Trees of the Future - Genetic Engineering as a Tool for Improving Feedstock

Amy L. Klocko
Steven H. Strauss
Oregon State University

OSU Oregon State UNIVERSITY

Advanced Hardwood Biofuels Northwest

Presentation overview

- Rationale
- Poplar improvement: what are the options?
- Regulatory and social concerns
- Data: what do we know so far?
- Future prospects

Rationale

- Genetic engineering (GE): a valuable tool for economics and sustainability

- Improved feedstock yield (biomass)
- Feedstock quality modification
  - Increase in product formation (fermentability)
  - Input reduction (pesticides, irrigation)
  - Valuable co-products (chemicals, bioplastics, enzymes)

- Social, marketplace, and regulatory limits on use of GE are severe (e.g., FSC system)

- Gene flow to certified and wild stands a major issue

We know lots of information about poplar genetics

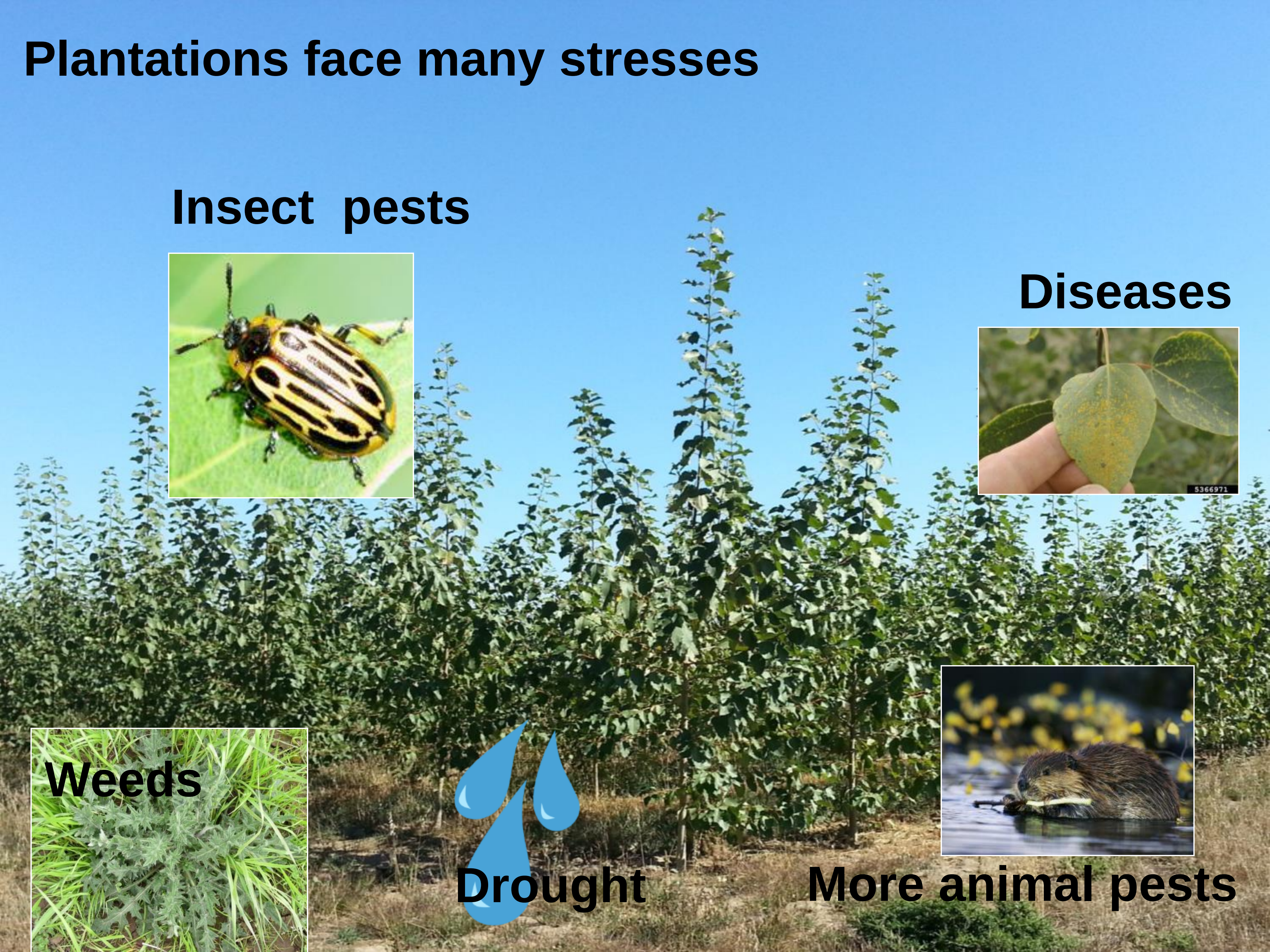
- **First ever sequenced tree genome was *Populus trichocarpa***
- **Lots of naturally existing genetic diversity**

RESEARCH ARTICLES

The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray)

G. A. Tuskan,[1,3]* S. DiFazio,[1,4]† S. Jansson,[5]† J. Bohlmann,[6]† I. Grigoriev,[9]† U. Hellsten,[9]† N. Putnam,[9]† S. Ralph,[6]† S. Rombauts,[10]† A. Salamov,[9]† J. Schein,[11]† L. Sterck,[10]† A. Aerts,[9] R. R. Bhalerao,[5] R. P. Bhalerao,[12] D. Blaudez,[13] W. Boerjan,[10] A. Brun,[13] A. Brunner,[14] V. Busov,[15] M. Campbell,[16] J. Carlson,[17] M. Chalot,[13] J. Chapman,[9] G.-L. Chen,[2] D. Cooper,[6] P. M. Coutinho,[19] J. Couturier,[13] S. Covert,[20] Q. Cronk,[7] R. Cunningham,[1] J. Davis,[22] S. Degroeve,[10] A. Déjardin,[23] C. dePamphilis,[18] J. Detter,[9] B. Dirks,[24] I. Dubchak,[9,25] S. Duplessis,[13] J. Ehlting,[7] B. Ellis,[6] K. Gendler,[26] D. Goodstein,[9] M. Gribskov,[27] J. Grimwood,[28] A. Groover,[29] L. Gunter,[1] B. Hamberger,[7] B. Heinze,[30] Y. Helariutta,[12,31,33] B. Henrissat,[19] D. Holligan,[21] R. Holt,[11] W. Huang,[9] N. Islam-Faridi,[34] S. Jones,[11] M. Jones-Rhoades,[35] R. Jorgensen,[26] C. Joshi,[15] J. Kangasjärvi,[32] J. Karlsson,[5] C. Kelleher,[6] R. Kirkpatrick,[11] M. Kirst,[22] A. Kohler,[13] U. Kalluri,[1] F. Larimer,[2] J. Leebens-Mack,[21] J.-C. Leplé,[23] P. Locascio,[2] Y. Lou,[9] S. Lucas,[9] F. Martin,[13] B. Montanini,[13] C. Napoli,[26] D. R. Nelson,[36] C. Nelson,[37] K. Nieminen,[31] O. Nilsson,[12] V. Pereda,[13] G. Peter,[22] R. Philippe,[6] G. Pilate,[23] A. Poliakov,[25] J. Razumovskaya,[2] P. Richardson,[9] C. Rinaldi,[13] K. Ritland,[8] P. Rouzé,[10] D. Ryaboy,[25] J. Schmutz,[28] J. Schrader,[38] B. Segerman,[5] H. Shin,[11] A. Siddiqui,[11] F. Sterky,[39] A. Terry,[9] C.-J. Tsai,[15] E. Uberbacher,[2] P. Unneberg,[39] J. Vahala,[32] K. Wall,[18] S. Wessler,[21] G. Yang,[21] T. Yin,[1] C. Douglas,[7]‡ M. Marra,[11]‡ G. Sandberg,[12]‡ Y. Van de Peer,[10]‡ D. Rokhsar[9,24]‡

https://en.wikipedia.org/wiki/Populus_trichocarpa


Plantations face many stresses
Insect pests
Diseases
5366971
Weeds
Drought
More animal pests

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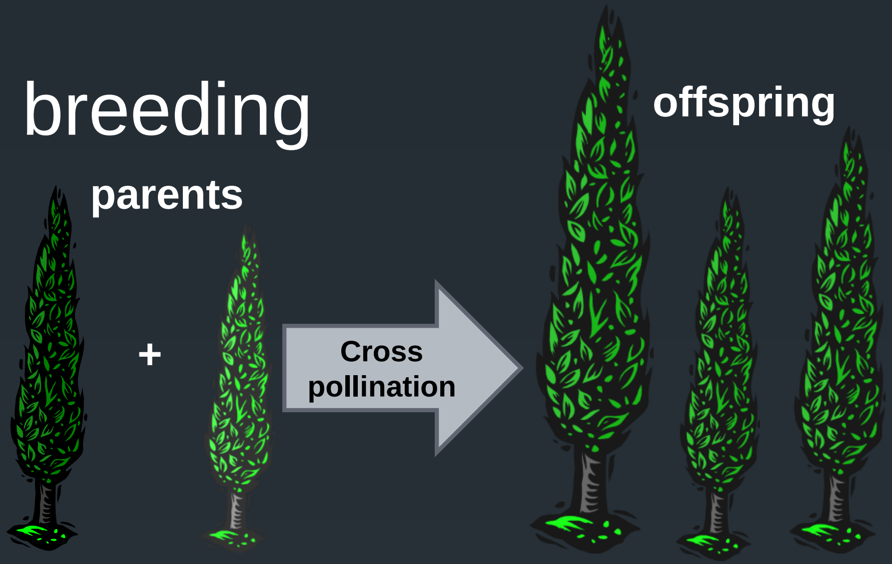
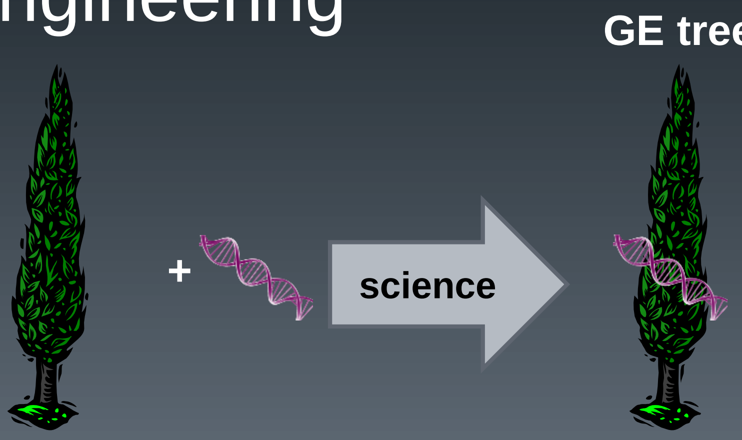
Poplar improvement: how to obtain a better tree

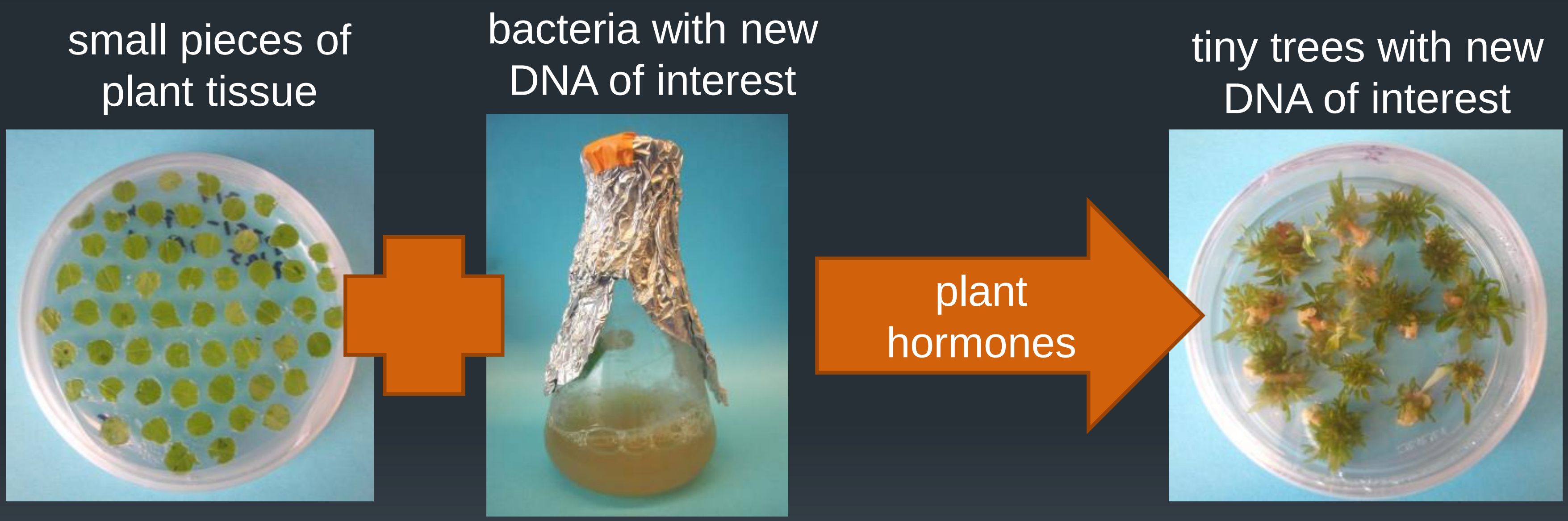
- Option 1: traditional breeding



- Option 2: genetic engineering



Genetic engineering can quickly add specific traits to trees



New DNA can encode a new trait of interest (insect resistance) or alter a native gene

GE can add traits faster and more specifically than traditional breeding

Genetic engineering had led to biofuel feedstock benefits in poplar

New Phytologist

Research

Designed for deconstruction – poplar trees altered in cell wall lignification improve the efficacy of bioethanol production

Shawn D. Mansfield[1], **Kyu-Young Kang**[1] **and Clint Chapple**[2]

[1]Department of Wood Science, The University of British Columbia, Vancouver, BC, V6T 1Z4, Canada; [2]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA

Author for correspondence:
Shawn D. Mansfield
Tel: +1 604-822-0196
Email: shawn.mansfield@ubc.ca

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Key words: bioethanol, hydrolysis, lignin, monomer composition, organosolv, poplar, steam explosion.

Summary

- There is a pressing global need to reduce the increasing societal reliance on petroleum and to develop a bio-based economy. At the forefront is the need to establish a sustainable, renewable, alternative energy sector. This includes liquid transportation fuel derived from lignocellulosic plant materials. However, one of the current limiting factors restricting the effective and efficient conversion of lignocellulosic residues is the recalcitrance of the substrate to enzymatic conversion.
- In an attempt to assess the impact of cell wall lignin on recalcitrance, we subjected poplar trees engineered with altered lignin content and composition to two potential industrial pretreatment regimes, and evaluated the overall efficacy of the bioconversion to ethanol process.
- It was apparent that total lignin content has a greater impact than monomer ratio (syringyl : guaiacyl) on both pretreatments. More importantly, low lignin plants showed as much as a 15% improvement in the efficiency of conversion, with near complete hydrolysis of the cellulosic polymer.
- Using genomic tools to breed or select for modifications in key cell wall chemical and/or ultrastructural traits can have a profound effect on bioenergy processing. These techniques may therefore offer means to overcome the current obstacles that underpin the recalcitrance of lignocellulosic substrates to bioconversion.

New Forests (2016) 47:653–667
DOI 10.1007/s11056-016-9536-6

CrossMark

Improved growth and weed control of glyphosate-tolerant poplars

Kori Ault[1] · **Venkatesh Viswanath**[1,4] · **Judith Jayawickrama**[1] · **Cathleen Ma**[1] · **Jake Eaton**[2] · **Rick Meilan**[1,5] · **Grant Beauchamp**[2,6] · **William Hohenschuh**[3] · **Ganti Murthy**[3] · **Steven H. Strauss**[1]

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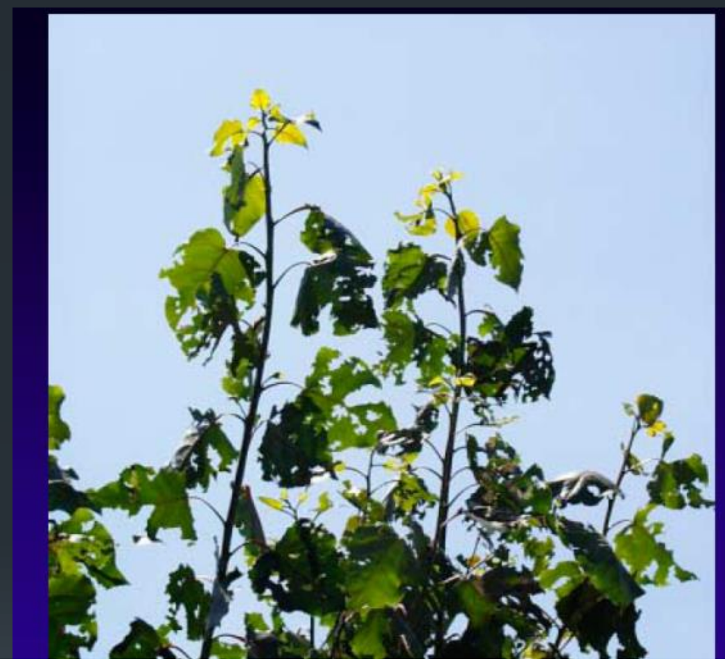
Abstract We studied the impact of glyphosate tolerance on weed control and tree growth in field-grown transgenic poplars. Using *Agrobacterium*-mediated transformation, we produced 94 transgenic transformation events in four hybrid genotypes (three *Populus trichocarpa* × *P. deltoides* and one of *P. trichocarpa* × *P. nigra*). These lines were screened for high levels of tolerance in two plantations in Oregon. Based on screening results, we propagated four lines from two hybrid genotypes to study their value for weed control and productivity in a 2-year management trial in eastern Oregon, comparing conventional weed control at the time of the study to methods that included over-the-top applications of glyphosate during the growing season. Herbicide tolerance was stable in all of the trees over the 2-year period. Weed control, based on weed abundance, was substantially improved in the over-the-top application. Growth of the trees, as measured by stem volume index, was correspondingly improved; transgenic trees grew approximately 20 % faster than the transgenic and non-transgenic control trees. An exploratory life-cycle analysis of the embodied greenhouse-gas benefits for a coppice bioenergy plantation

Genetic engineering can be used to obtain high value traits in poplar

Insect resistant

Not insect resistant



28

NRC Research Press

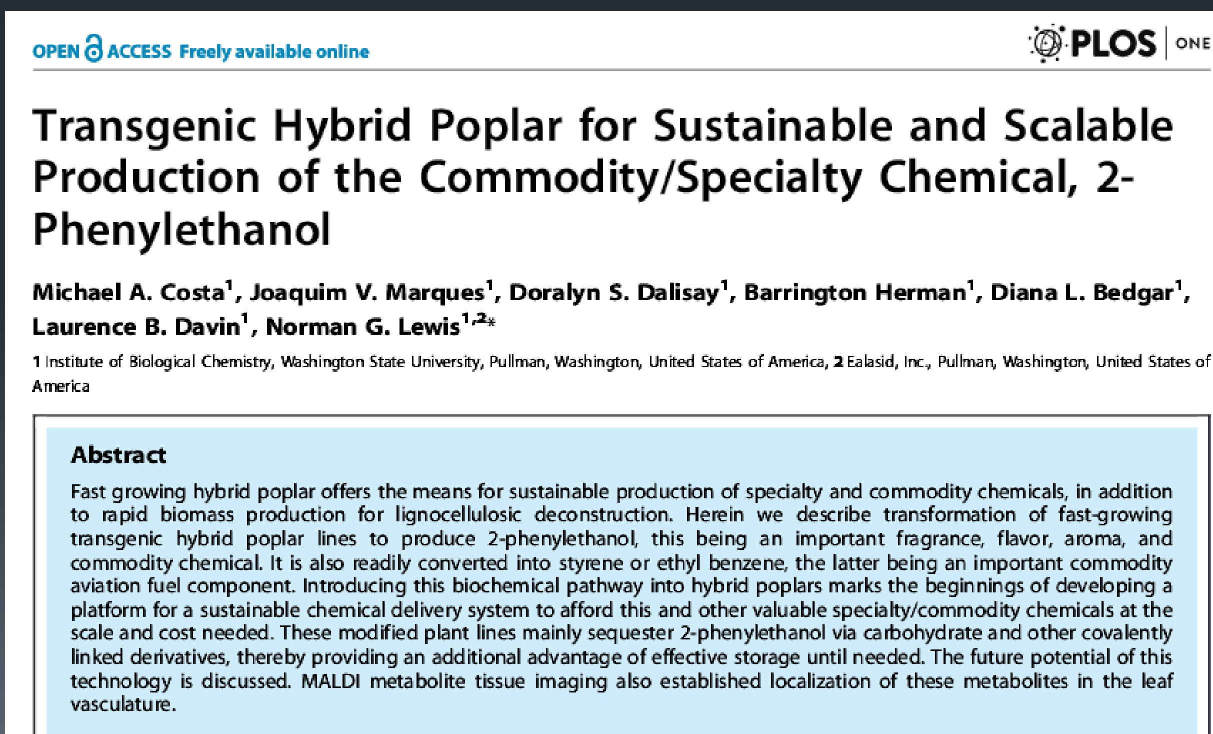
ARTICLE

Bt-Cry3Aa transgene expression reduces insect damage and improves growth in field-grown hybrid poplar

Amy L. Klocko, Richard Meilan, Rosalind R. James, Venkatesh Viswanath, Cathleen Ma, Peggy Payne, Lawrence Miller, Jeffrey S. Skinner, Brenda Oppert, Guy A. Cardineau, and Steven H. Strauss

Genetic engineering can make poplar trees with specialty chemicals

- Trees produce 2-phenylethanol, a chemical with multiple uses and a high-value
- This chemical is normally found in roses and other flowers


OPEN ACCESS Freely available online — PLOS ONE

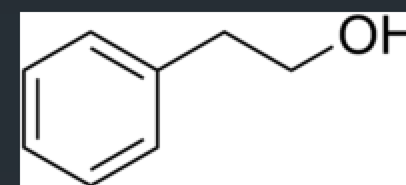
Transgenic Hybrid Poplar for Sustainable and Scalable Production of the Commodity/Specialty Chemical, 2-Phenylethanol

Michael A. Costa[1], Joaquim V. Marques[1], Doralyn S. Dalisay[1], Barrington Herman[1], Diana L. Bedgar[1], Laurence B. Davin[1], Norman G. Lewis[1,2]*

1 Institute of Biological Chemistry, Washington State University, Pullman, Washington, United States of America, 2 Ealasid, Inc., Pullman, Washington, United States of America

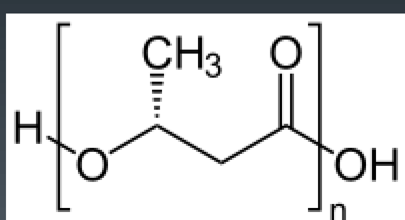
Abstract

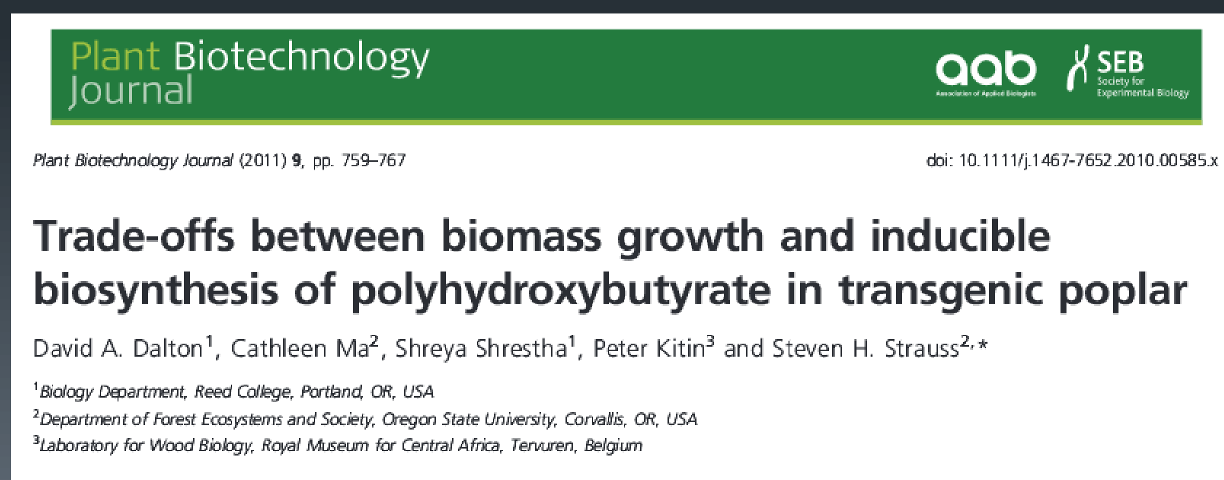
Fast growing hybrid poplar offers the means for sustainable production of specialty and commodity chemicals, in addition to rapid biomass production for lignocellulosic deconstruction. Herein we describe transformation of fast-growing transgenic hybrid poplar lines to produce 2-phenylethanol, this being an important fragrance, flavor, aroma, and commodity chemical. It is also readily converted into styrene or ethyl benzene, the latter being an important commodity aviation fuel component. Introducing this biochemical pathway into hybrid poplars marks the beginnings of developing a platform for a sustainable chemical delivery system to afford this and other valuable specialty/commodity chemicals at the scale and cost needed. These modified plant lines mainly sequester 2-phenylethanol via carbohydrate and other covalently linked derivatives, thereby providing an additional advantage of effective storage until needed. The future potential of this technology is discussed. MALDI metabolite tissue imaging also established localization of these metabolites in the leaf vasculature.



Genetic engineering can create trees with valuable co-products

- Addition of 3 genes from bacterial to poplar can lead to the formation of biodegradable plastic in trees
- Current methods have a tradeoff between polymer production and plant growth





Plant Biotechnology Journal

Plant Biotechnology Journal (2011) **9**, pp. 759–767

doi: 10.1111/j.1467-7652.2010.00585.x

Trade-offs between biomass growth and inducible biosynthesis of polyhydroxybutyrate in transgenic poplar

David A. Dalton[1], Cathleen Ma[2], Shreya Shrestha[1], Peter Kitin[3] and Steven H. Strauss[2,*]

[1]*Biology Department, Reed College, Portland, OR, USA*
[2]*Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR, USA*
[3]*Laboratory for Wood Biology, Royal Museum for Central Africa, Tervuren, Belgium*

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There are public and social concerns over the use of GE trees

iForest
Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor1441-008
(Early View)

Public attitudes towards the use of transgenic forest trees: a cross-country pilot survey

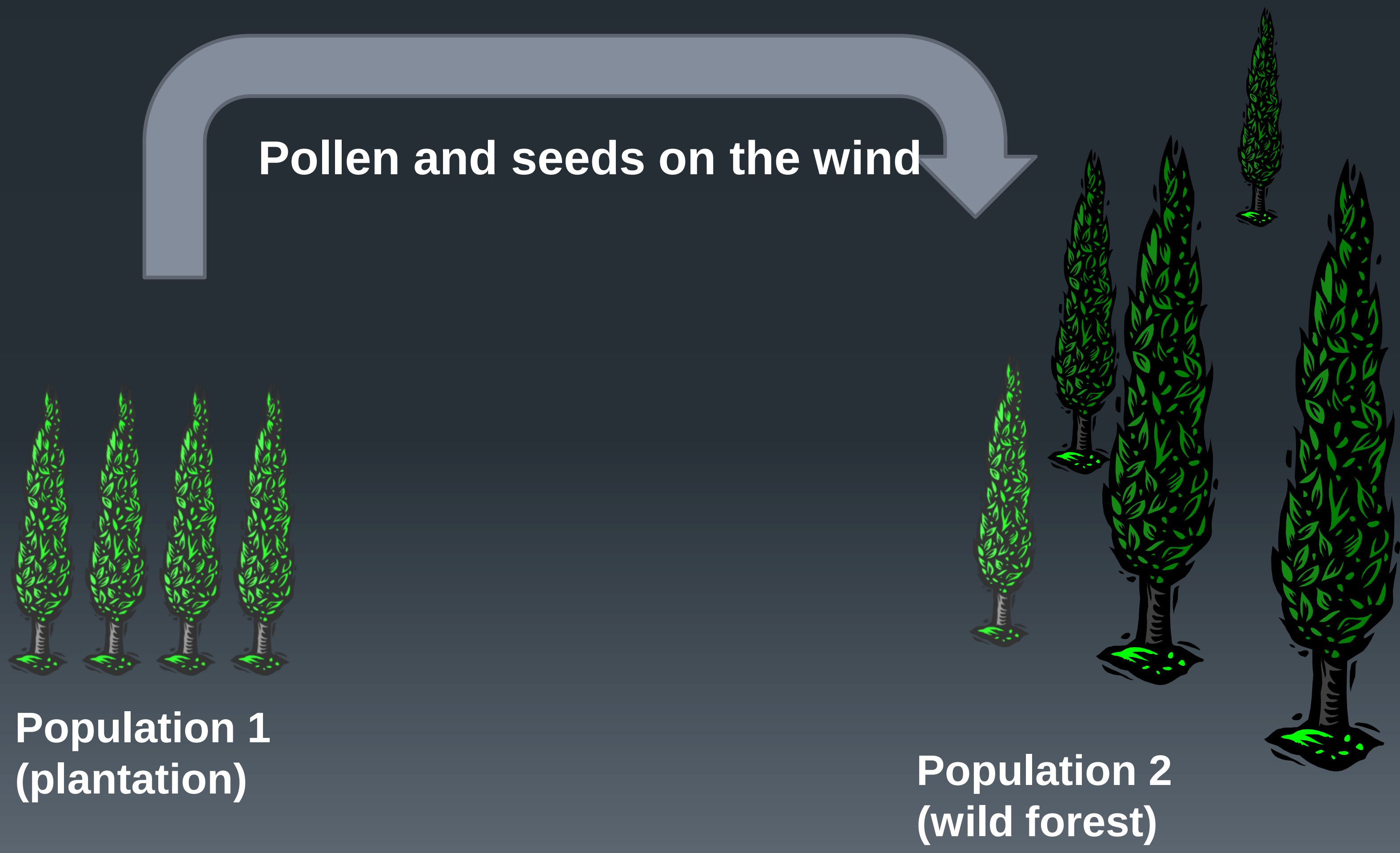
Vassiliki Kazana [1], Lambros Tsourgiannis [2], Valasia Iakovoglou [1], Christos Stamatiou [1], Alexander Alexandrov [3], Susana Araújo [4], Sasa Bogdan [5], Gregor Bozic [6], Robert Brus [7], Gerd Bossinger [8], Anastasia Boutsimea [1], Nevenka Celepirović [9], Helena Cvrčková [10], Matthias Fladung [11], Mladen Ivankovic [9], Angelos Kazaklis [1], Paraskevi Koutsona [1], Zlata Luthar [12], Pavlina Máchová [10], Jana Malá [10], Kostlend Mara [13], Milan Mataruga [14], Jana Moravcikova [15], Donatella Paffetti [16-22], Jorge AP Paiva [17], Dimitiros Raptis [1], Conchi Sanchez [18], Sandra Sharry [19], Terezia Salaj [15], Mirjana Šijačić-Nikolić [20], Noemi Tel-Zur [21], Ivaylo Tsvetkov [3], Cristina Vettori [22], Nieves Vidal [18]

Information on public attitudes towards the use of transgenic trees in forest plantations is important in the decision-making process and policy implementation for safe tree development, particularly at the EU level. In Europe, the use of transgenic forest trees is very limited and therefore such information is completely lacking. To address this issue within the FP0905 European COST Action on the Biosafety of Transgenic Forest Trees a pioneer cross-country pilot survey on public attitudes towards the use of transgenic forest trees was conducted using young population as a focus group. This was decided mainly because this focus group represents the future consumers, policy makers or developers. Specifically, the survey aimed to: i) assess the level of young people's knowledge about transgenic forest trees, ii) identify issues of concern to them regarding the cultivation of transgenic forest trees and iii) explore whether they approve or disapprove of the use of transgenic forest trees in plantations. Purposive sampling was performed and university students of different disciplines were included in the research as sampling subjects. In total, 1868 completed questionnaires from 15 European and non-European countries were analyzed. The young educated people that took part in the survey appeared to approve of the use of transgenic forest trees in plantations and would be willing to buy forest transgenic products. The potential loss of biodiversity due to a risk of gene flow between transgenic and wild trees was seen as the safety issue of most concern when considering the commercial release of transgenic forest trees. However, a serious perceived lack of knowledge about potential benefits and risks of the cultivation of transgenic forest trees was recorded in most of the countries. K-means clustering was implemented on respondents' positive responses to identify potential country patterns. No differences in patterns of public attitude towards the acceptance of the commercial growing of transgenic forest trees were observed between European and non-European countries. Extended research on public attitude issues towards the use of transgenic forest trees is strongly recommended as a basis for policy implementation on safe tree development.

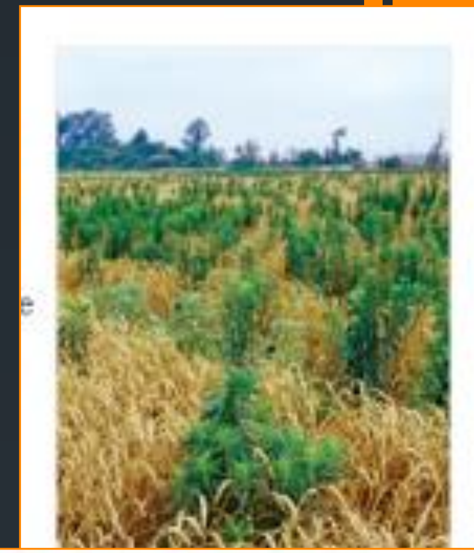
Keywords: GM Forest Trees, Public Awareness, Public Acceptance, k-means Clustering, University Students

"The potential loss of biodiversity due to **a risk of gene flow** between transgenic and wild trees was seen as the safety issue of most concern when considering the commercial release of transgenic forest trees."

Gene flow: the movement of pollen and/or seeds into new populations



Lesson from crops: weed control measures used with resistant crops led to selection for resistant weeds




nature biotechnology

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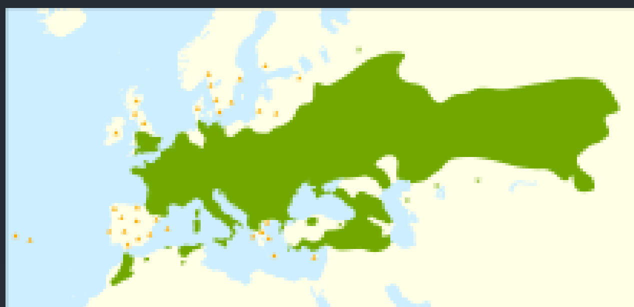
NATURE BIOTECHNOLOGY | NEWS

Glyphosate resistance threatens Roundup hegemony

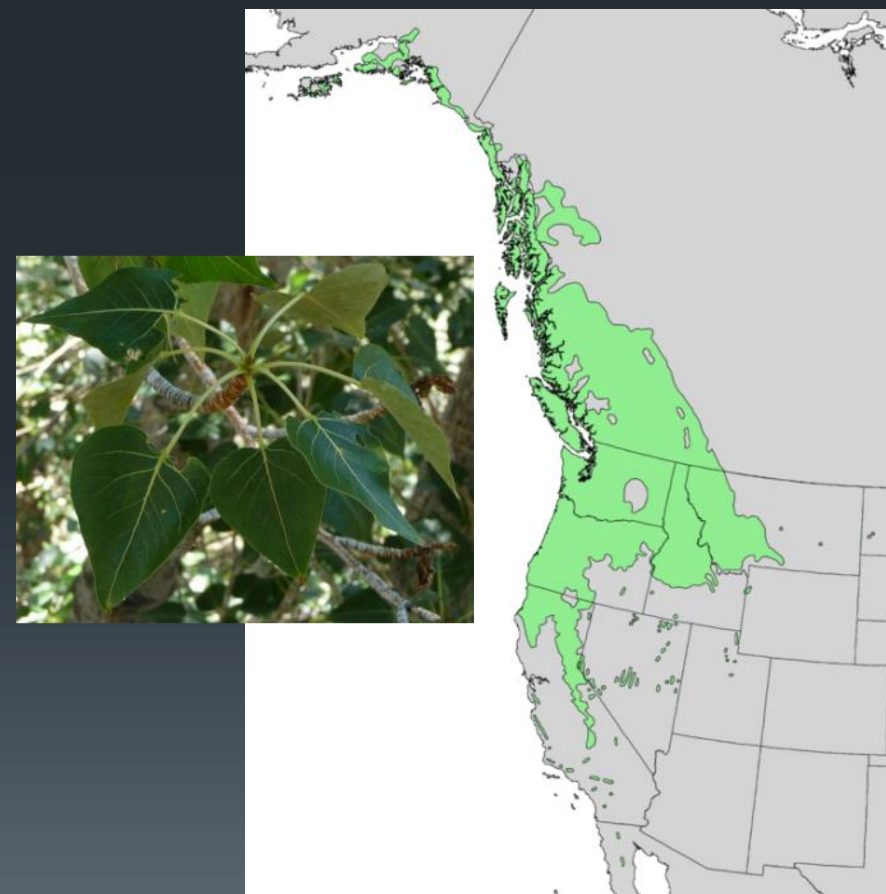
We want to avoid similar problems with any potential future use of GE trees

Many poplar hybrids are crosses between exotic species

Populus nigra



Populus trichocarpa



Populus maximowiczii

Populus deltoides

Forest stewardship council: concern over potential ecological impacts of exotic tree species

“The use of exotic species shall be carefully controlled and actively monitored to avoid adverse ecological impacts”

Traditional breeding has not yet been able to achieve a solution to this issue
GE methods can prevent spread by pollen and/or seeds

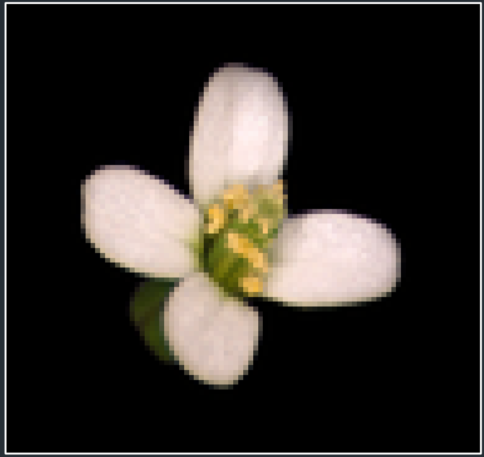
Genetic containment as a possible solution to prevent spread of GE and exotic species

Goal: create non-flowering trees

Pollen and seeds can spread for miles on the wind

Long-distance impacts across many boundaries

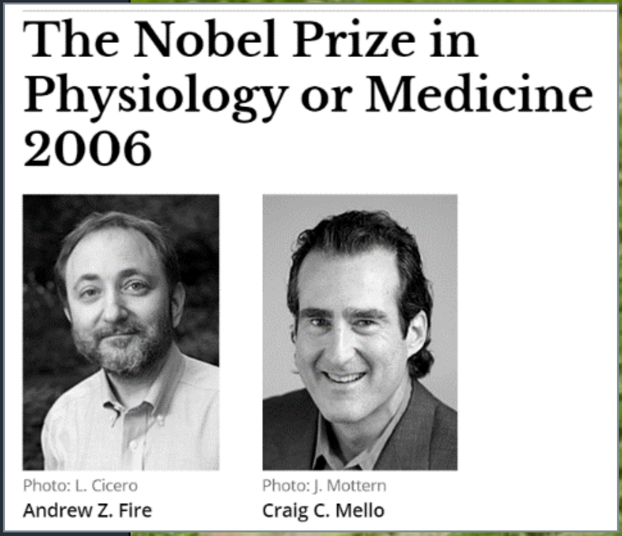
We are targeting fundamental regulators of flowering by RNA interference (RNAi)



Scientists have identified key genes for the development of flowers

New Phytologist
Volume 201, Issue 3, pages 717-732, 25 OCT 2013 DOI: 10.1111/nph.12555
http://onlinelibrary.wiley.com/doi/10.1111/nph.12555/full#nph12555-fig-0001

Gene suppression with RNAi (RNA interference) has already produced diverse traits




The New York Times
Business Day
In a Bean, a Boon to Biotech

A new federal push to purge artery-clogging trans fats from foods could be just what the doctor ordered — not only for public health but for the unpopular biotechnology industry, specifically, two developers of genetically modified crops.

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Strauss lab RNAi trees are undergoing field testing

RNA interference again the key floral regulator LFY led to female sterile trees

Containment of transgenic trees by suppression of *LEAFY*

To the Editor:

Field studies and commercial use of genetically engineered (GE) trees have been limited, in large part owing to concerns over transgene flow into wild or feral tree populations[1–4]. Unlike other crops, trees are long-lived, weakly domesticated and their propagules can spread over several kilometers[5]. Although male sterility has been engineered in pine, poplar, and eucalyptus trees grown under field conditions by expression of the barnase RNase gene in anther tapetal cells[6,7], barnase can reduce rates of genetic transformation and vegetative growth[6]. Furthermore, barnase expression may not be fully stable[8]. Bisexual sterility would allay concerns over seed dispersal, could be used to control invasive exotic trees, and might increase wood production[9]. We report the use of RNA interference (RNAi) to suppress expression of the single-copy *LEAFY* (*LFY*) gene to produce sterility in poplar.

RNAi has been used to reduce gene expression in many plant species[10,11], and the reduction in gene expression that RNAi confers is highly stable in trees under field conditions[12]. *LFY* is required for the early stages of male and female floral organ formation in plants, and encodes a transcription factor that promotes floral meristem identity[13,14]. In *Arabidopsis thaliana*, loss of *LFY* function results in the formation of vegetative structures instead of floral meristems, whereas reduction of *LFY* expression decreases floral abundance and results in partial conversion of floral organs to leaf-like structures[13,14]. We selected *LFY*

http://hardwoodbiofuels.org/news-fall2016-poplar-reproduction/

Klocko et al. Nature Biotechnology 2016

RNAi-LFY trees appear to be female sterile

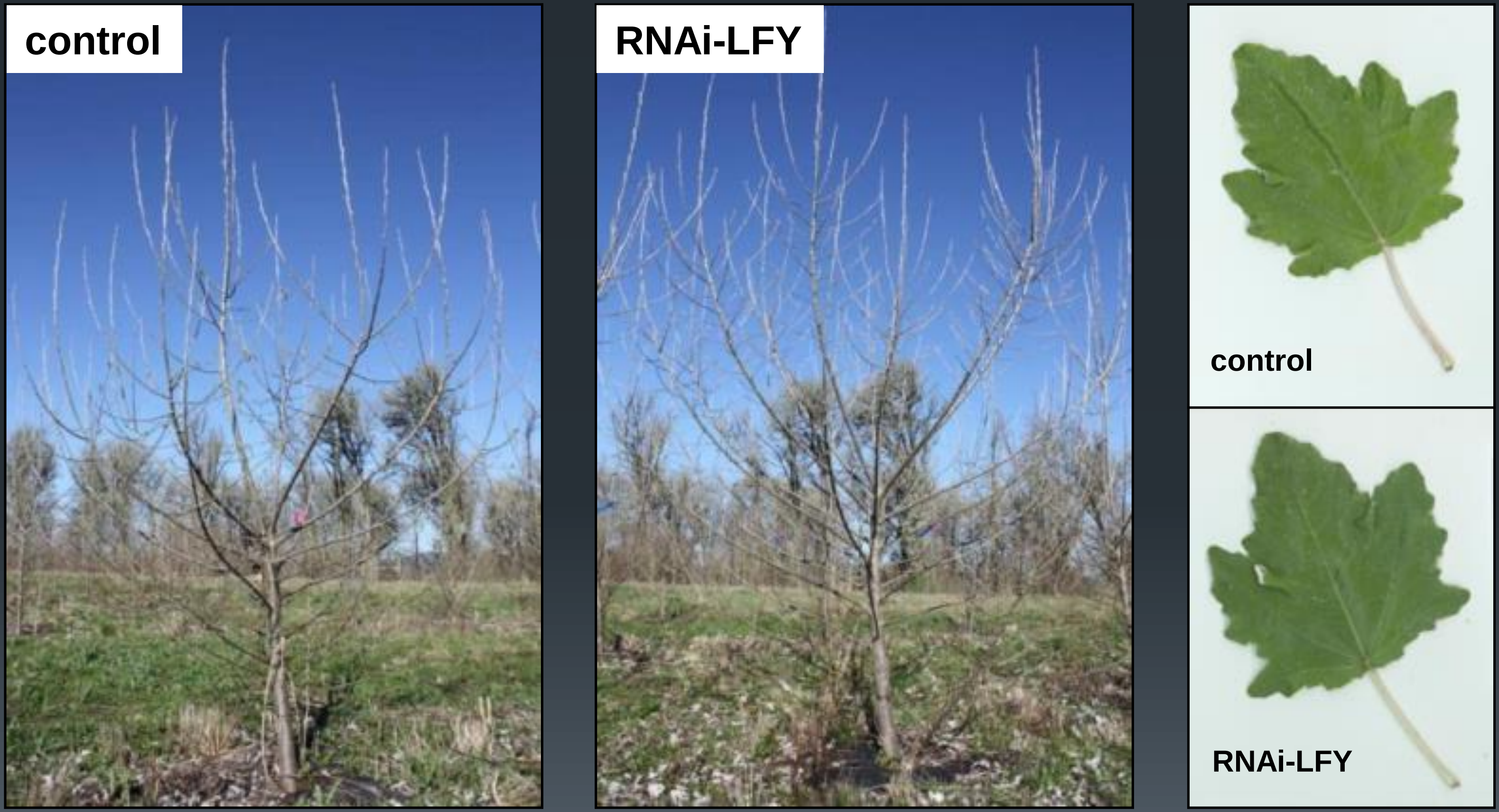
WILD TYPE

RNAi-LFY

Flowers fail to enlarge and were seedless

Klocko et al. Nature Biotechnology 2016

Female sterile RNAi-LFY trees have normal vegetative performance

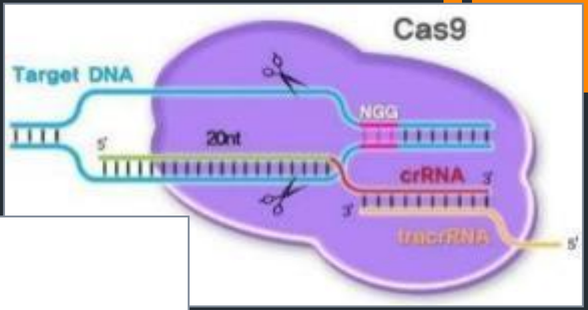


Klocko et al. Nature Biotechnology 2016

Sterile trees can still be propagated by vegetative cuttings

Healthy new trees were started by rooting branches of sterile trees

We are also trying the latest and greatest method for targeting floral genes



Science magazine names CRISPR ‘Breakthrough of the Year’

By Robert Sanders | DECEMBER 18, 2015

In its year-end issue, the journal *Science* chose the CRISPR genome-editing technology invented at UC Berkeley 2015’s Breakthrough of the Year.

A runner-up in 2012 and 2013, the technology now revolutionizing genetic research and gene therapy “broke away from the pack, revealing its true power in a series of spectacular achievements,” wrote *Science* correspondent John Travis in the Dec. 18 issue. These included “the creation of a long-sought ‘gene drive’ that

The CRISPR method can be used to create organisms not regulated as GE by the USDA

These mushrooms have a precise change to a single mushroom gene

No extra DNA/genes are present

Similar applications in trees might be acceptable by the FSC or other regulators

IN FOCUS NEWS

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a hand-

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

Upcoming Strauss lab research:

- Scaled-up field test of RNAi-LFY trees
- Greenhouse and field tests of trees with floral genes targeted by CRISPR

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Future use of genetic containment in poplar trees could benefit biofuel plantations

- All AHB field sites are non-GE hybrids of poplar species
- If exotic species become a concern or if GE varieties are tested, containment could be use to
  - Prevent flowering if fields have early flowering or aren’t harvested as planned
  - Prevent movement of pollen and seeds to wild populations, feral trees, or other plantations

Future prospects

- GE traits can provide considerable value and benefits to poplar plantations
- Obstacles prevent commercial use – gene flow is a major concern
- Our research established that sterility can be a solution
- New developments in gene editing technology (CRISPR) offer possibilities for tree containment

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Kori Ault

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Advanced **Hardwood Biofuels** Northwest

USDA
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National Institute of Food and Agriculture

TBGRC