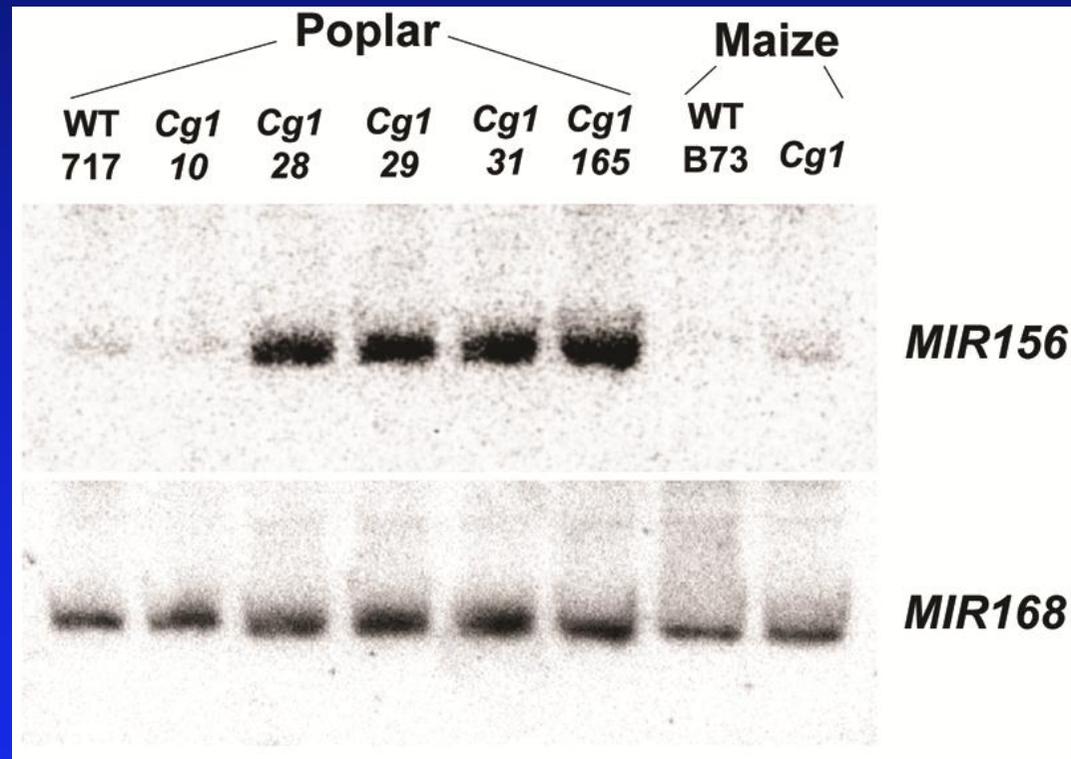
- ◆ **Cg1 encodes tandem miR156 genes**
 - Originally identified in a mutant screen of maize (*Zea mays*)
 - Targets highly conserved SBP box
 - Controls fate of meristems and lateral organs
- ◆ **The maize *cg1* mutant exhibited:**
 - Adventitious root formation
 - Shoot proliferation
 - Sterility
- ◆ **Over-expression of *Cg1* in heterologous, herbaceous plant species results in several consistent phenotypes**
 - Multiple stems
 - Faster growth
 - Lower lignin
 - Higher carbohydrate levels
 - No or delayed flowering

Cg1 OVER-EXPRESSION IN POPLAR

- ◆ Transformed *Cg1* into poplar
 - Hybrid aspen genotype INRA 717-1B4 (*Populus tremula* x *P. alba*)
 - Under control of the constitutive 35S promoter
- ◆ 30 independent lines (unique transformational events) regenerated *in vitro* in the presence of a selection agent
- ◆ Transgene presence verified via standard PCR
- ◆ *Cg1* message was processed to produce a mature miRNA (next slide)
- ◆ So far, transgenic poplar phenotypes are consistent with what has been seen in other species

SMALL RNA NORTHERN BLOT

Cg1 transcript processed into mature miRNA in poplar the same way as in maize



35S::*Cg1* POPLAR PHENOTYPE

Figure 1. Comparison of wild-type hybrid aspen clone INRA 717-1B4 and 35S:*Cg1* transgenic plants. A) Whole plants at 7 months of age. Wild-type (left-hand plant) and 35S:*Cg1* transgenic (right-hand plant). Ruler = 1 m. B) An ~40-cm apical shoot removed from one-year-old wild-type 717 (left-hand shoot) and 35S:*Cg1* (right-hand shoot) plants. Leaves were removed to reveal syleptic branching.

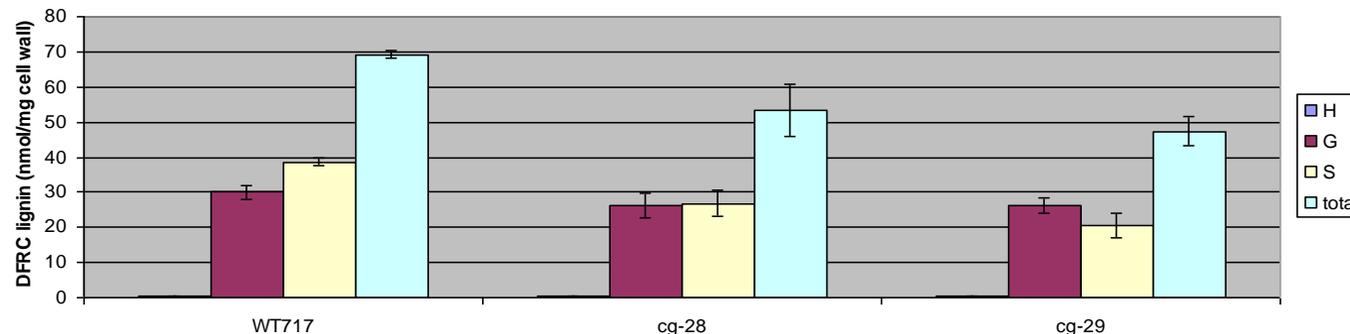
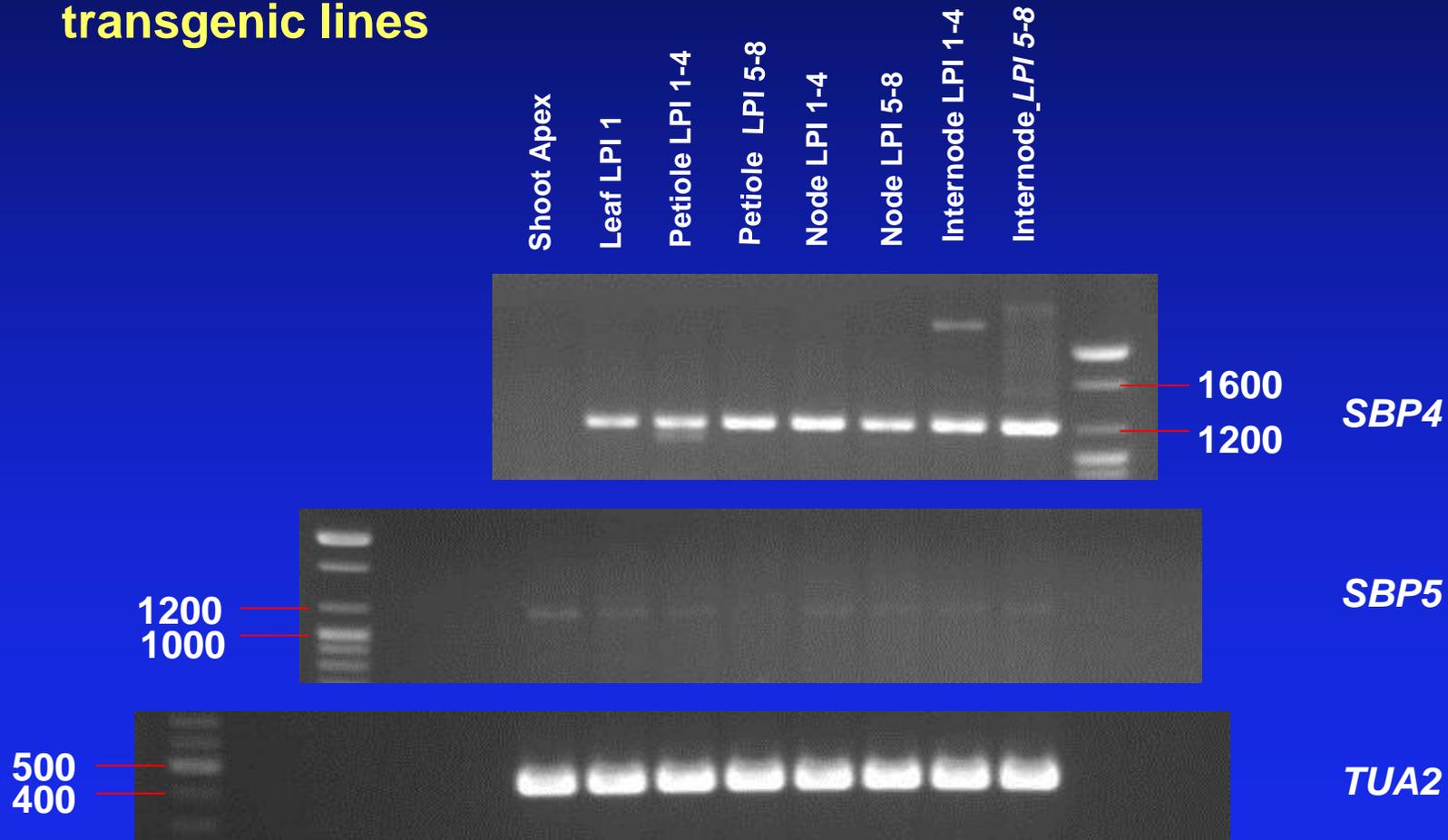


Figure 2. Analysis of total lignin and monolignol composition on three biological replicates of the wild type and two transgenic lines. H, coumaryl monomer; G, guaiacyl monomer; S, syringyl monomer. Bar heights represent mean monolignol/lignin content and brackets represent one standard deviation of the mean.

Reference: Rubinelli, P.M., Chuck, G., Li, X., and Meilan R. 2012. Constitutive expression of microRNA *Corngrass1* in poplar affects axillary meristem outgrowth, internode length, and lignin quantity and composition. *Biomass and Bioenergy* (in press).

EXPRESSION PATTERNS OF *SBP* GENES

- ◆ 14 poplar genes contain an SBP box
 - Potential targets of *Cg1*
 - Begun looking at their expression levels in various tissues of our transgenic lines



REGULATORY AFFAIRS

- ◆ Decisions concerning GE trees are based largely on the technique(s) used to produce them.
- ◆ They should be made on a case-by-case basis, considering the gene(s) being used, the biology of the species being engineered, and where the trees will be deployed.
- ◆ Regulatory hurdles contribute to industry's reluctance to support research that is needed to advance the technology.
- ◆ To foster investment and expedite progress, it will be necessary to implement regulatory reforms rather than requiring innovators to jump through unjustified regulatory hoops that are based on hypothetical or unfounded risks.
- ◆ Revision of the regulatory framework will involve engaging lawmakers and attracting a spectrum of stakeholders to take part in scientific and public debates.

Reference: Meilan, R., Harfouche, A., and Nehra, N. 2012. Risk Assessment of Biotech Trees: A Path Forward. *Journal of Forestry* 110(3):171-172.

SUMMARY

- ◆ **There is likely to be growing demand for woody feedstocks**
 - **Electrical generation**
 - **Aviation fuel sector**
- ◆ **Need to attract growers**
 - **Economic models (region-specific) for predicting profitability**
- ◆ **Economic model under construction**
 - **Incorporation data from field studies**
 - **Predictive tool for potential growers**
- ◆ **Work being done to improve poplar's utility**
 - **Conventional breeding and genetic engineering**
 - **Not just lignin modification**
 - **Transgene confinement essential**
- ◆ **Regulatory impediments**

QUESTIONS?



Photo provided by Jake Eaton, GreenWood Resources