Purpose-Grown Trees:
Providing Socio-economic Value

Maud Hinchee
ISCHP 2011
October 17, 2011



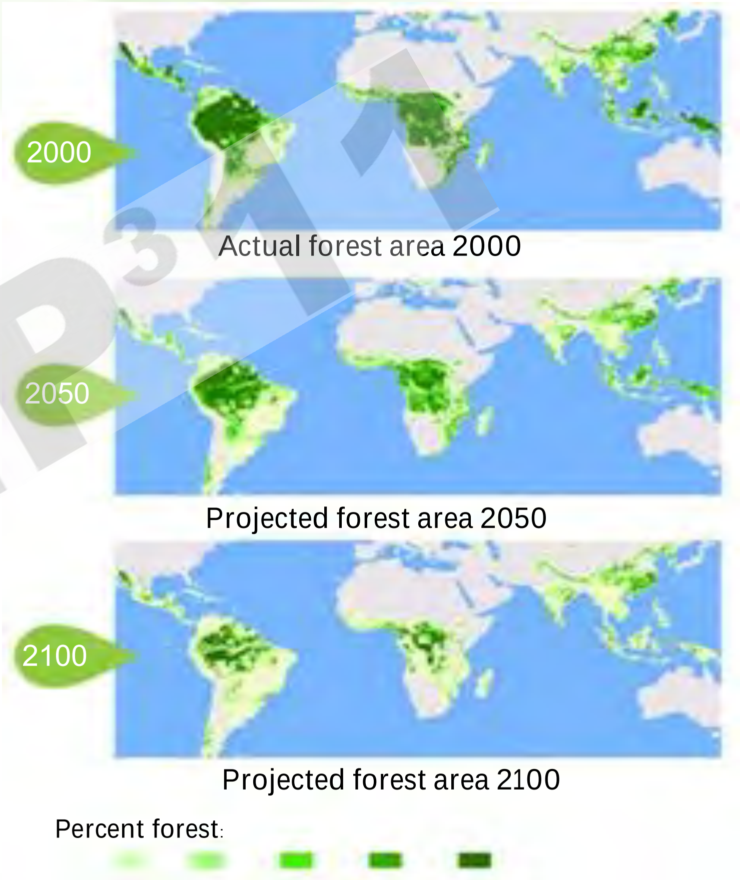


Forests: What future do we want?

•Secure and healthy forests have helped stabilize the climate throughout the world.

•Responsibly managed plantations supply fibre for materials and energy, deliver important ecosystem services, and share the landscape with wild forests, towns, productive farms, and nature reserves.

•Maintaining forests is a cornerstone of national and international policies.



Forest area in 2000 and projected forest area in 2050 and 2100, As calculated by the Living Forests Model under a Do Nothing Scenario, in which demand for land increases to supply a growing global population with food, fibre and fuel, and historical patterns of poorly planned and governed exploitation of forest resources continue.

The living forests vision

How do we halt forest loss and balance the potential implications for human well-being, economic development, and the wider environment?

- We are currently exceeding the Earth's biocapacity to produce renewable resources and absorb CO_2 by 50 per cent. *

- To eliminate this ecological overshoot, we need to balance human demand with the regenerative capacity of the planet.

- If we maintain current resource use, we will need the equivalent of two planets by 2030.

*WWF 2011 – Living Forests Report

Can we sustain net zero deforestation as human population rises?

- The WWF Living Forests Model suggests that achieving net zero deforestartion beyond 2030 will *require higher productivity* across large, often suboptimal, areas of land
  - Requiring hundreds of millions of farmers and foresters changing to more sustainable and productive practices- a task of an unprecedented scale.

- Improved productivity can bring its own environmental costs, including salinization, erosion, depleted aquifers, increased energy use, pollution and biodiversity loss.
  - low-input, knowledge-based intensification will be required

Tree genetic improvement, biotechnology & zero net deforestation

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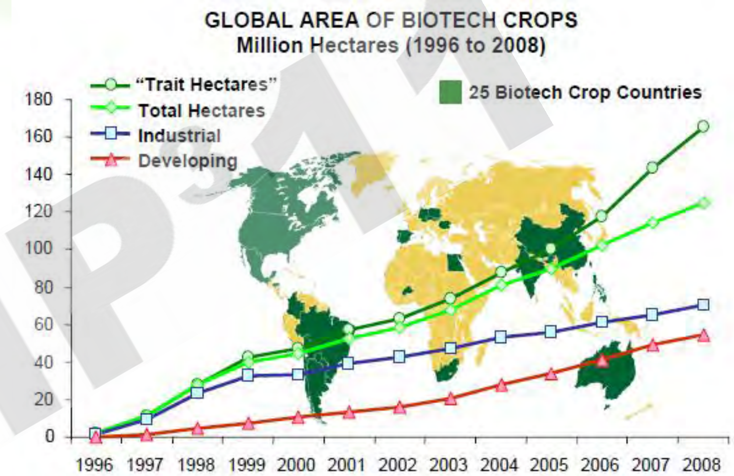
To meet the demands on our forest resources, we will need to maximize the efficiency of our existing resource areas.

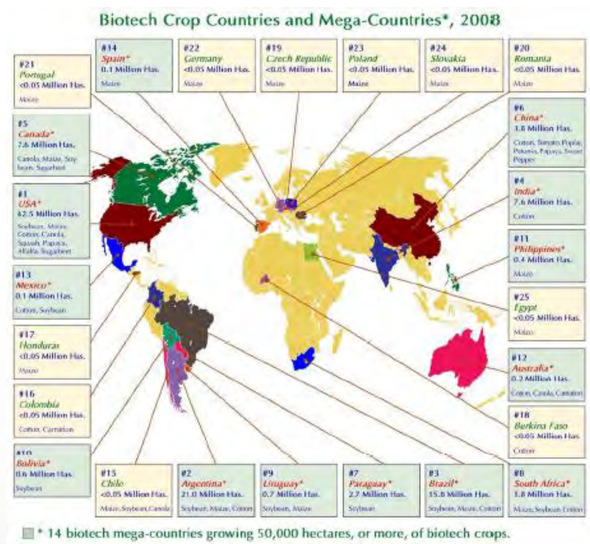
"Biotechnology provides powerful tools for the sustainable development of agriculture, fisheries and forestry, as well as the food industry. When appropriately integrated with other technologies for the production of food, agricultural products and services, biotechnology can be of significant assistance in meeting the needs of an expanding and increasingly urbanized population in the next millennium."

Source: FAO

Biotech has successfully been introduced in agriculture throughout the world

- First ag biotech crop planted in 1996
- Significant increase in biotech crop production since then
  - 25 countries
  - > 300 million acres planted in 2008
  - 2 billion cumulative acres planted
  - Including key agricultural crops
    - 70% Soybean
    - 46% Cotton
    - 24% Maize
    - 20% Canola
- Regulatory path in place in key markets
- Increasing public acceptance





Source: Global Status of Commercialized Biotech/GM Crops, 2008

Tree Biotechnology

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- The application of biotechnology to commercial plantation forestry offers many opportunities to increase the productivity and quality of the crop
- Biotechnology contributes to the goals of producing more wood per acre, improved forest health, sustainability and lower greenhouse gas emissions.

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Through increased per acre productivity, forest biotechnology will play a significant role in meeting global mandates on:

- Renewable Energy
- Climate Change
- Food Security

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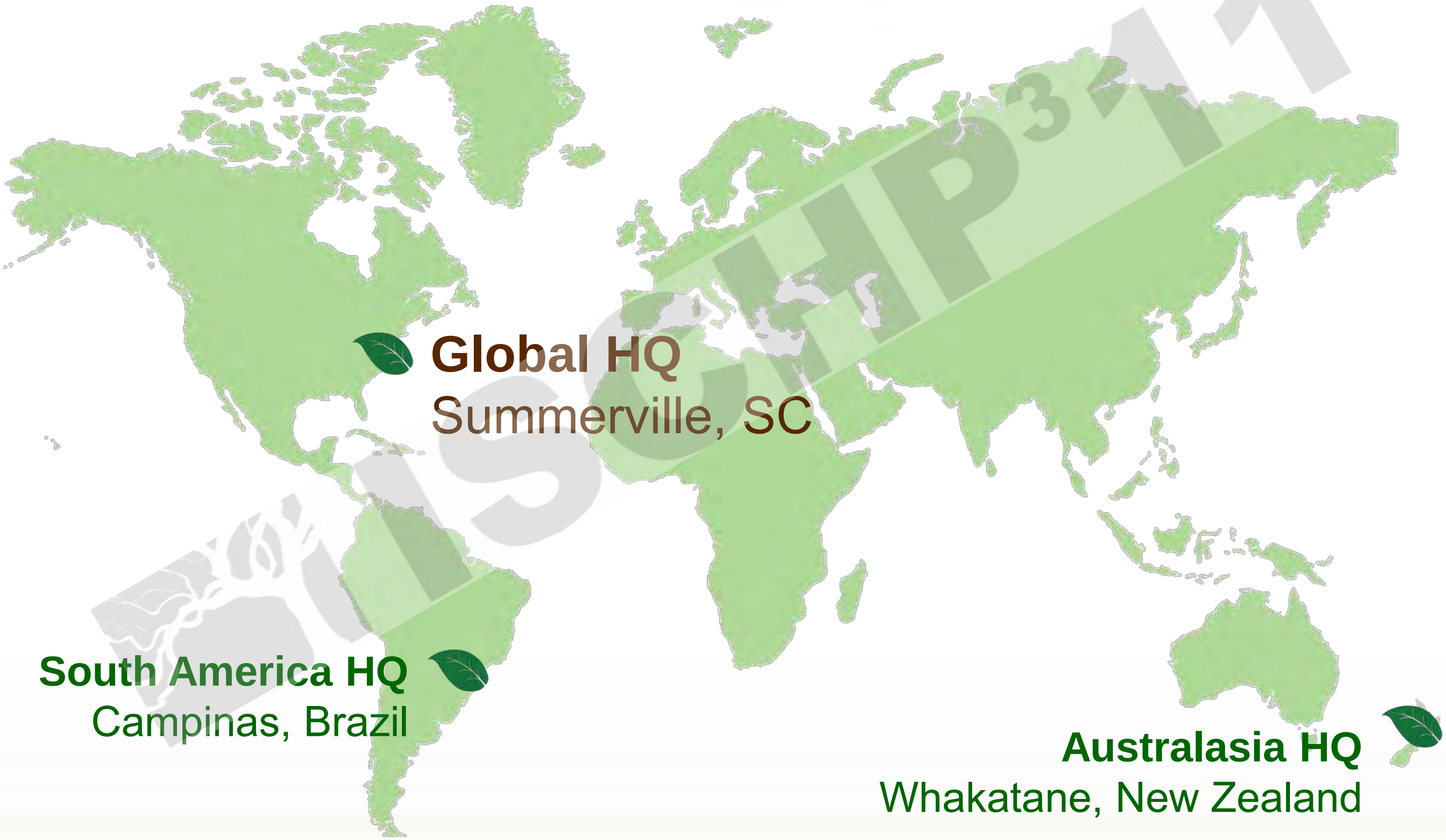
ArborGen Vision:

We are dedicated to providing superior performing trees for wood, fiber and energy, while helping conserve the world's native forests in all their beauty, diversity and complexity.

Our Mission:

We will deliver superior performing trees through innovative science and world class customer service – every tree, every time.

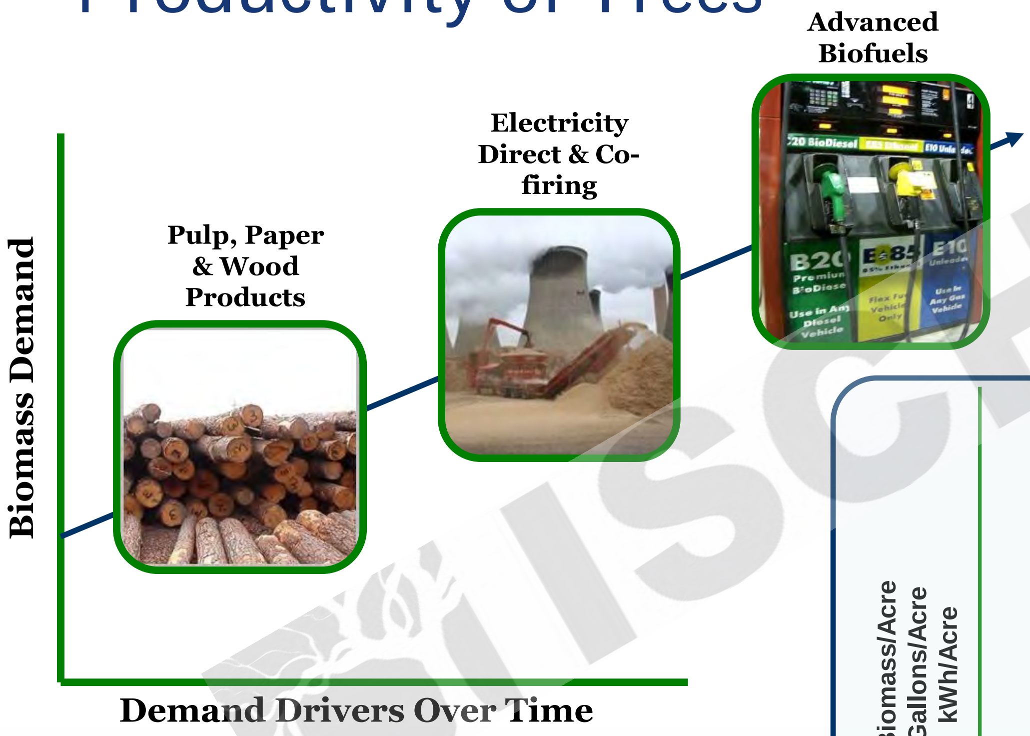
ArborGen: Global Leader in Tree Improvement



Who We Are: Focused on the Future of Forestry

- Leading producer of purpose grown trees
  - Produce nearly 300 million seedlings per year
  - Drawing on 50+ years of forestry and technology experience
  - Multi-national team of dedicated conservationists, biologists, foresters, researchers and scientists
- Technology leader
  - Innovative product platform: Pine and hardwood
  - Pipeline of world-class elite germplasm
  - More forestry field / regulatory trials than any other companies

As Demand for Woody Biomass Increases, ArborGen is Focused on Improving the Productivity of Trees




The world doesn’t just need more trees,
we need trees that *can do more*.

Our approach: Providing better, more sustainable purpose grown trees

- Conventional tree improvement
  - Breeding and selection
- Accelerated improvements through advanced genetic technologies
  - Hybrids
  - Advanced propagation technologies
  - Introduced traits

ArborGen products and R&D pipeline

- More than 100 species of Pine and Hardwood for traditional planting stock
  - Loblolly, Slash, Longleaf Pine
  - Oak, Elm, Birch, Poplar, Black Walnut
- Biotech product portfolio
  - Eucalyptus, Loblolly Pine, Populus
- Trait introductions
  - Yield Enhancement, Improved Wood Quality, Pollen Control, Stress Resistance

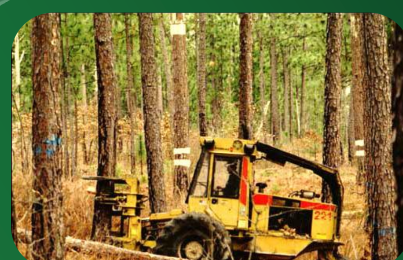
Our research provides solutions to improving wood production per acre

Improved Germplasm

- *Conventional Breeding*
- *Varietal Technology*

Management Systems

- *Increased Densities*
- *Shortened Rotations*
- *Other Silvicultural Improvements*



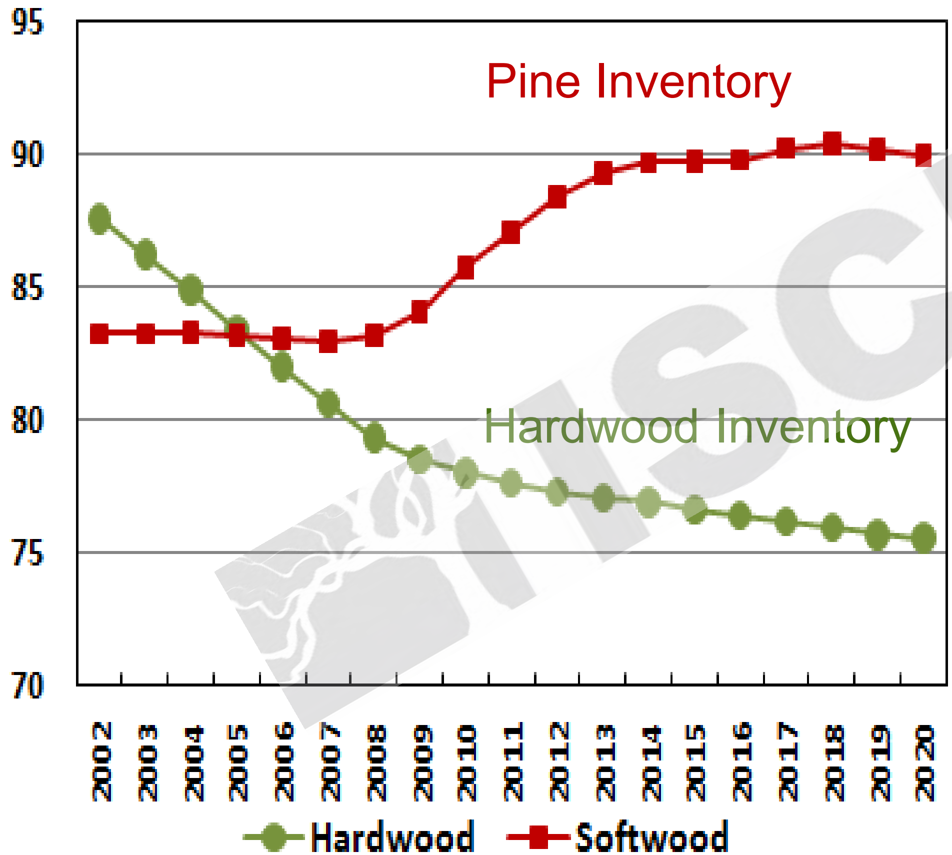
Biotech Improvements

- *Improved Growth*
- *Shorter Rotation*
- *Stress Tolerance*
- *Improved Processing*
- *Improved Wood Quality*

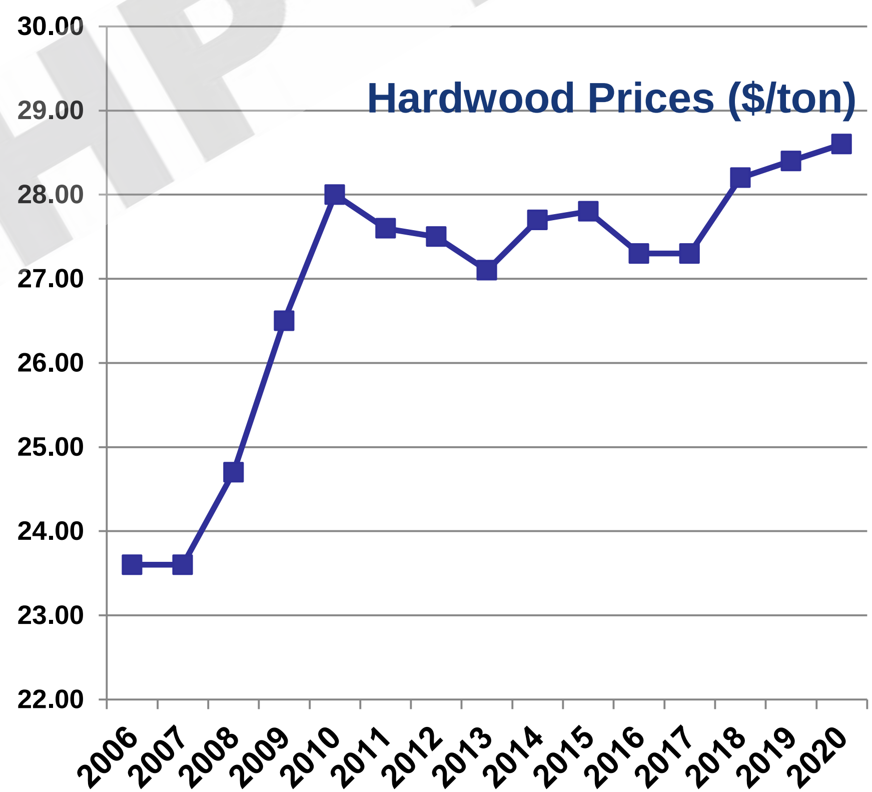
Productivity improvements increase the amount of biomass that can be produced from existing forestland to supply both existing and newly emerging demand

The traditional pulp and paper industry the Southeastern (SE) US is facing price constraints from limited hardwood availability

US South Private Operable Growing Stock Inventory 2002-2020 (billion cubic feet)



Hardwood Pulpwood Delivered Prices Adjusted for Inflation ($2009)



Source: RISI 2010. North American Timber Forecast

Brazilian operational Eucalyptus clones can grow well in non-freeze challenged regions of the Southeastern US

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- *Eucalyptus grandis* x *E. urophylla hybrid* (EH1)

48 Months Growth
in
Central Florida:
56 feet tall
6.4 inch diameter

Pulpwood Yield Potential:
7.5 – 12.5
dry tons/ac/yr

Biomass Yield Potential:
27 – 33
dry tons/ac/yr

Chilling tolerant Eucalyptus species and freeze tolerant *E. urograndis*

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- Provide significant biomass productivity
- Coppicing ability allows for the production of multiple harvest crops from a single planting

Chilling Tol. *E. benthamii in South Carolina*

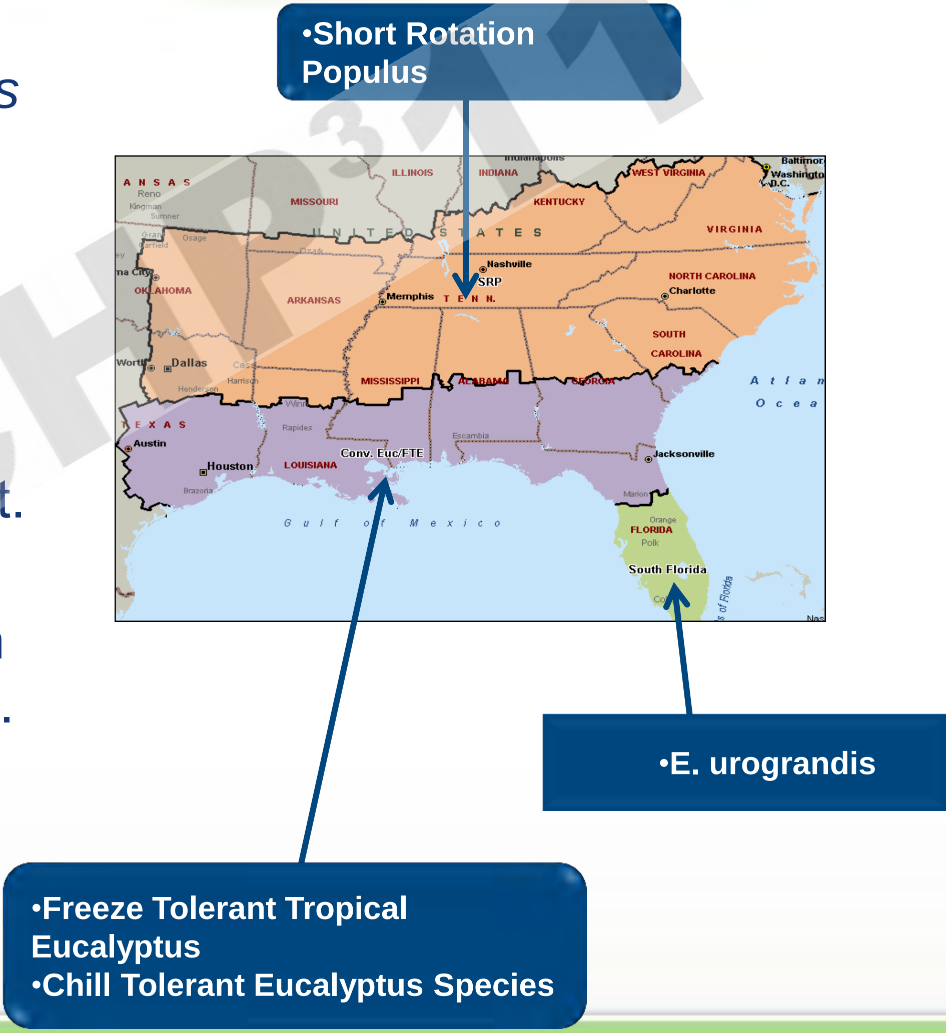
14 to 18 green tons/acre/year
4 year rotation
3 harvests

Biotech *E. urograndis* In Alabama

14-33 green tons/acre/year
4 yr rotation
3 harvests

Addressing the Southeast U.S. need for purpose grown hardwood: *AGEH427 Freeze Tolerant Eucalyptus*

- AGEH427 is a *Eucalyptus urograndis* hybrid (*Eucalyptus urophylla X Eucalyptus grandis*).
- Genetically enhanced to tolerate freeze events with minimal damage.
- Currently grown on a research scale on various sites along the Gulf Coast.
- Yields15 green tons/acre/year within the identified deployment zone when challenged with winter freeze events.
- AGEH427 wood has excellent qualities for pulping and bioenergy, and is quite suitable for mulch and other products.



Freeze tolerance gene

- CBF gene and mechanism
  - Cold responsive transcription factor in plants[1]
  - Induces cold protection pathway
  - Found across all plant species, but plants adapted to tropical climates don't properly express pathway[2]
  - First demonstration in transgenic Arabidopsis that over expression of inserted CBF genes can promote cold and freeze tolerance[3]
- Expression can be controlled by a cold inducible promoter
  - Rd29a – Arabidopsis promoter that drives gene expression in response to cold and other abiotic stress[4]

[1]Jaglo-Ottosen, KR, et al. 1998. Science 280:104-106.
[2]Jaglo, KR, et al.. 2001 Plant Physiol. 127:910-917.
[3]Cook, D., et al.. 2004. PNAS 101:15243-15248.
[4]Naruksa, Y., et al. 2003. Plant Journal 34:137-148.

How does CBF2 generate freeze tolerance?

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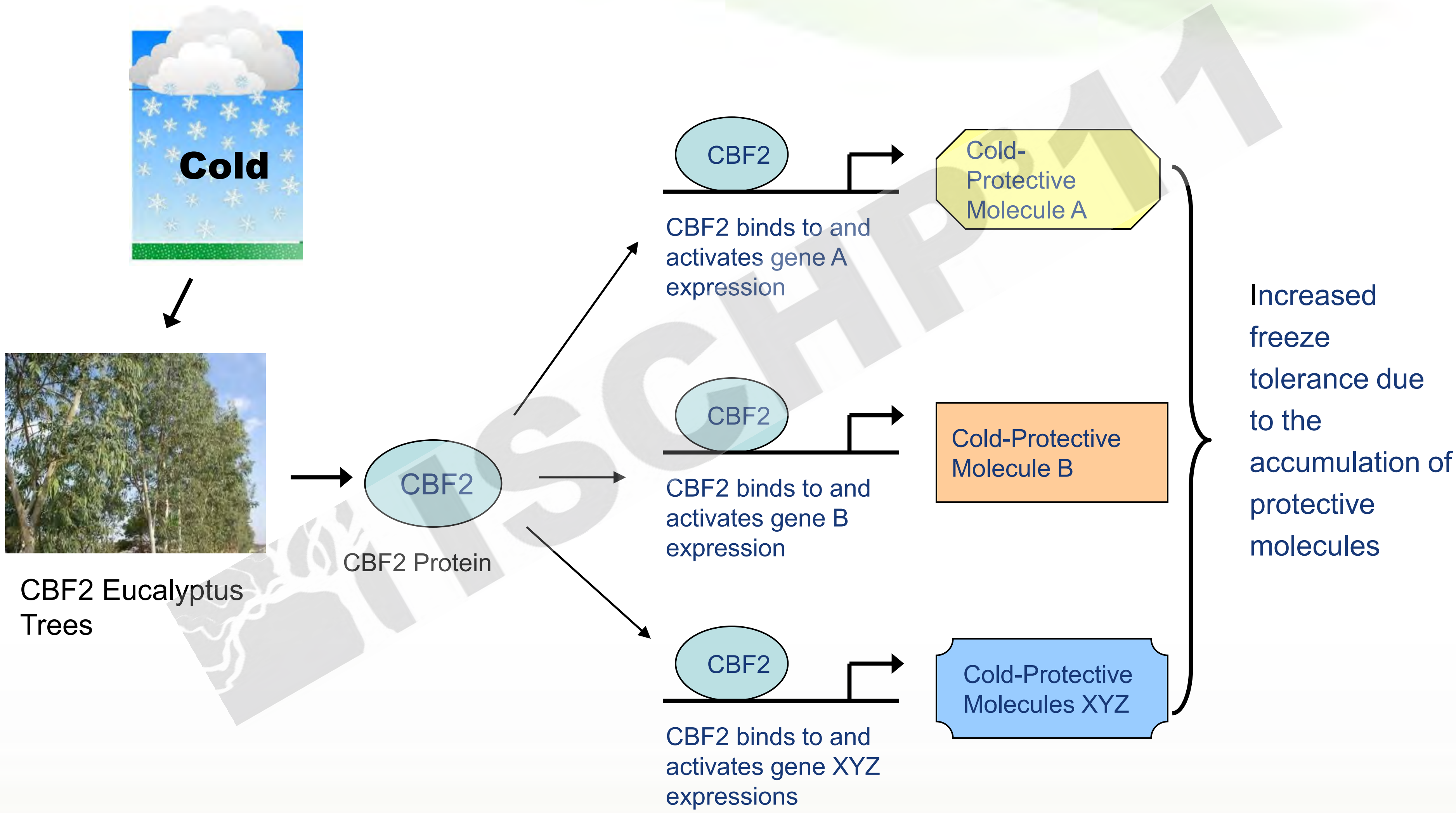

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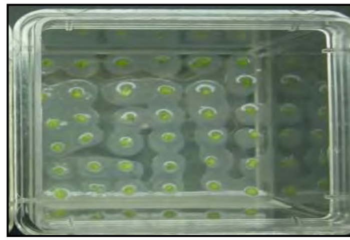
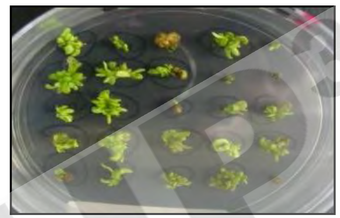
Single insert, backbone-free transgenic lines produced in *E. urograndis* EH1

Varietal stock



Agrobacterium transformation



Regeneration

Transgenic line selection

Propagation

Rooted plants

Field trials

Initial cold chamber testing

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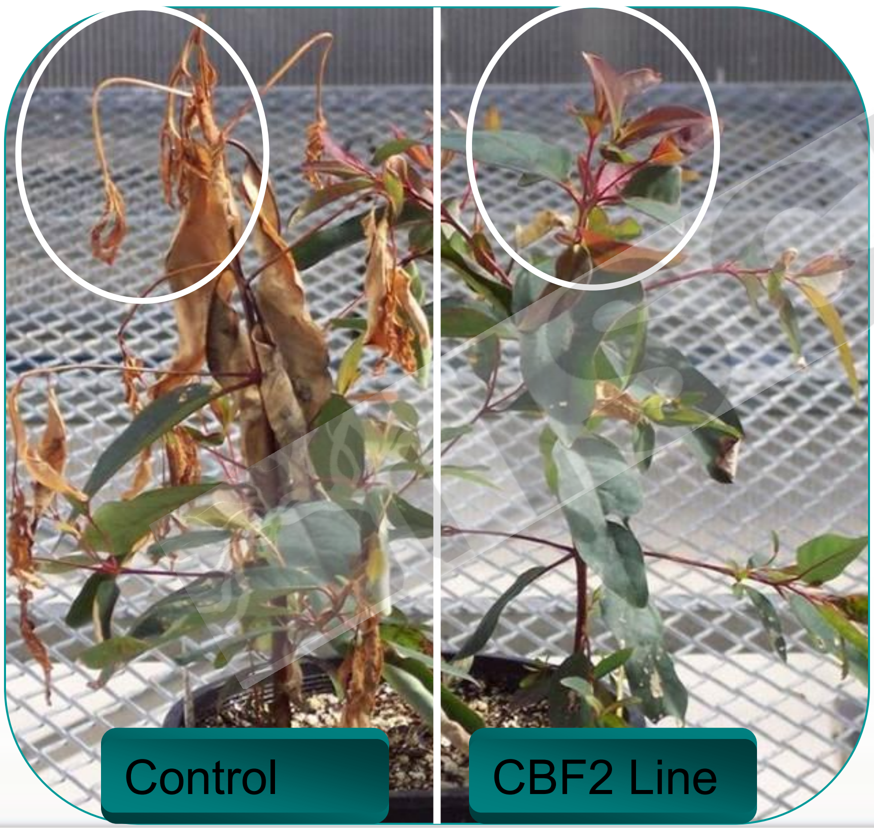
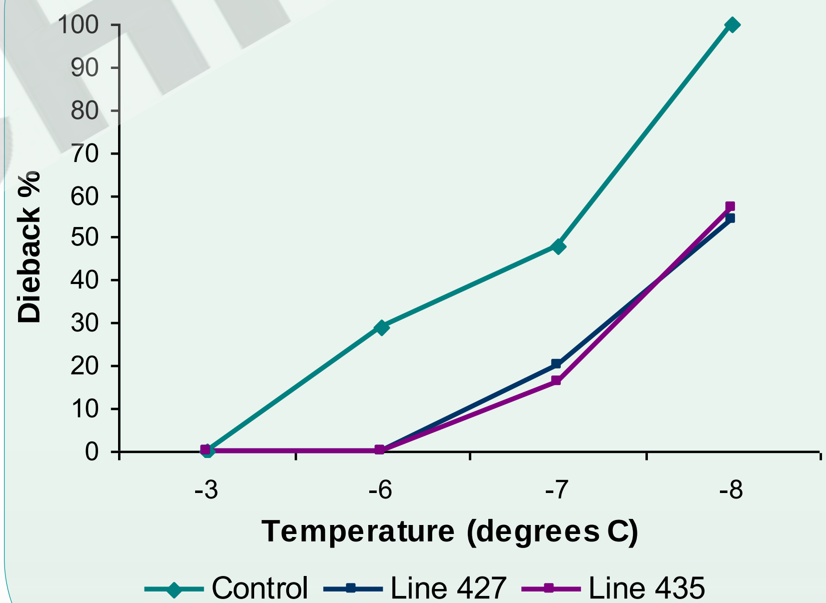
- Rd29a::CBF2 confers freeze tolerance in chamber tests
- Transgenic lines have a 3ºC improvement in tolerance





Freeze tolerant Eucalyptus field performance demonstrated

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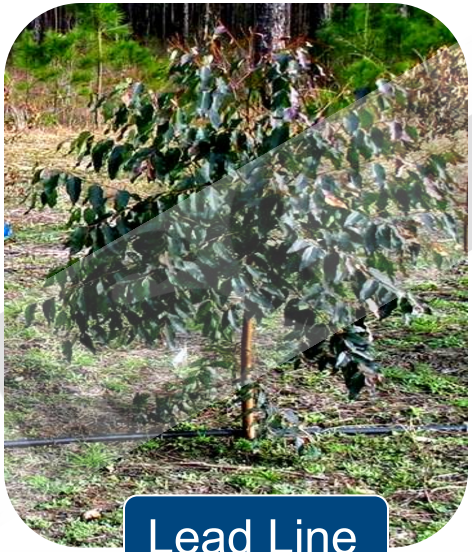
Field results indicate freezing tolerance to ~16°F (-8°to -9°C)
Height @ 48 months – 56 feet, DBH @ 48 months 6.4 inches

Control



Lead Line

Lead Lines + Control

Commercial performance further validated under severe winter conditions

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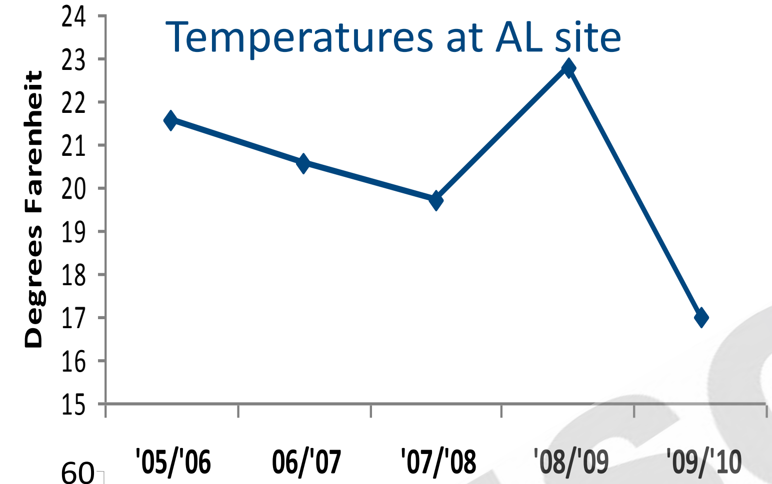

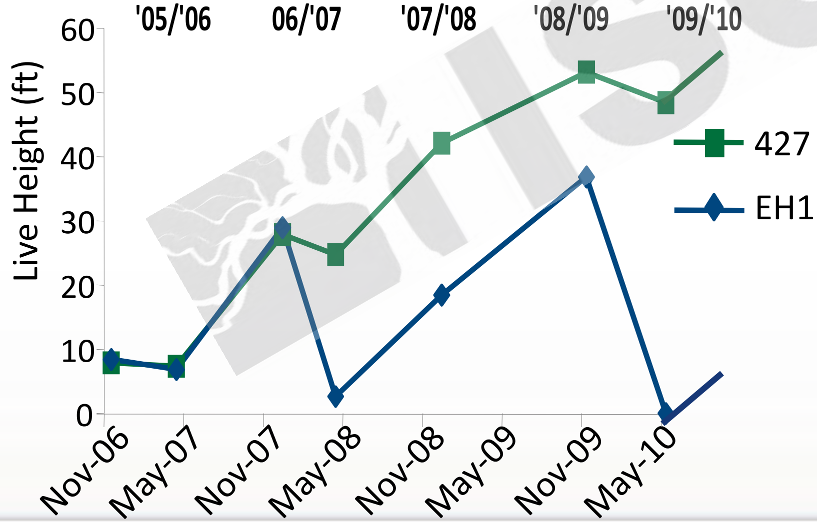

Control - EH1

FTE Line 427

AL Site, May, 2010

Risk of gene flow from biotech Eucalyptus into native trees is unlikely

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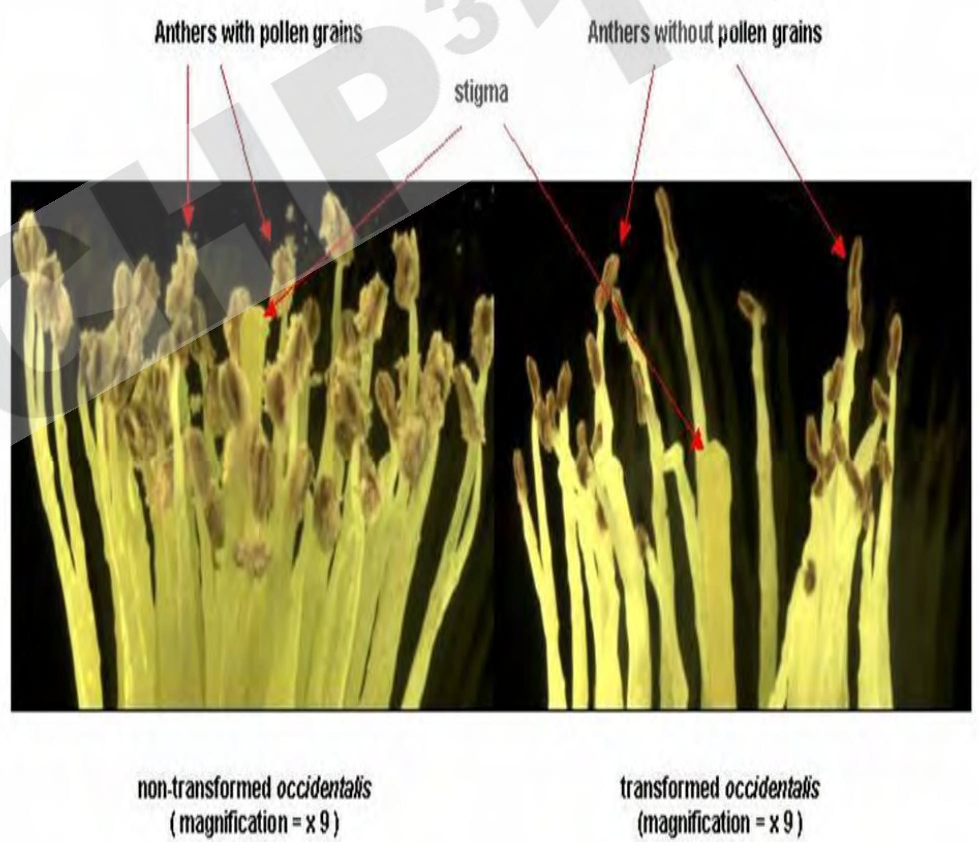
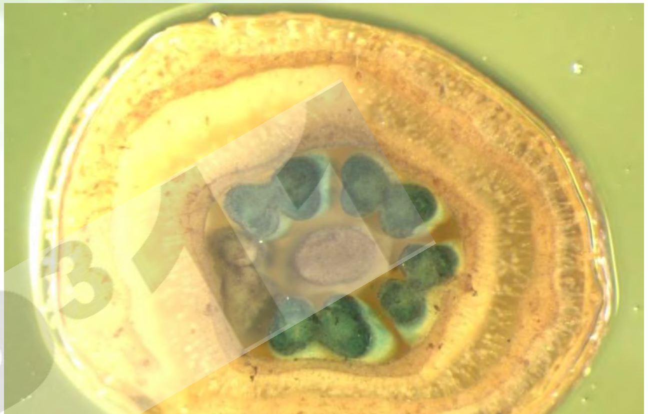
- Limited natural reproduction
  - In-breeding depression results in no or low seed set
  - Poor seed germination
  - No natural vegetative propagation
- No sexually compatible native species
- Pollen control gene in AGEH427



ArborGen Pollen Ablation for Multiple Tree Species


Developing Tobacco Flower

- *Pinus radiata* anther specific promoter
  - PrMC2

- Modified *Bacillus amyloliquefaciens* barnase coding sequences
  - Single amino acid substitutions were generated: barnaseH102E, barnaseK27A, barnaseE73G and barnaseF106S.
  - Relative activity estimated by *E. coli* modified barnase transformants (w/o barstar) colony growth characteristics (colony size and number)
  - strongest to weakest (no colonies up to 1.0mm colonies after 24hrs)

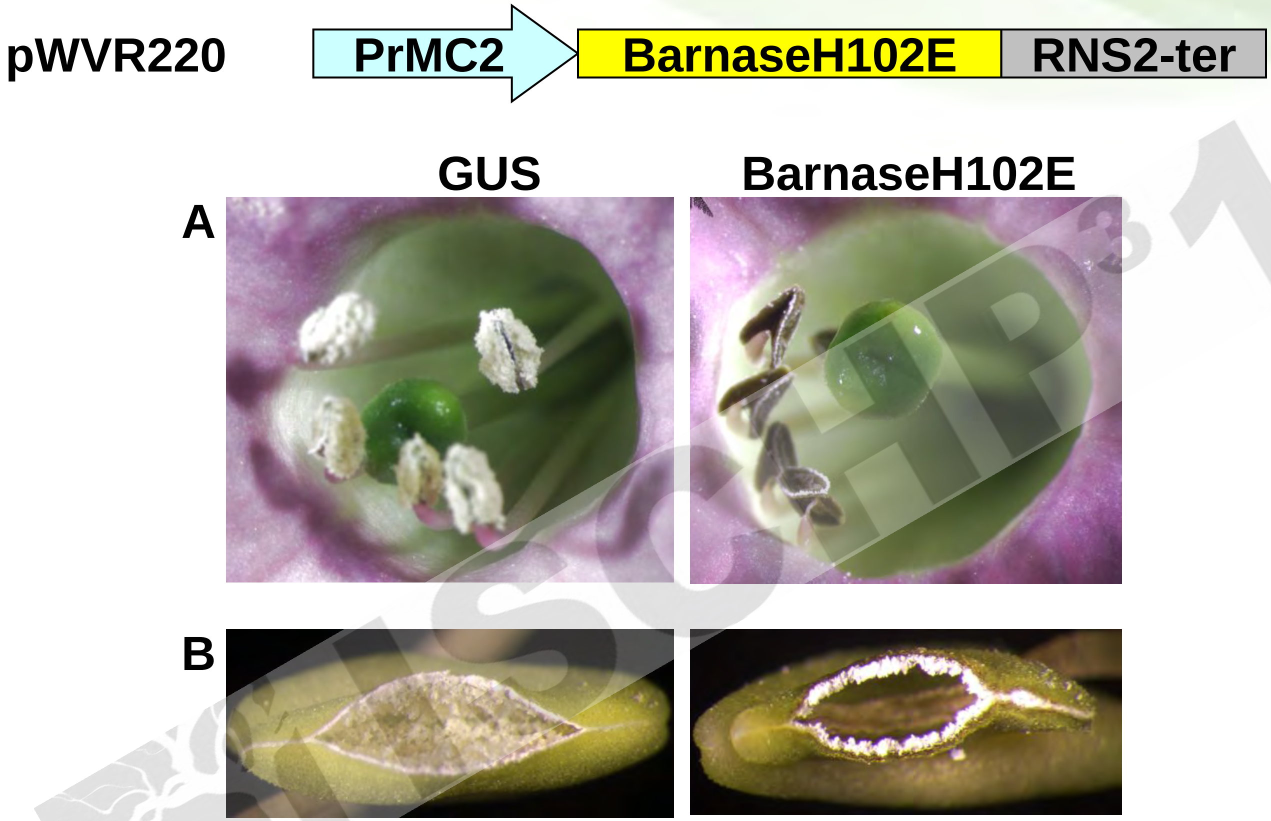
barnaseK27A >

barnaseF106S >

barnaseE73G >

barnaseH102E = barnaseH102Y

First Demonstrated in Tobacco



- All 18 barnaseH102E tobacco transgenic lines containing pWVR220 did not produce pollen.
- All 12 GUS tobacco lines produced normal pollen.

Demonstrated in Eucalyptus

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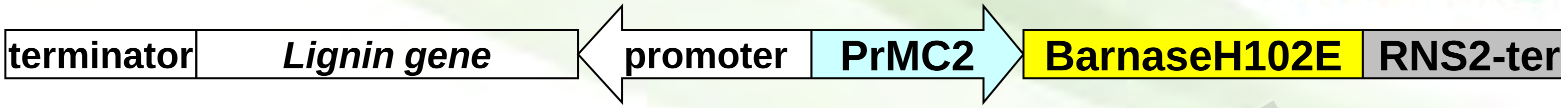
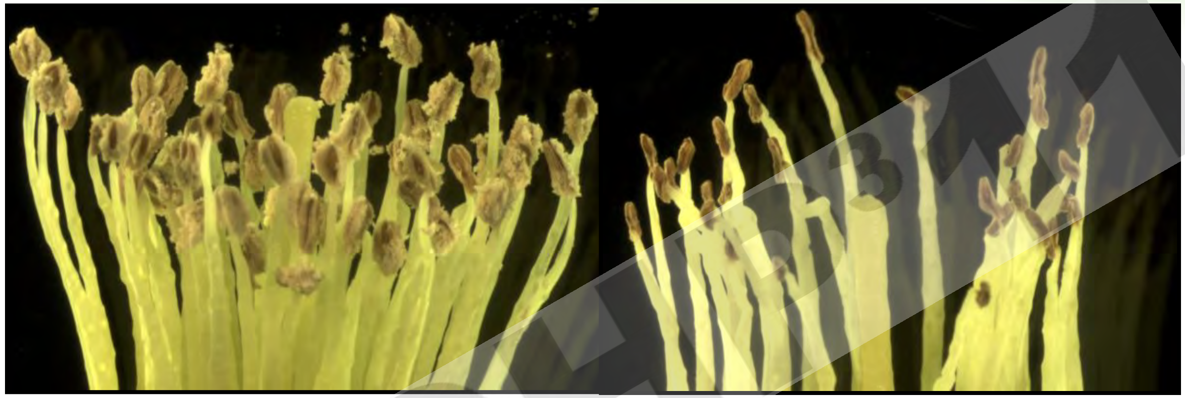
pARB598

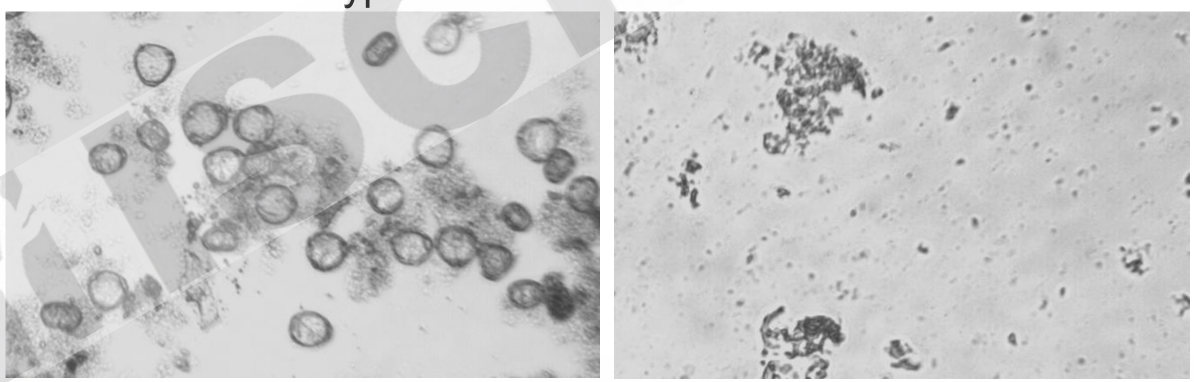


Anthers at Anthesis



Wild-type BarnaseH102E

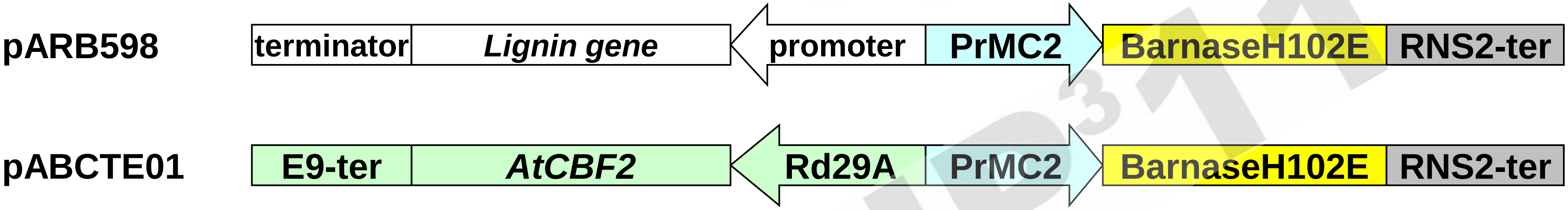
Microscopic view of material In anthers prior to anthesis



Wild-type BarnaseH102E

- 23 *Eucalyptus occidentalis* lines produced
- Flowering occurred 4 months after planting in greenhouse
- 22 of the 23 lines had completed pollen control; all wild-type trees produced pollen.

Results from field grown AGE427 Eucalyptus and lignin modified Eucalyptus



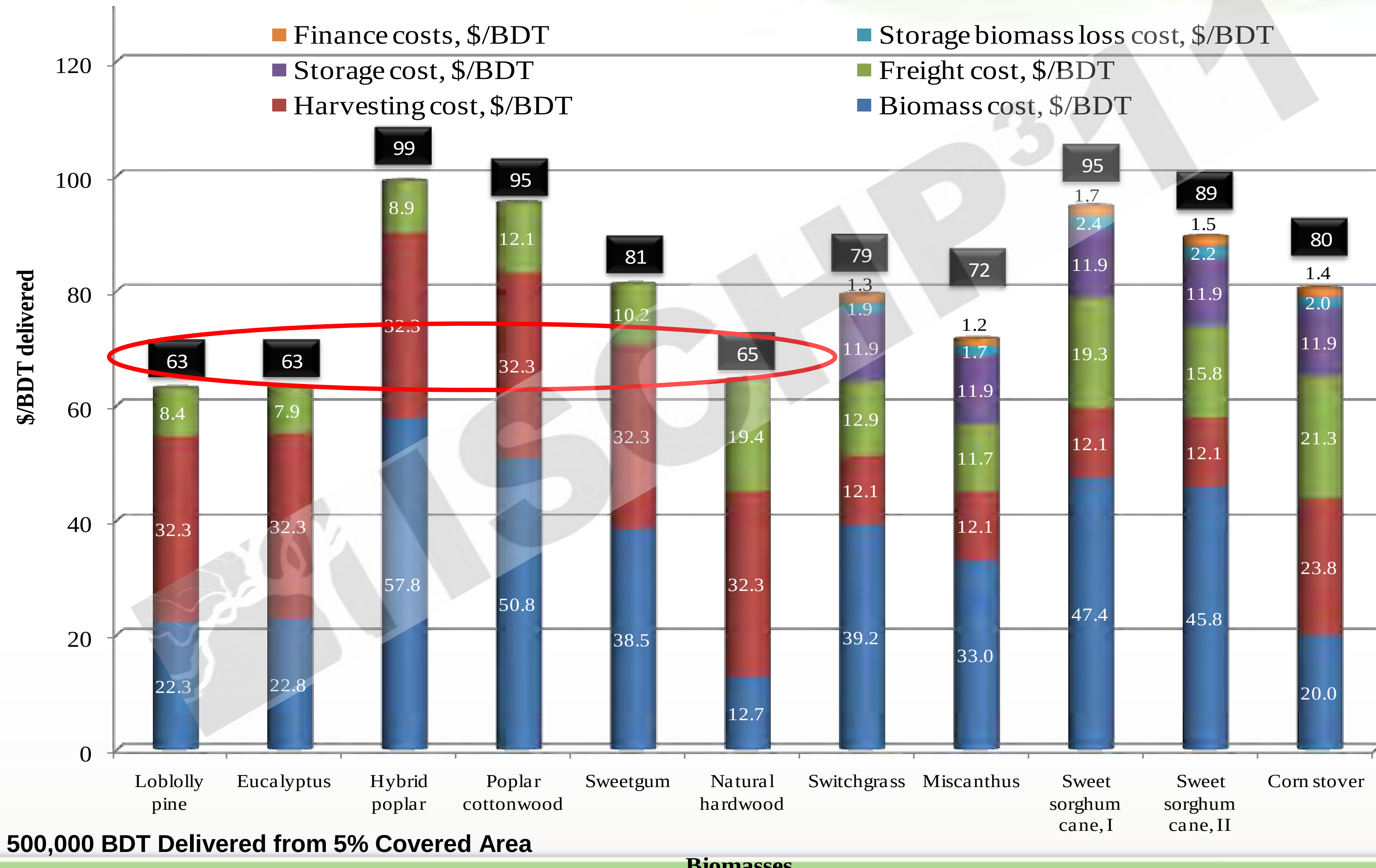
Field Location	Construct	# of Lines / Total # of Trees Studied	# of Lines with Pollen-less Phenotype	Did Pollen-less Lines Show Stable Expression?
Central Florida	pARB598 or pARB599	29 / 87	27	Yes
Southern Alabama	pABCTE01	12 / 96	12	Yes

Environmental Considerations



- **Land Use**: Eucalyptus plantations in the Southeast US would be planted on land already in pine plantations or on marginal land
- **Invasive Characteristics**: *E. grandis x E. urophylla* species and hybrids do not show aggressive invasive characteristics; IFAS score of 3 – noninvasive.
- **Hydrology**: Uses less water per unit weight of biomass produced than many agricultural crops

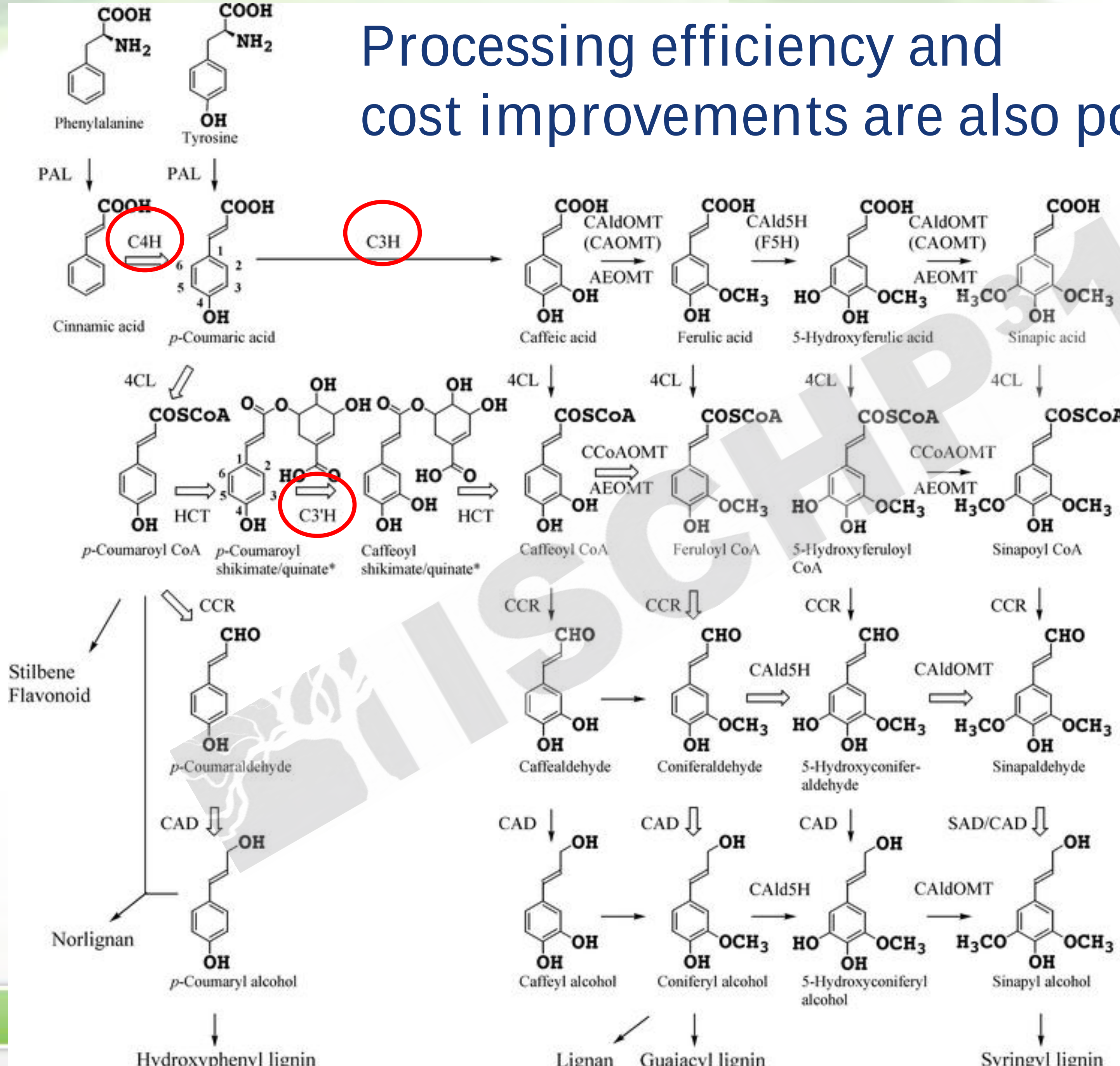
Purpose grown Eucalyptus will have the economic benefit of low costs per ton



* 500,000 BDT Delivered from 5% Covered Area

Source: NCSU Wood to Ethanol Consortium

Processing efficiency and cost improvements are also possible

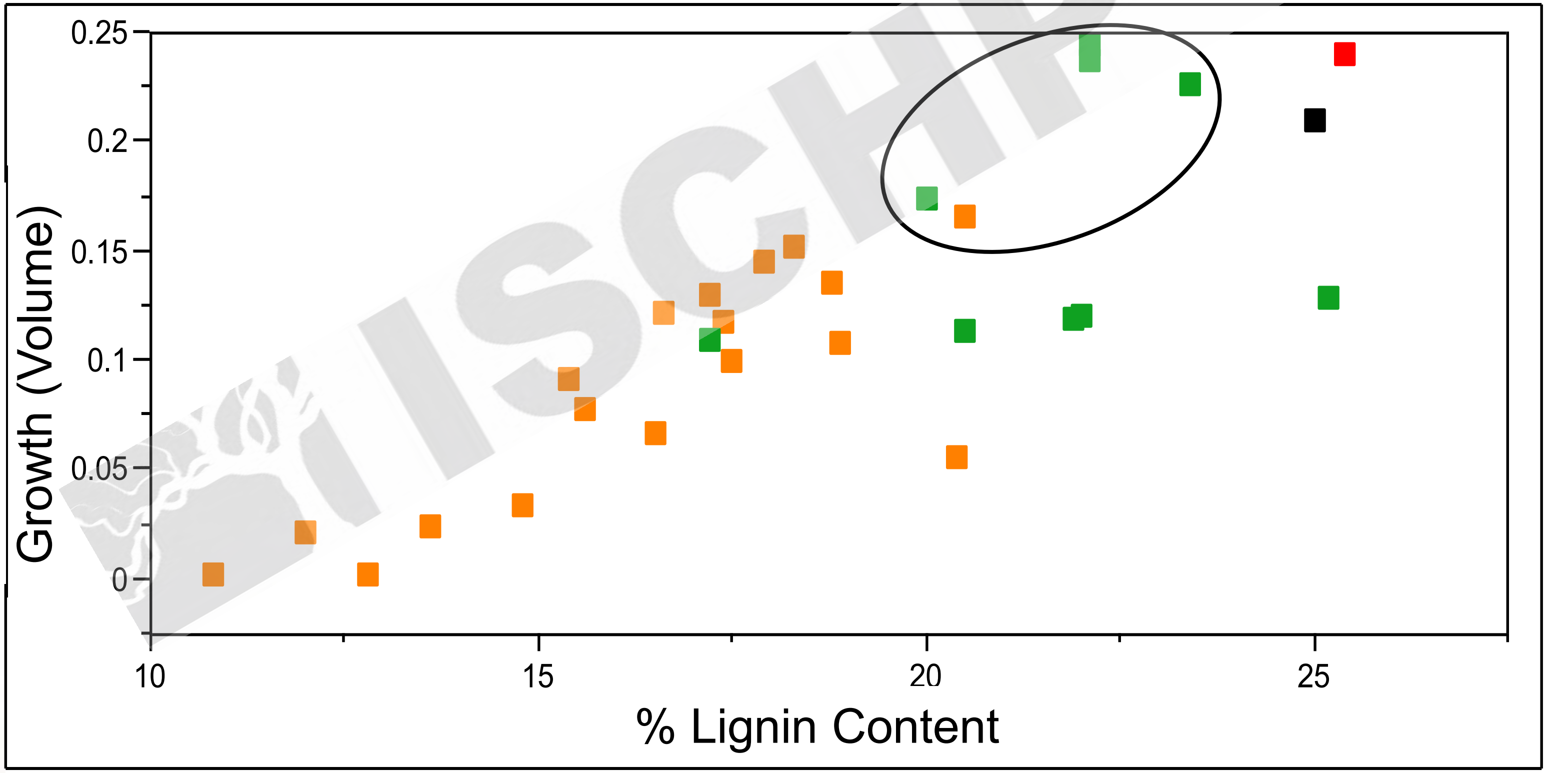


Reduced lignin through reduced C3H and C4H enzymatic activity

Hisano et al (2009) *In vitro* Cellular & Developmental Biology-Plant, 43, 3, 306-313,

Reduced Lignin in Eucalyptus

- Reducing expression of C4H, an early step in lignin biosynthesis, can decrease lignin content by as much as 20% with normal or near-normal growth.

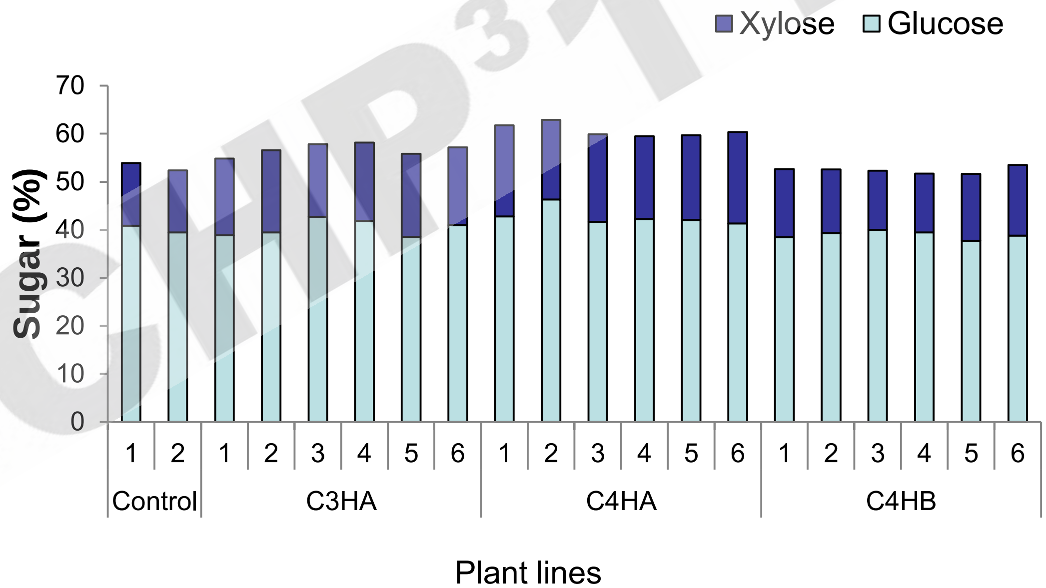


C3H and C4H down-regulated eucalyptus have similar or slightly higher carbohydrate levels as controls

- Sugar levels similar in control, C3H and C4H lignin reduced Eucalyptus

  - Small variation in Glucose between controls and lignin reduced Eucalyptus (37.8 - 46.3%)

  - Small variation in Total Sugar (51.7- 62.9%)



Glucose & Xylose content (Wet Chemistry)

NREL LAP Technical Report
NREL/TP-510-42618 Revised June 2010

With lignin reduction, more sugars can be released for bioconversion to biofuels

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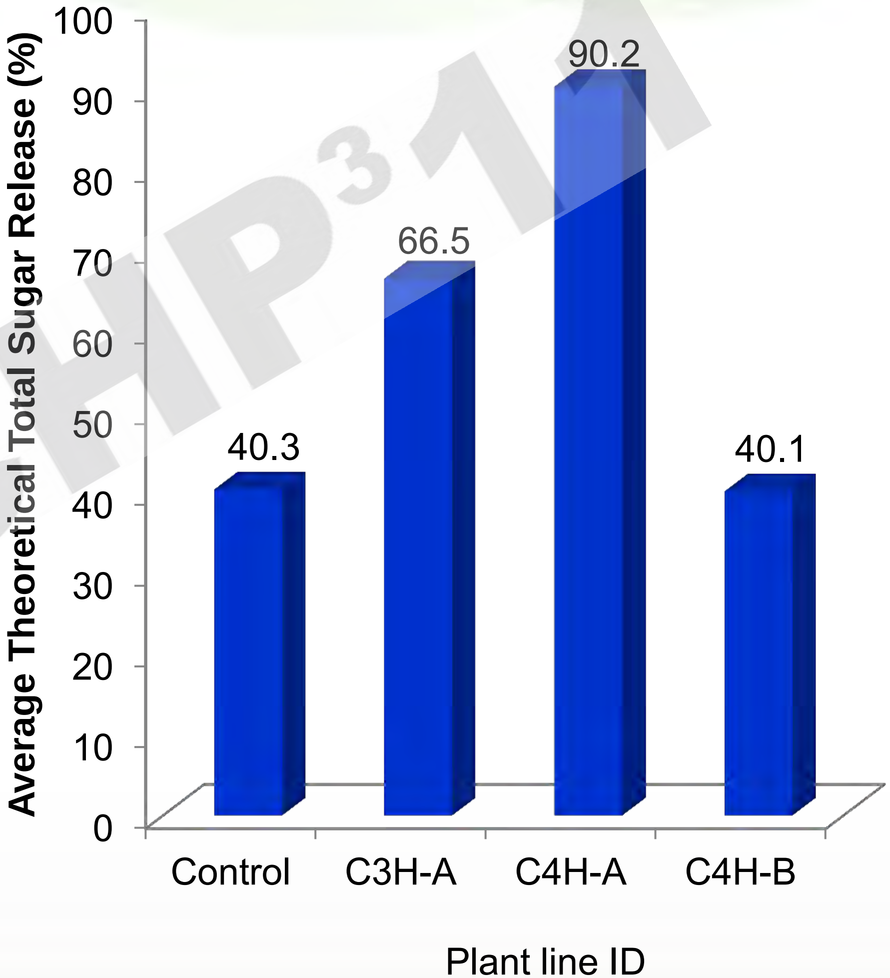
Large increase in sugar release

Line ID	With Pretreatment (%)
Euc control	40
Euc C3HA	56-72
Euc C4HA	83-99
Euc C4HB	36-45
SWG control (T0)	35
SWG COMT (T0)	30-55
SWG control (T1)	42
SWG COMT (T1)	52
Alfalfa control	45
Alfalfa - various	45-80



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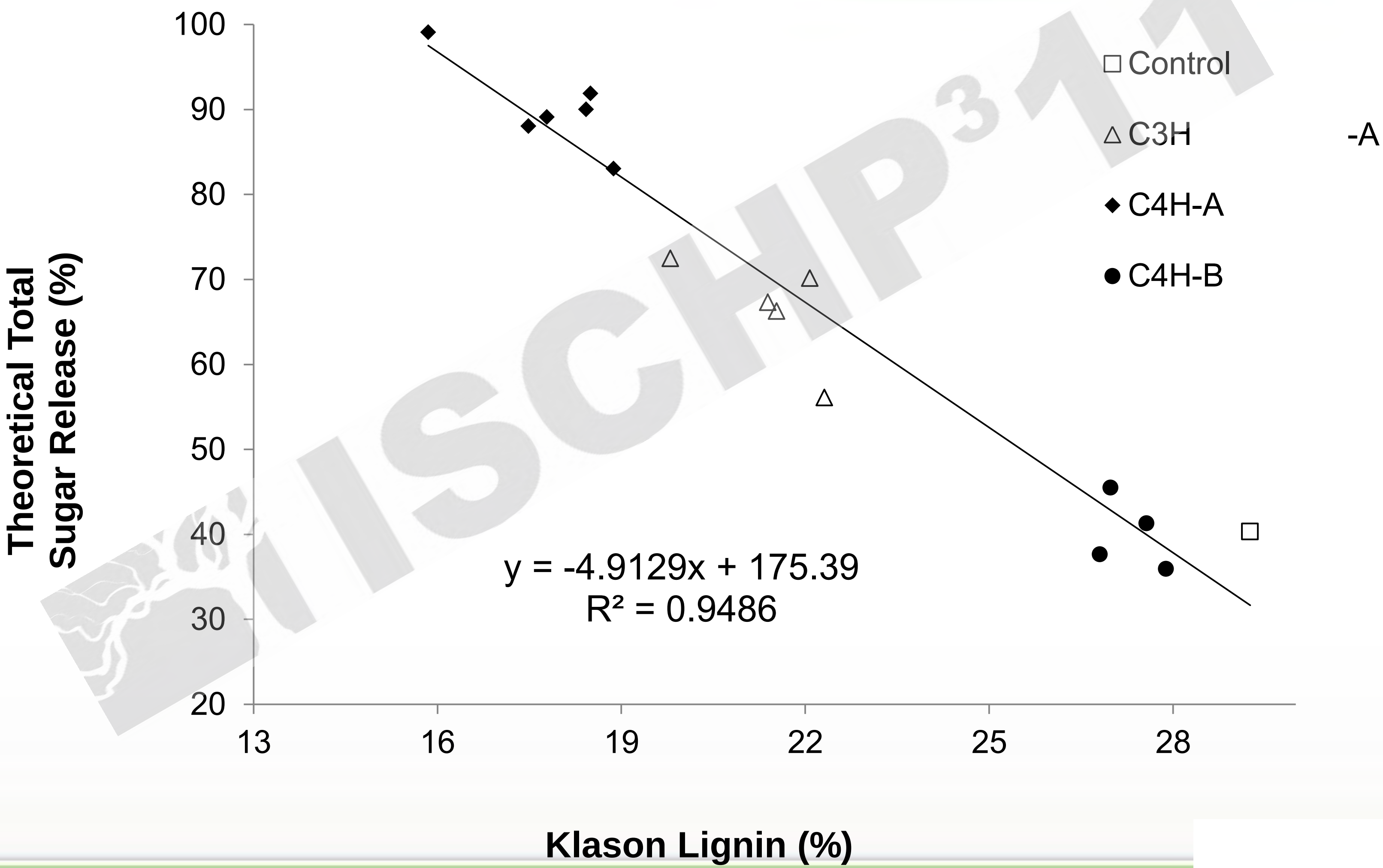
There is a strong correlation between Klason Lignin and theoretical total sugar release (pretreated)



C4H reduced eucalyptus is a great candidate feedstock for biofuels

- Low lignin mediated by C4H reduction greatly reduce biomass recalcitrance
- Sugar release correlates well with:
  - glucose content
  - lignin content
- As C4H reduced eucalyptus grew similarly to the control; its output could be:
  - - 10 dry tons/acre/year
  - - 1,000 gallons of fuel/acre

Altered lignin composition can Improve pulping efficiency

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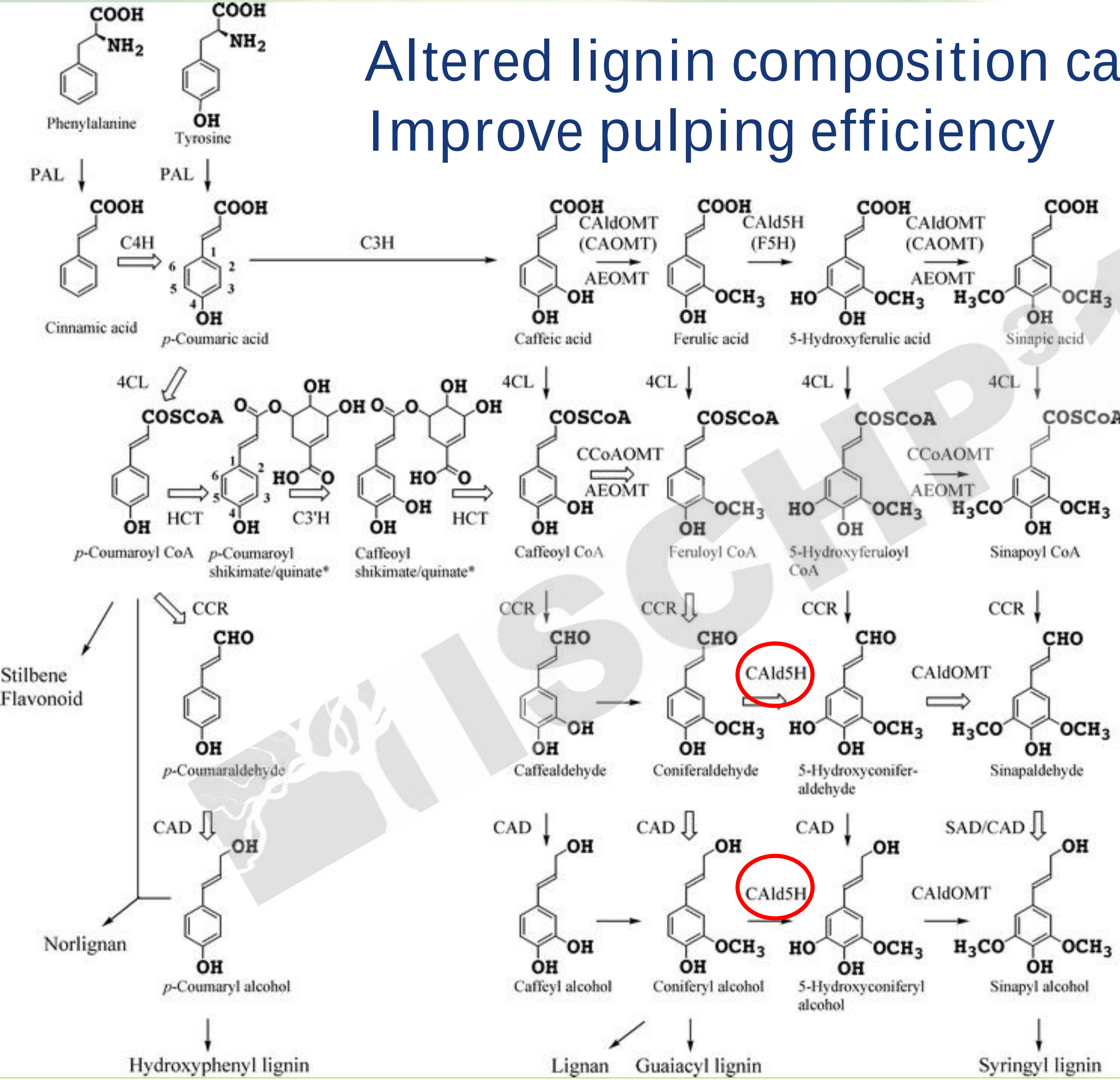


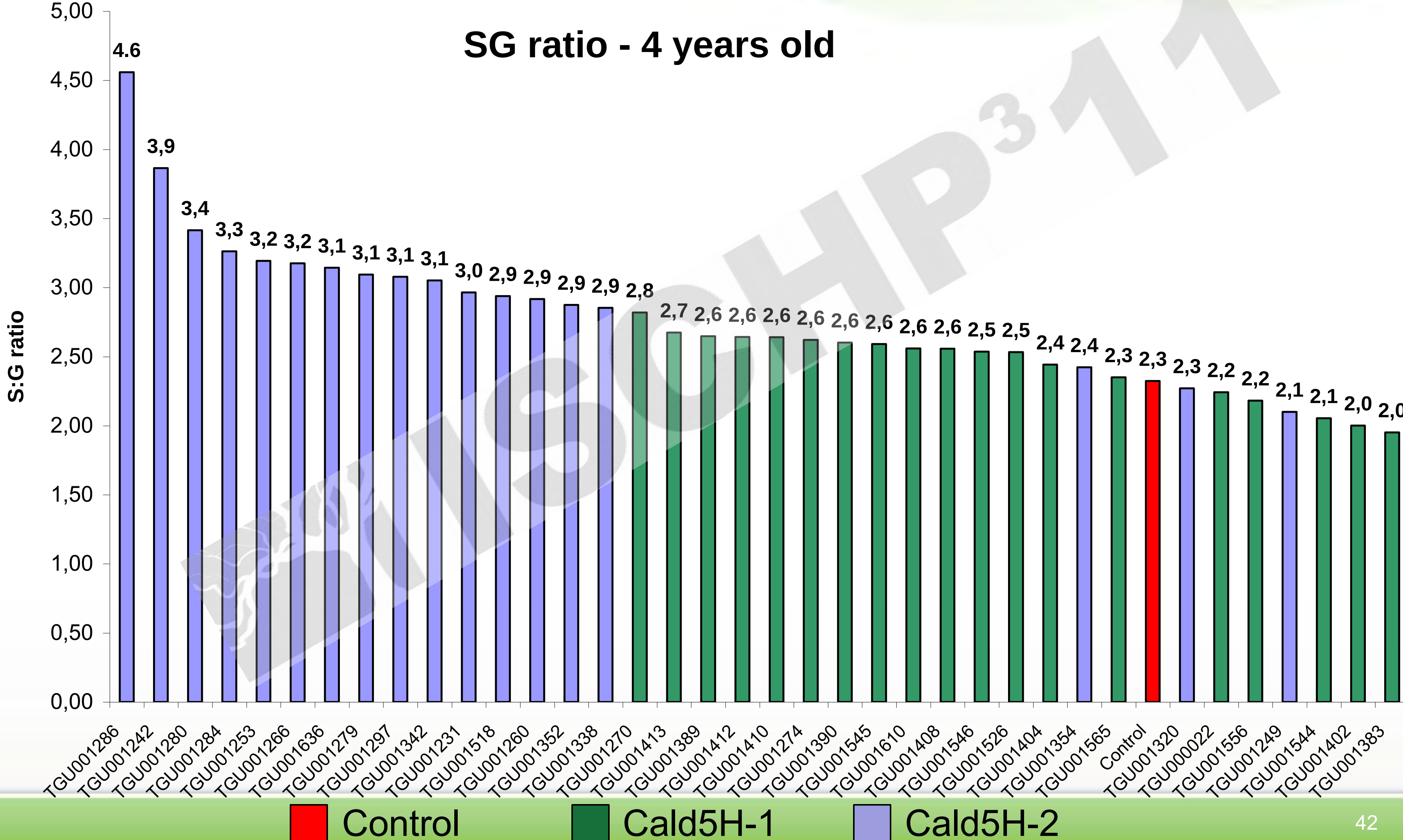
Increased syringyl lignin due to overexpression of Cald5H

Hisano et al (2009) *In vitro* Cellular & Developmental Biology-Plant, 43, 3, 306-313,

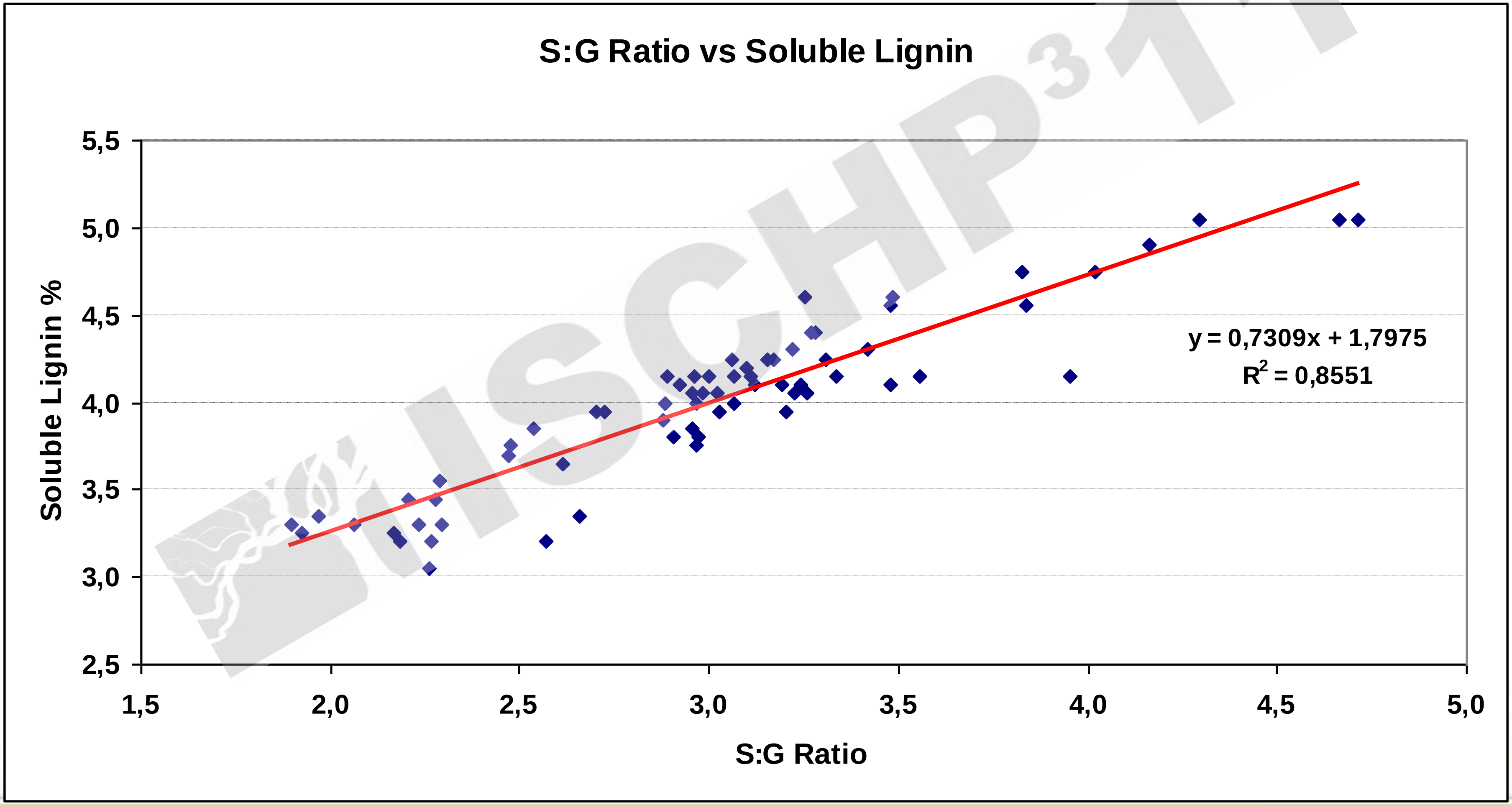
Kraft pulping process requires alkali (sodium hydroxide and sodium sulfite) to remove lignin

- The key to improving pulp production is the development of wood with **novel properties**
  - low lignin content or a higher proportion of reactive lignin
  - Lowers the kraft energy and chemical intensity limits

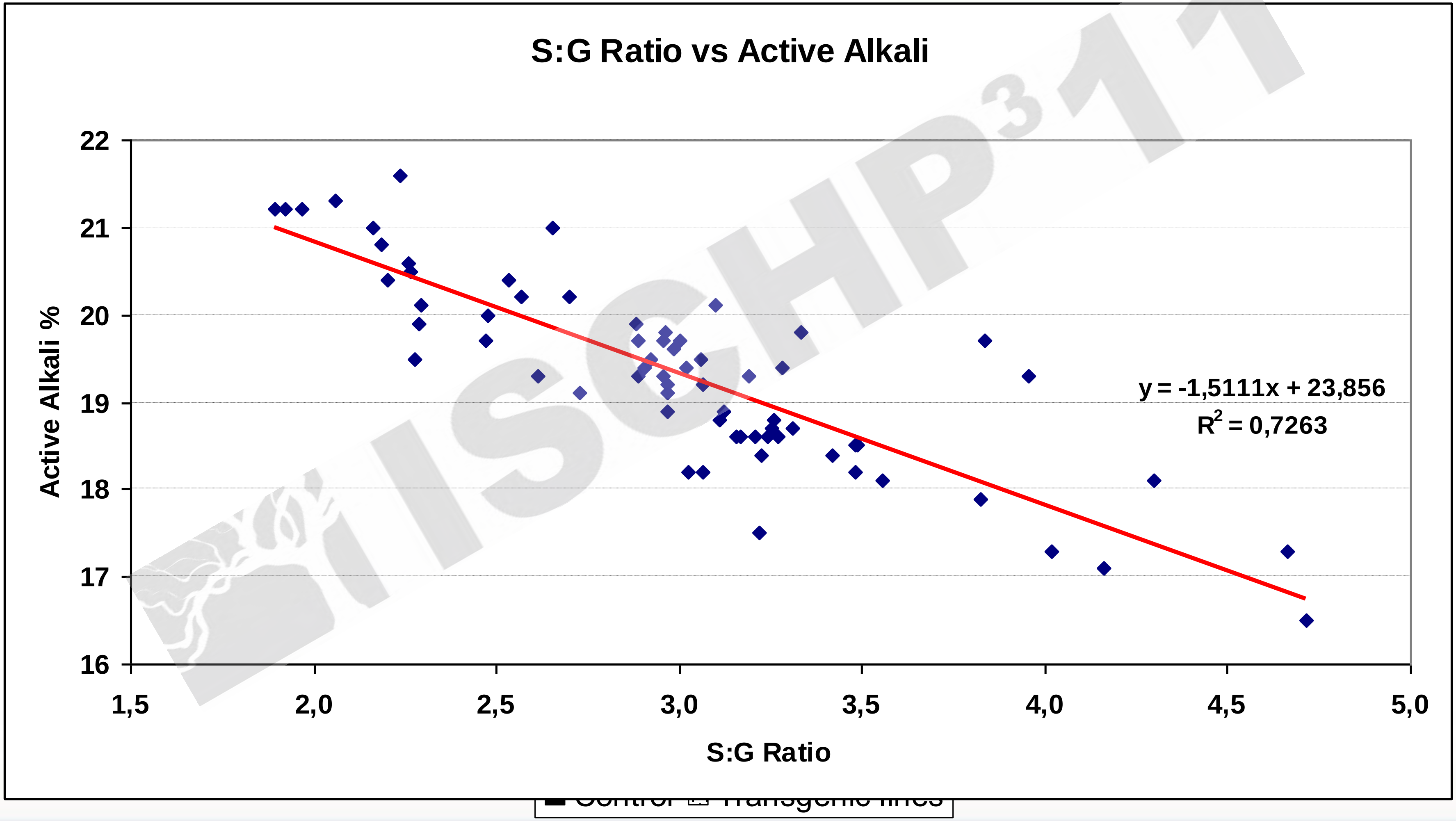
Over-expressing a coniferaldehyde 5-hydroxylase (cald5H) gene in *Eucalyptus* increases S lignin relative to G lignin



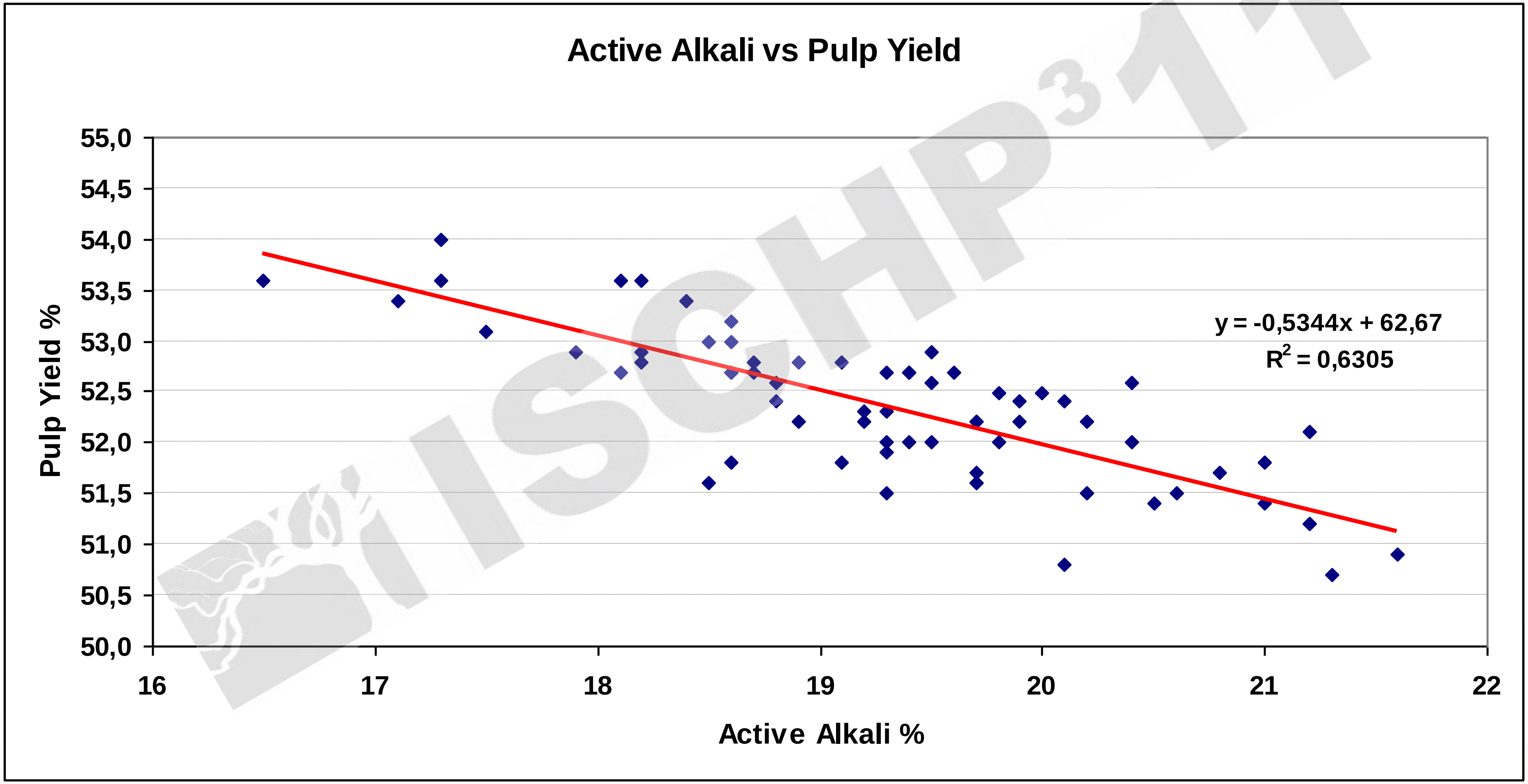
Increased S:G ratio affects the amount of soluble lignin relative to insoluble lignin



Less chemicals (active alkali) needed to produce pulp from wood with a 15% reduction in chemical cost



Reduced alkali use correlates to higher pulp yields



The Eucalyptus Example: Biotechnology can provide benefits for renewable bioproducts from wood

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- Fast growing short rotation Eucalyptus provides:
  - Biomass crop which can generate 33 green tons (16 dry tons) per acre per year
- Cold tolerance
  - Enables the possibility of growing this crop in the Southeastern US
- Reduced lignin
  - Enables cost-effective economics for biofuel generation from wood
- Altered lignin
  - Increases yield and reduces the cost of generating pulp

ARBORGEN®

More Wood. *Less Land*.®
And more sustainable bioproducts!

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