Crop biotechnology for enhancing carbon uptake and greenhouse gas mitigation in plantation trees: Status and challenges

Presented at Rockefeller conference on “How can Agrogenomics Help to Address Climate Change?”
November 2021

Steve Strauss, University Distinguished Professor
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Agenda

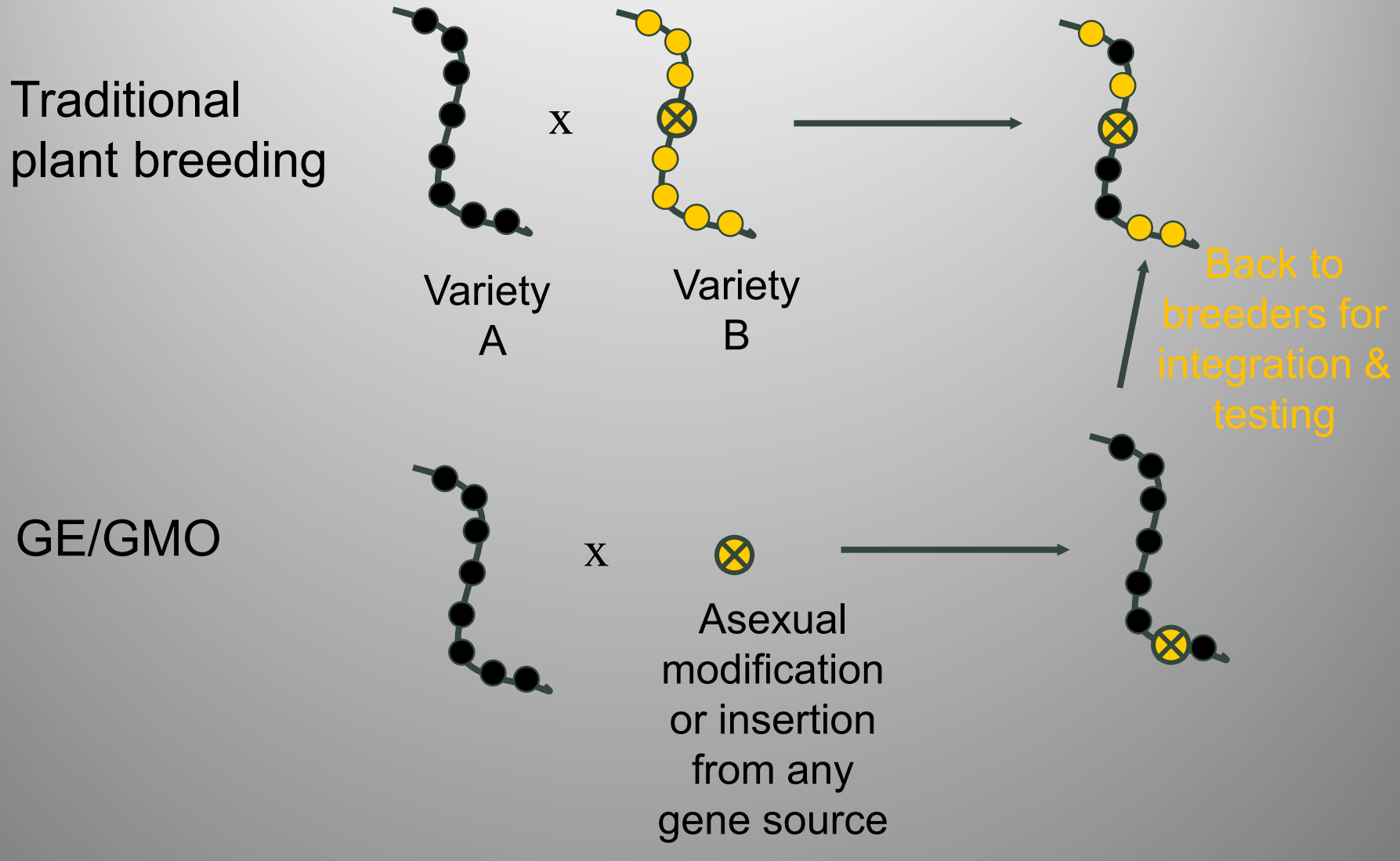
- Definitions, aims, and context
- Biological constraints and opportunities
- Social constraints
- What is needed moving forward

Aim: Try to connect basic biotech ideas and methods to practical outcomes in the context of intensive forestry systems

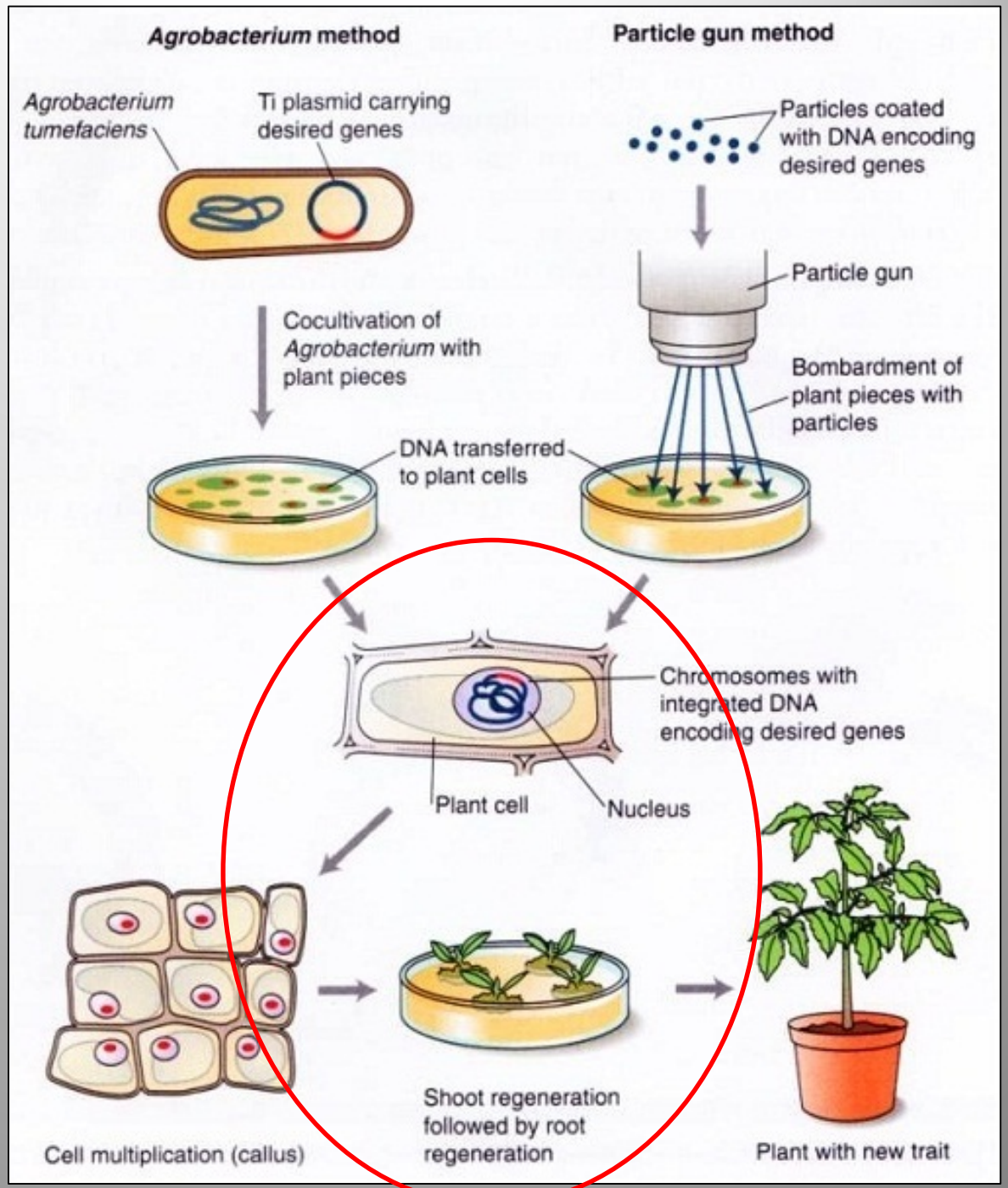
Illustrated with examples from my laboratory's work, old and new – a microcosm

Most focus on biomass growth or allocation as indexes of carbon uptake

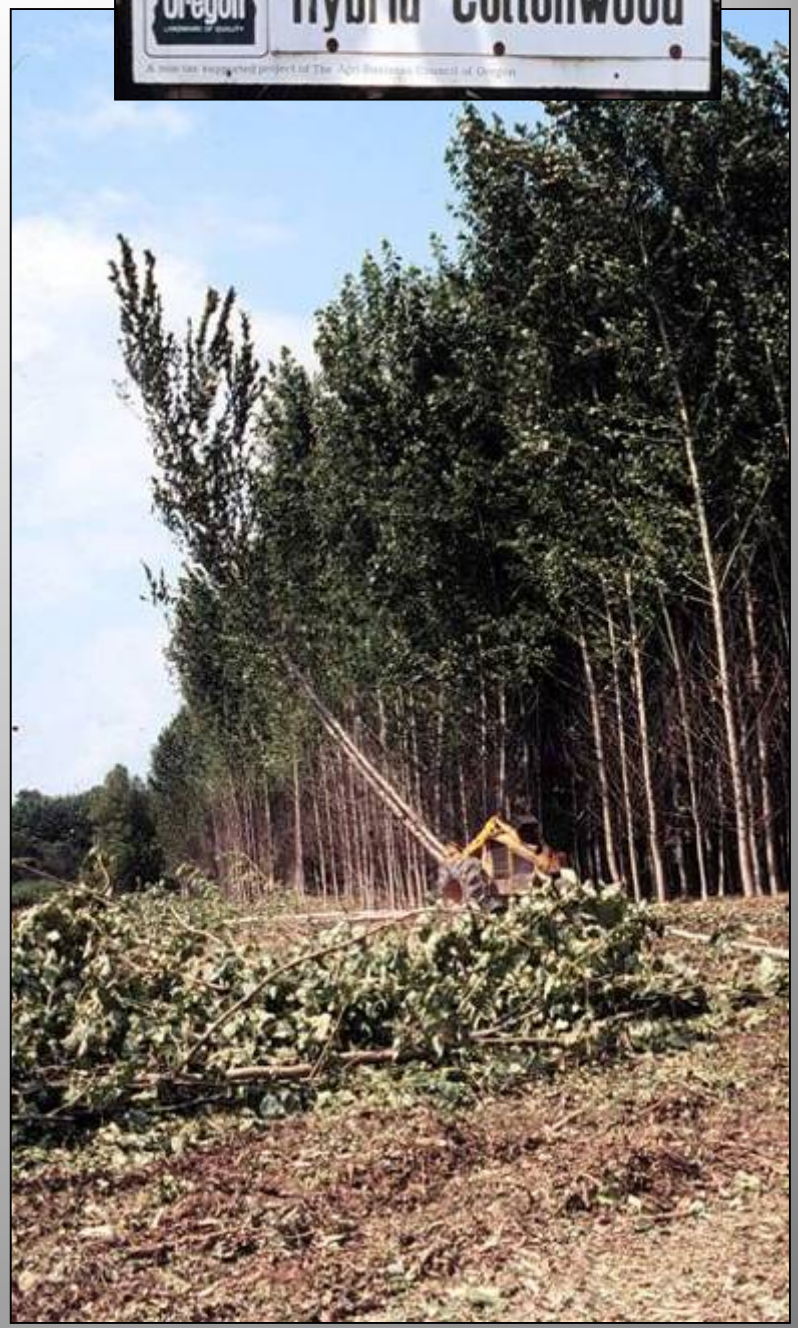
Gene edit/GMO (GE) = “biotech” for the purpose of this talk – not genomic breeding



Overview of steps to create a GE plant



Poplar plantations are examples of my research ecosystem



Eucalypts in Brazil another example of the relevant ecosystem for this talk

Super productive due to conventional breeding – exotics, clones, hybrids, continued cycles of infusion and testing

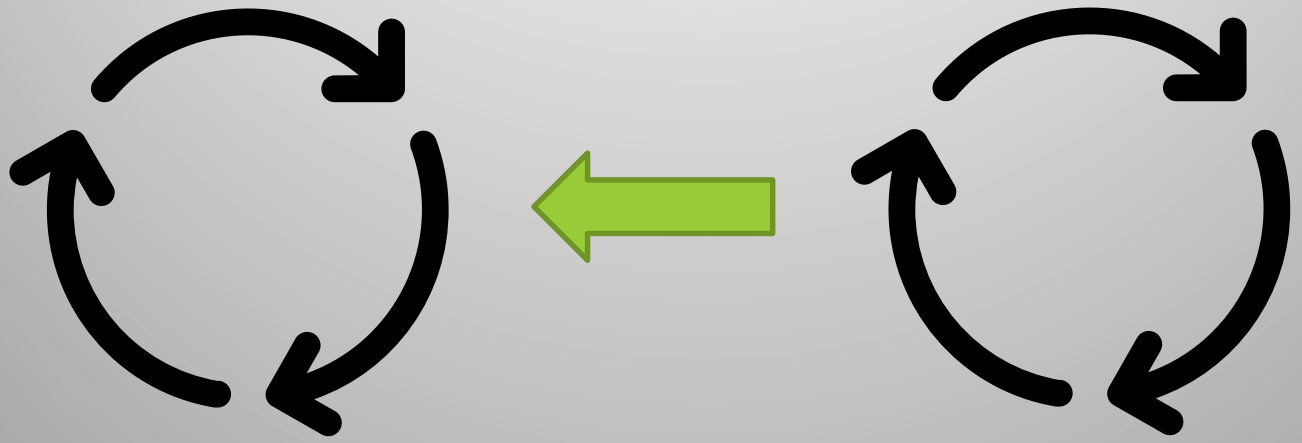
Biotech for wild forest trees?

American chestnut and other wild forest species under serious threat worldwide – but most are not amenable to biotech due to inadequate research, regulatory and market obstacles, and recalcitrance to transformation/editing

Relationship of breeding and biotech

Breeding populations

Biotech innovations



Polygenic:
Growth rate and adaptation

Oligogenic:
Specific modifications and novel traits

These need to be integrated in a way that does not slow down conventional breeding, with its growing power due to physiological and genomic innovations

Key issues for integration of breeding and biotech

- Difficulty of adding new traits to breeding populations
  - Efficiency of transformation and gene editing, trait control, rapid flowering, gene action
- Effect of physiology-modifying traits on breeding advances and performance (pleiotropy, genetic diversity)
- Serious costs and constraints to doing the needed field research and breeding
  - Public and private sector investments
  - Regulations and market restrictions

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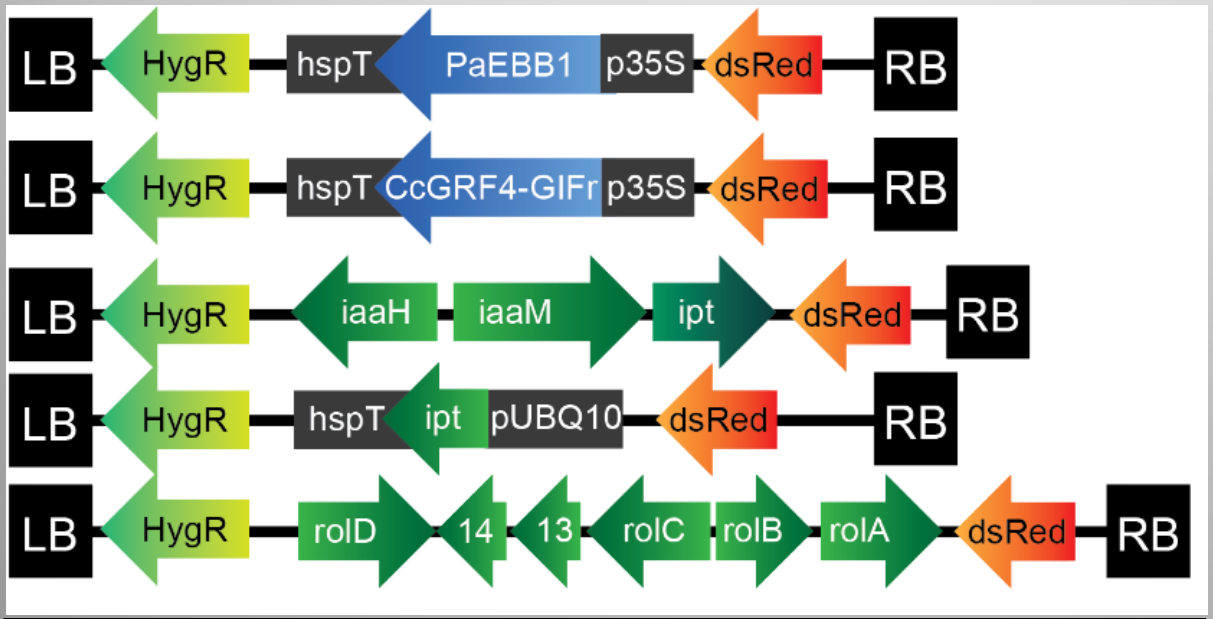
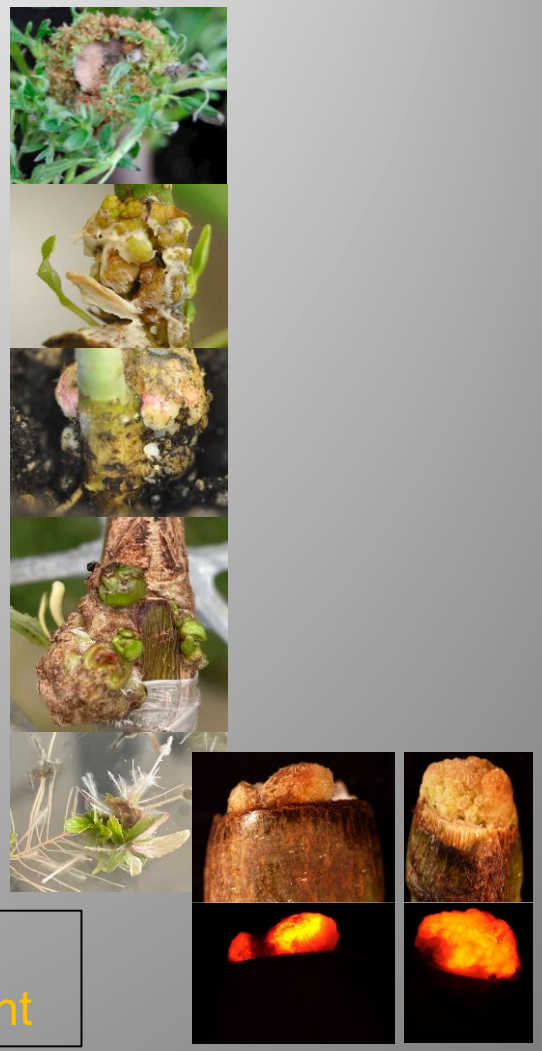
Developmental biology and transformation are growing together – but it's a "long strange trip"

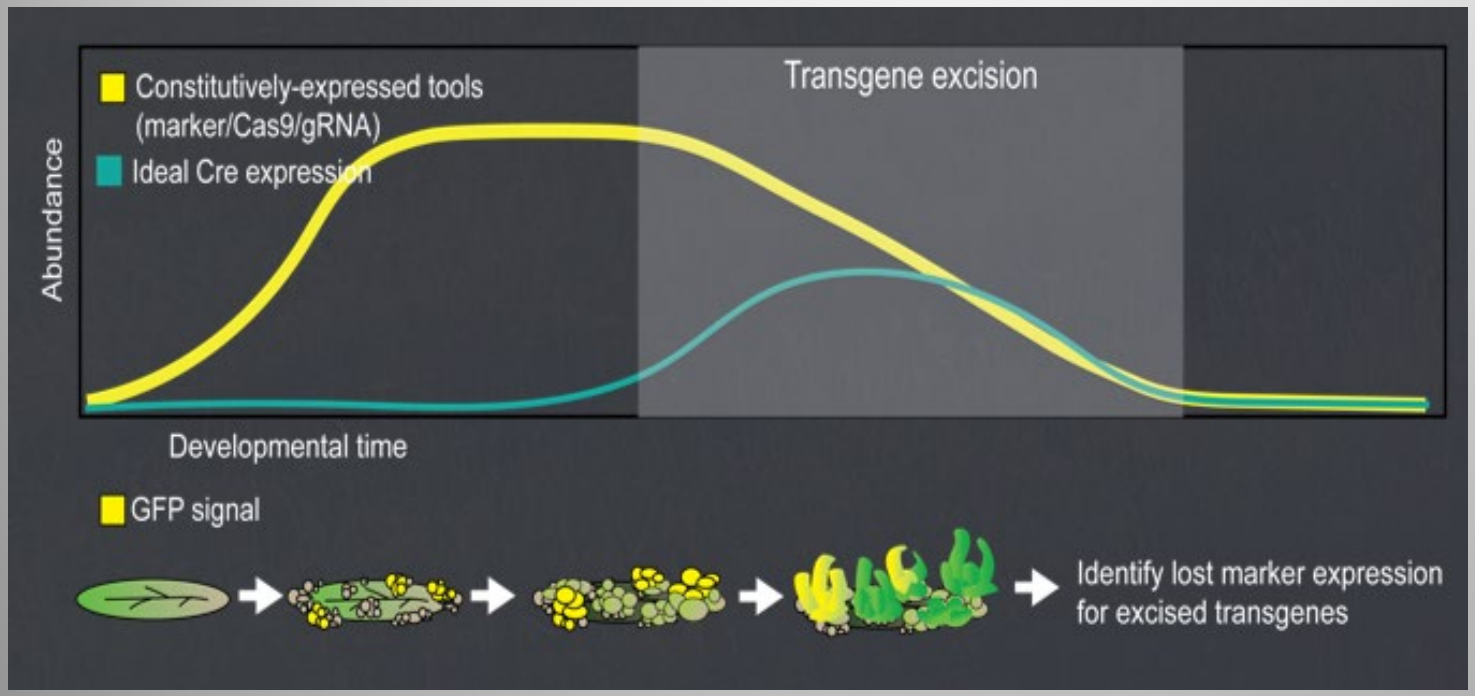


Some of the current work in my laboratory to develop DEV-gene facilitated *in planta* transformation for woody plants

Most of the time these silver-bullet genes do not work, or depend strongly on genotype, physiology, and environment

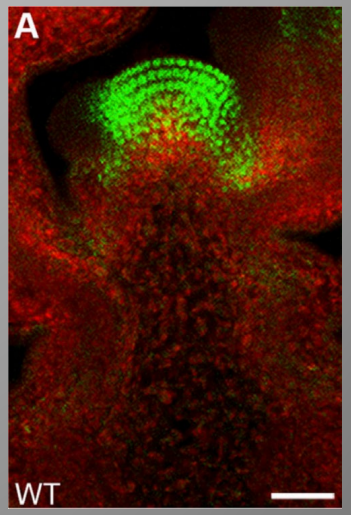


Our trait control toolkit is deficient – a case study of meristem specific excision

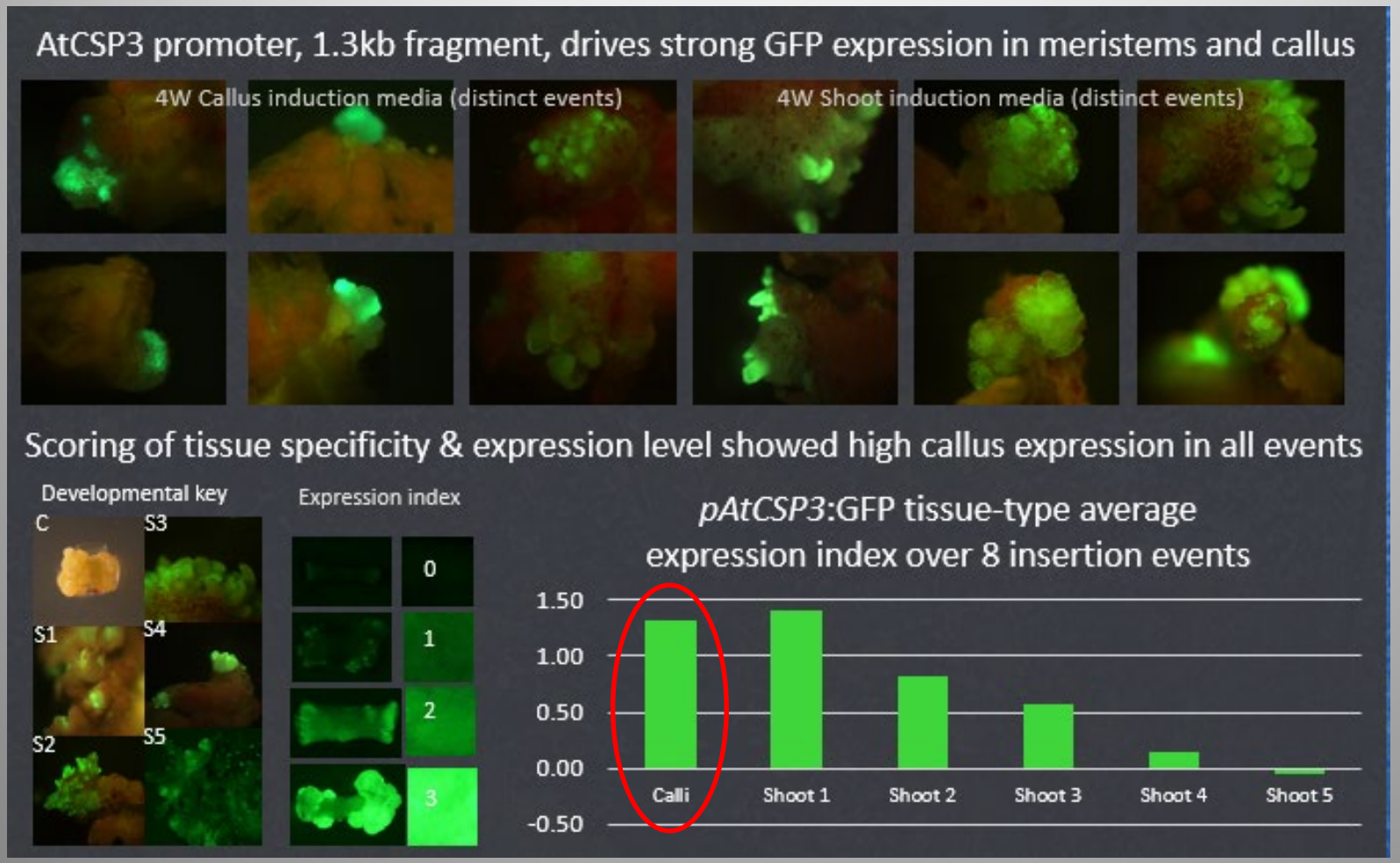


Tested *WUSCHEL*, *SHOOT MERISTEMLESS*, and *CSP3* promoters -- that are strongly meristem dominant in Arabidopsis -- in transgenic poplars using promoter:GFP fusions

STM:GFP

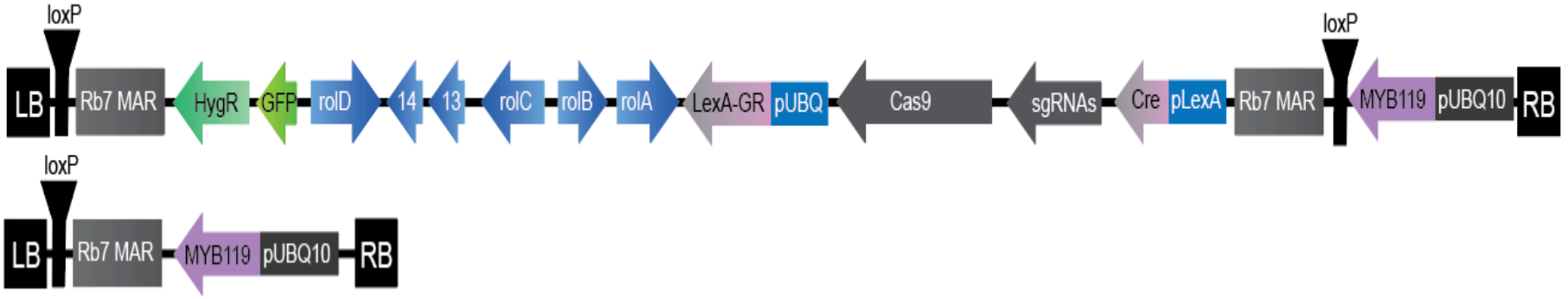


Meristem dominant expression but also strong callus expression



Cre recombinase excision efficiency, with added Dex induction handle, extremely low – likely due to target methylation

Essential for large, vir-launched T-DNAs from systems like GAANTRY to create “normal” chromosomal environment?



Construct with elements to promote transgenic tissue growth, stabilize expression, give inducible Cre expression, do directed gene editing at two gene targets, and overexpress purple pigment genes

Our experience: “off the shelf” transformation, gene expression control and excision systems, work poorly or not at all

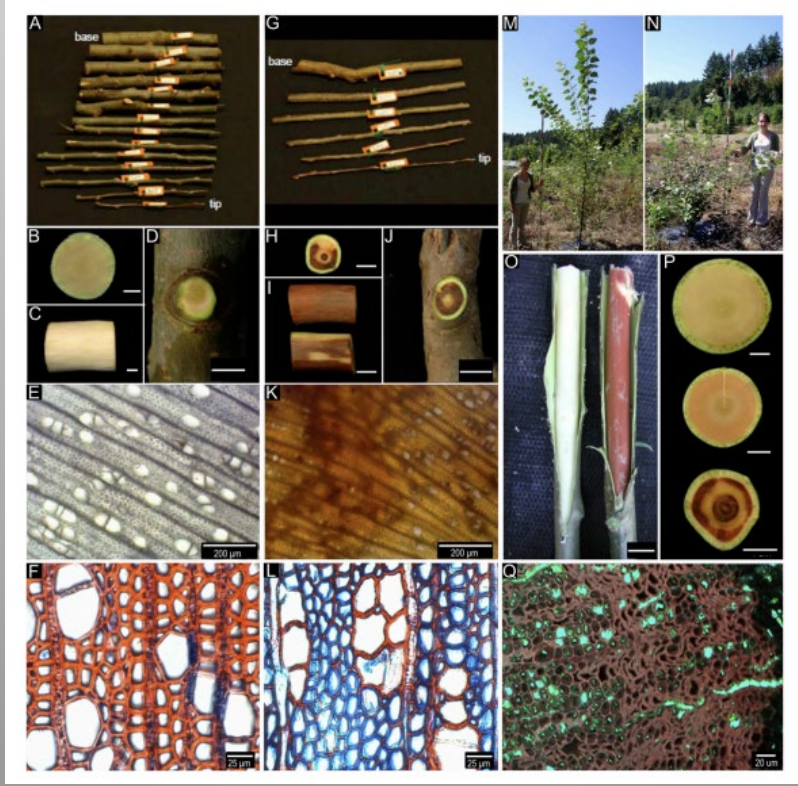
Much more translational “synthetic biology” research needed to take good ideas, proof-of-concept methods, and make them reliable technology in important crops

Greenhouse to field: Some victories, many disappointments

Antisense Down-Regulation of *4CL* Expression Alters Lignification, Tree Growth, and Saccharification Potential of Field-Grown Poplar[1][W][OA]

Steven L. Voelker, Barbara Lachenbruch, Frederick C. Meinzer, Michael Jourdes, Chanyoung Ki, Ann M. Patten, Laurence B. Davin, Norman G. Lewis, Gerald A. Tuskan, Lee Gunter, Stephen R. Decker, Michael J. Selig, Robert Sykes, Michael E. Himmel, Peter Kitin, Olga Shevchenko, and Steven H. Strauss*

Plant Physiology®, October 2010, Vol. 154, pp. 874–886,



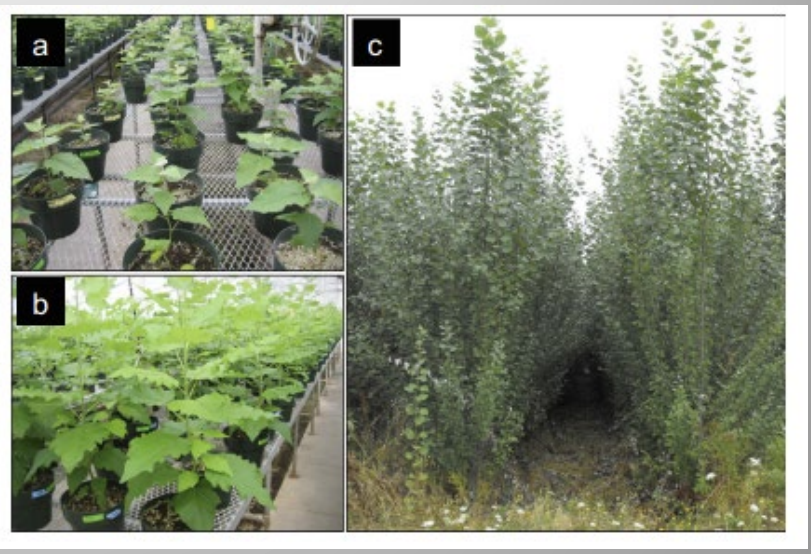
Modification of gibberellin activity modifies growth and biomass allocation – but highly variable effects in greenhouse vs. field

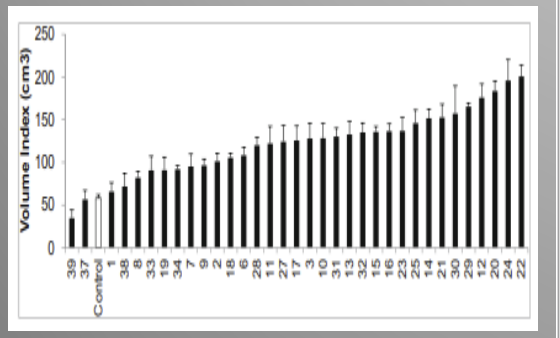
Tree Genetics & Genomes (2015) 11:127
DOI 10.1007/s11295-015-0952-0

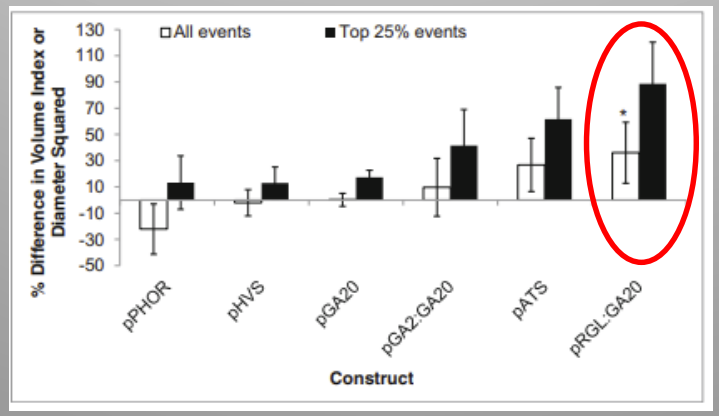
ORIGINAL ARTICLE

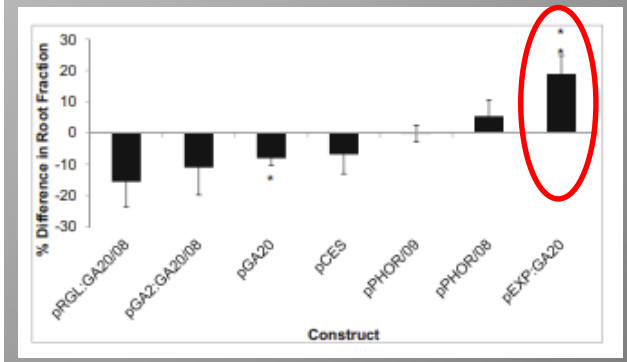
Recombinant DNA modification of gibberellin metabolism alters growth rate and biomass allocation in *Populus*

Haiwei Lu[1] · Venkatesh Viswanath[1,4] · Cathleen Ma[1] · Elizabeth Etherington[1,5] · Palitha Dharmawardhana[1,6] · Olga Shevchenko[1,7] · Steven H. Strauss[1] · David W. Pearce[2] · Stewart B. Rood[2] · Victor Busov[3]









Much metabolic engineering innovation needed for major, novel products

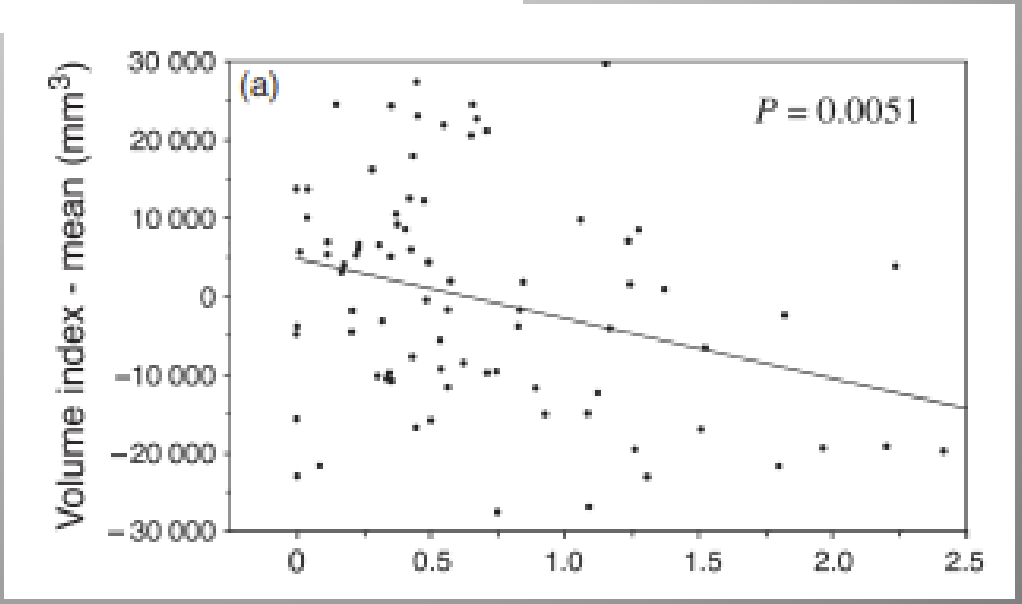
Plant Biotechnology Journal

Plant Biotechnology Journal (2011) **9**, pp. 759–767 doi: 10.1111/j.1467-7652.2010.00585

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*



Field trials can pleasantly surprise too

High productivity in hybrid-poplar plantations without isoprene emission to the atmosphere

Russell K. Monson[a,b,1], Barbro Winkler[c], Todd N. Rosenstiel[d,1], Katja Block[c], Juliane Merl-Pham[e], Steven H. Strauss[f], Kori Ault[f], Jason Maxfield[d], David J. P. Moore[g], Nicole A. Trahan[g], Amberly A. Neice[g], Ian Shiach[g], Greg A. Barron-Gafford[h], Peter Ibsen[i], Joel T. McCorkel[j], Jörg Bernhardt[k], and Joerg-Peter Schnitzler[c,1]

[a]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721; [b]Laboratory of T
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Germany; [d]Department of Biology, Portland State University, Portland, OR 97207; [e]Research Unit Protein Scie
Neuherberg, Germany; [f]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR
Environment, University of Arizona, Tucson, AZ 85721; [h]School of Geography and Development, University of
Botany and Plant Science, University of California, Riverside, CA 92507; [j]Biospheric Sciences Laboratory, NASA
20771; and [k]Institute for Microbiology, Ernst-Moritz-Arndt University, 17487 Greifswald, Germany

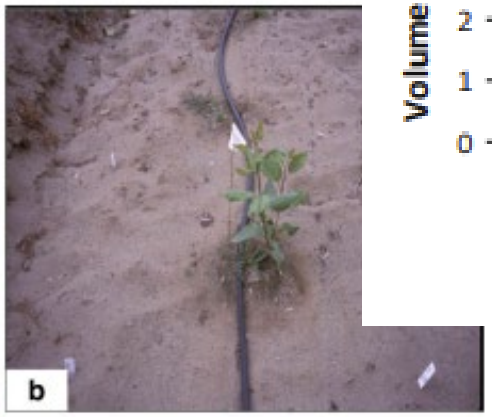
www.pnas.org/cgi/doi/10.1073/pnas.1912327117

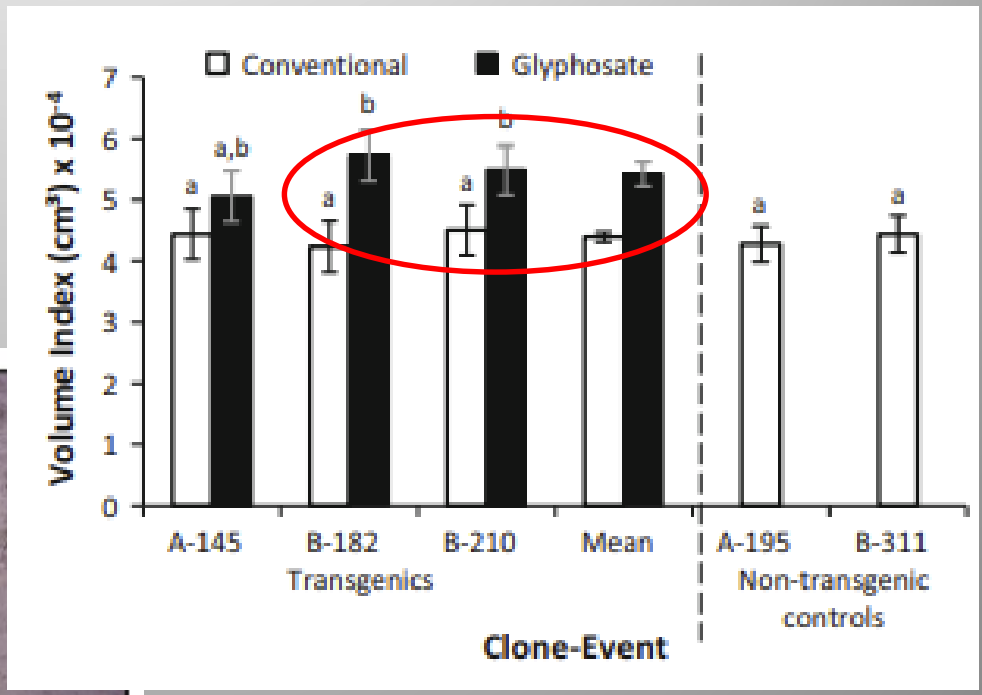
“Old” biotech traits can give large benefits – yield and water use (LCA) value of herbicide tolerance in poplar

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

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]



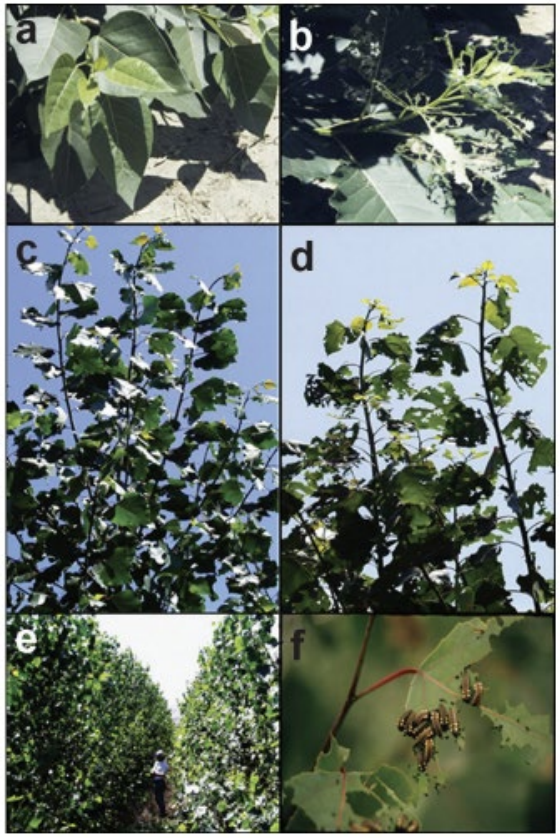


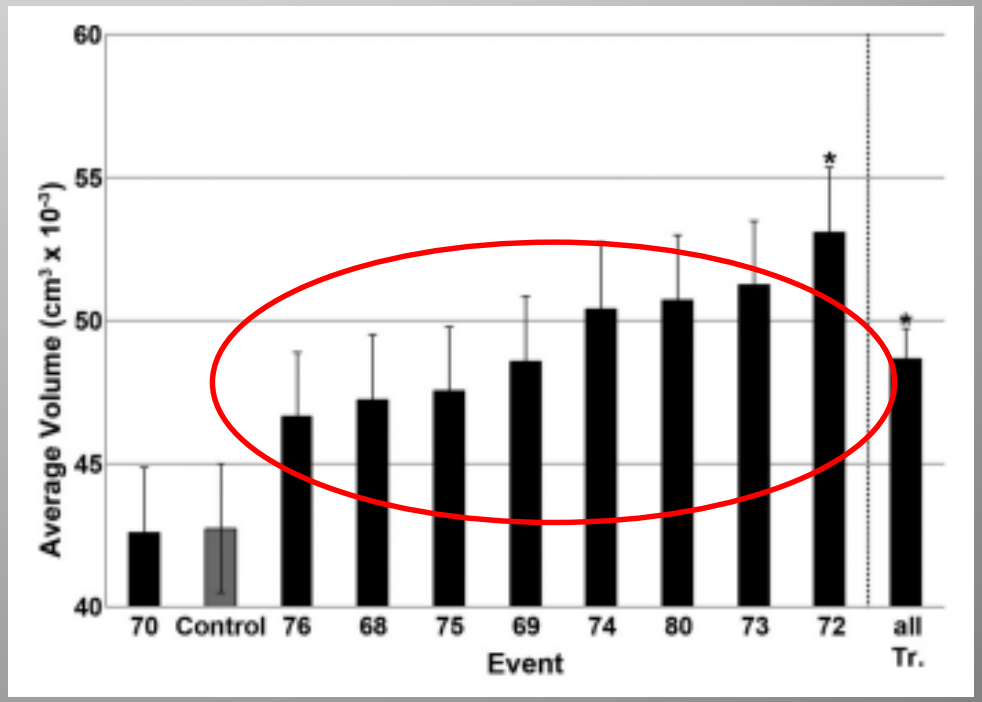
Large yield benefits from pest resistance genes in poplar

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

Can. J. For. Res. **44**: 28–35 (2014) dx.doi.org/10.1139/cjfr-2013-0270





RNAi suppression and gene editing powerful tools for genetic containment

Plant Biotechnology Journal
Open Access

Research Article | Open Access

Genetic containment in vegetatively propagated forest trees: CRISPR disruption of *LEAFY* function in *Eucalyptus* gives sterile indeterminate inflorescences and normal juvenile development

Estefania Elorriaga, Amy L. Klocko, Cathleen Ma, Marc du Plessis, Xinmin An, Alexander A. Myburg, Steven H. Strauss

First published: 27 March 2021 | https://doi.org/10.1111/pbi.13588 | Citations: 1

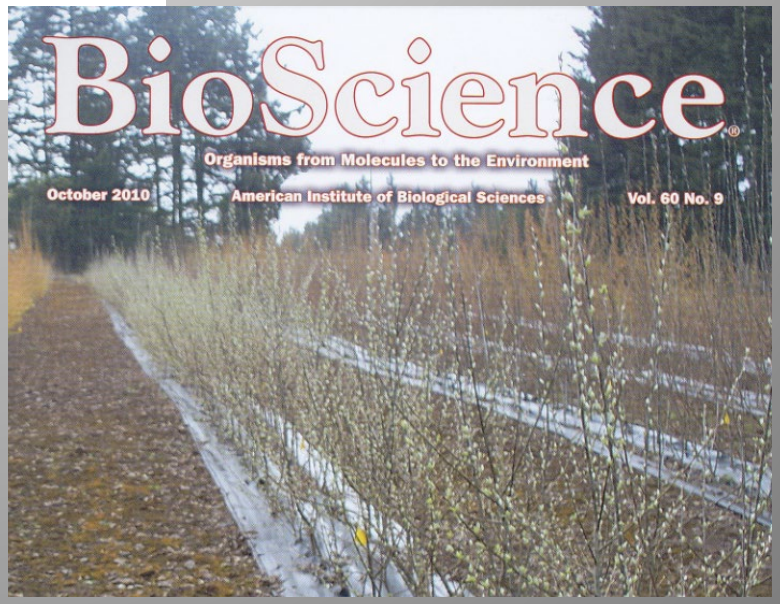
Agenda

- Definitions, aims, and context
- Biological opportunities and constraints
- Social constraints
- What is needed moving forward

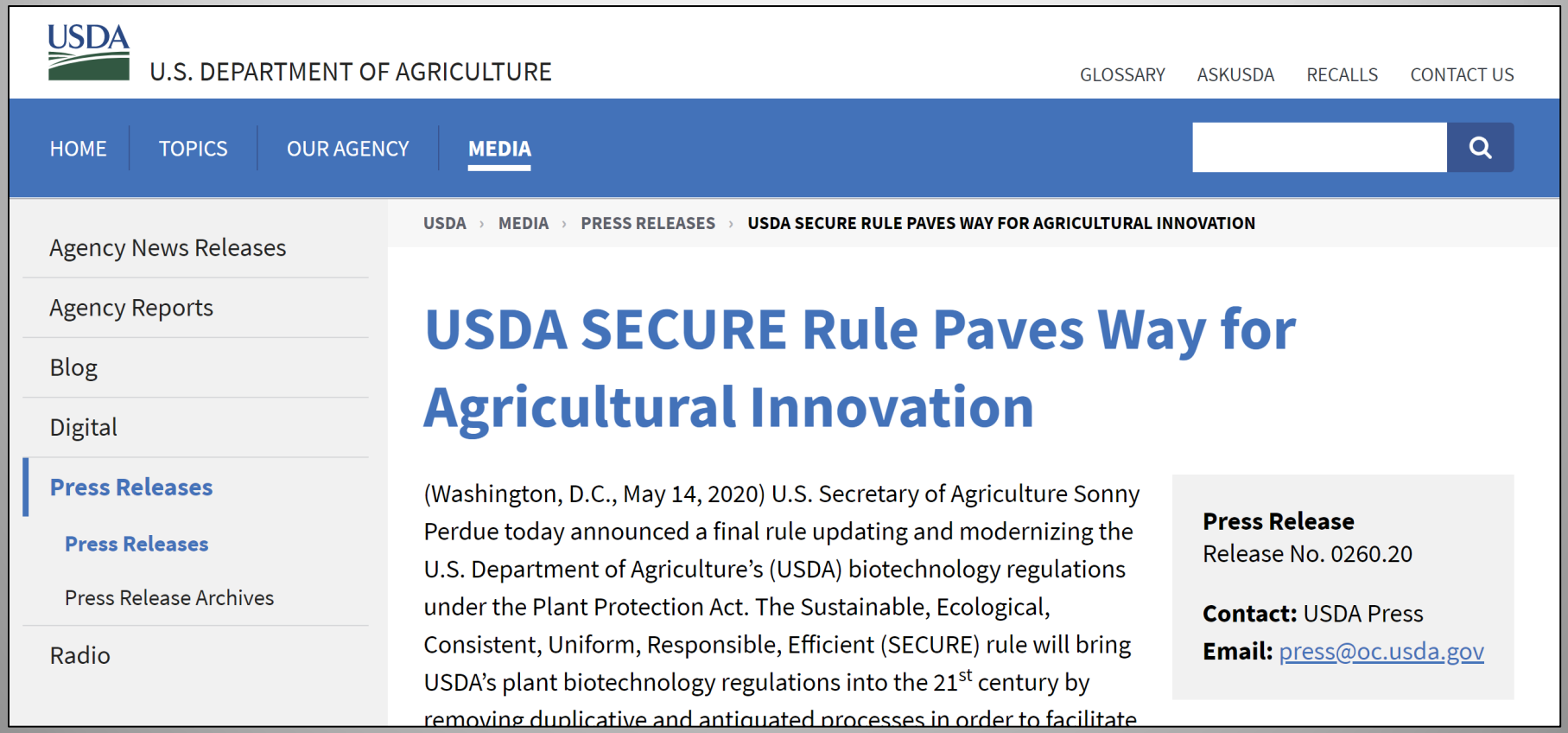
Regulations largely assume that all recombinant DNA insertions are guilty until proven innocent by extensive research

Far-reaching Deleterious Impacts of Regulations on Research and Environmental Studies of Recombinant DNA-modified Perennial Biofuel Crops in the United States

STEVEN H. STRAUSS, DREW L. KERSHEN, JOE H. BOUTON, THOMAS P. REDICK, HUIMIN TAN, AND ROGER A. SEDJO



The ~new 2020 USDA SECURE system is more enlightened – but improvement likely to be small



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USDA › MEDIA › PRESS RELEASES › USDA SECURE RULE PAVES WAY FOR AGRICULTURAL INNOVATION

USDA SECURE Rule Paves Way for Agricultural Innovation

(Washington, D.C., May 14, 2020) U.S. Secretary of Agriculture Sonny Perdue today announced a final rule updating and modernizing the U.S. Department of Agriculture's (USDA) biotechnology regulations under the Plant Protection Act. The Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient (SECURE) rule will bring USDA's plant biotechnology regulations into the 21st century by removing duplicative and antiquated processes in order to facilitate

Press Release
Release No. 0260.20

Contact: USDA Press
Email: press@oc.usda.gov

Classes vs. insertion events regulated, but all transgenics now brought under regulation, and very limited exemptions

There is still some movement for more ambitious changes – politically difficult

Agri Pulse
Agri-Pulse Communications, Inc.
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POLITICS FARM HANDS ENERGY FOOD LABOR CAMPAIGN 2020 RURAL TRADE ENVIRONMENT INNOVATION

NEWS POLITICS FOOD RURAL INNOVATION

House Ag members, witnesses back acceleration of biotech advances in US

10/26/21 3:20 PM *By Steve Davies*

KEYWORDS BIOTECHNOLOGY DAVID SCOTT GENE EDITING GLENN THOMPSON HOUSE AG COMMITTEE HOUSE AGRICULTURE COMMITTEE JIM BAIRD JIM COSTA STACEY PLASKETT TOM VILSACK

“Green certification” creates severe barriers to field research, markets for GMO and gene edited trees

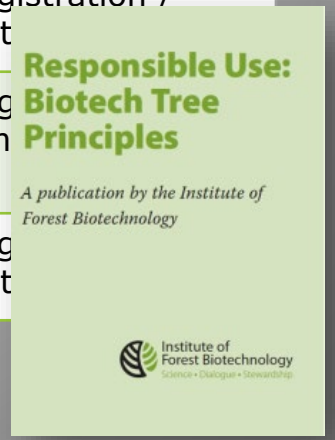
<u>A big deal</u>:
Many of the most highly managed forests and their products are certified

~500 million hectares,
~13% global forest area

Started by the Forest Stewardship Council, major principle:
“genetically modified trees are prohibited”

All major forest certification systems banned GE trees over time – no exemptions

System	Region	GM Tree Approach / Reason
PEFC : Programme for Endorsement of Forest Certification	International	**Banned** / Precautionary approach based on lack of data
FSC : Forest Stewardship Council	International	**Banned** / Precautionary approach based on lack of data
CerFlor : Certificação Florestal	Brazil	**Banned** via PEFC registration / No additional rationale
CertFor : Certficación Forestal	Chile	**Banned** via PEFC registration / No additional rationale
SFI : Sustainable Forestry Initiative	North America	**Banned** via PEFC registration / Awaiting risk-benefit data
ATFS : American Tree Farm System	USA	**Banned** via PEFC registration / No additional rat
CSA : Canadian Standards Association	Canada	**Banned** via PEFC reg Allows public to determ
CFCC : China Forest Certification Council	China	**Banned** via PEFC reg No additional rat



Adam Costanza, Institute for Forest Biotechnology

In 2001 and 2015, forest genetic and biotech scientists publicly criticized FSC for their complete ban on GMOs – because it does not allow field research or breeding with them on certified lands

…with little effect

Plantation Certification & Genetic Engineering

FSC's Ban on Research Is Counterproductive

Steven H. Strauss, Malcolm M. Campbell, Simon N. Pryor, Peter Coventry, and Jeff Burley

Traces of the emerald ash borer on the trunk of a dead ash tree in Michigan, USA. This non-native invasive insect from Asia threatens to kill most North American ash trees.

BIOTECHNOLOGY

Genetically engineered trees: Paralysis from good intentions

Forest crises demand regulation and certification reform

By Steven H. Strauss[1]*, Adam Costanza[2], Armand Séguin[3]

Intensive genetic modification is a long-standing practice in agriculture, and, for some species, in woody plant horticulture and forestry (*1*). Current regulatory systems for genetically engineered

recently initiated an update of the Coordinated Framework for the Regulation of Biotechnology (*2*), now is an opportune time to consider foundational changes.

Difficulties of conventional tree breeding make genetic engineering (GE) methods relatively more advantageous for forest trees than for annual crops (*3*). Obstacles

Although only a few forest tree species might be subject to GE in the foreseeable future, regulatory and market obstacles prevent most of these from even being subjects of translational laboratory research. There is also little commercial activity: Only two types of pest-resistant poplars are authorized for commercial use in small areas in China and two types of eucalypts, one approved in Brazil and another under lengthy review in the USA (*5*).

METHOD-FOCUSED AND MISGUIDED. Many high-level science reports state that the GE method is no more risky than conventional breeding, but regulations around the world essentially presume that GE is hazardous and requires strict containment

Petition created to end GMO ban by certification programs – implemented by Alliance for Science at Cornell University, USA

Endorsed by the largest scientific society of plant biologists in the world

American Society of Plant Biologists

ASPB has studied and endorsed the petition.

members to support a petition to change certification rules for forests to enable field research on biotech (gene edited, genetically engineered) trees. Amazingly, all of the private certification systems have a complete ban in place that extends to research, at a time when forest health is in growing crisis due to expanding pests and climate change. Biotech is not a panacea, but its also too powerful to ignore—and can sometimes provide powerful solutions where other approaches fail.The petition follows the release of a major report on The Potential for Biotechnology to Address Forest Health from the USA National Academy of Sciences that has identified biotechnologies as a key tool for helping to manage forest health and associated pest epidemics.

ASPB has studied and endorsed the petition.

Letter published in *Science* about it (September 2019)

Certification for gene-edited forests

Forest certification bodies were established to provide consumers with confidence that they are purchasing
sourced wood products. Over
hectares of forests, or about
l forest area, are certified
rgest certification systems
ver, certification bodies have
excluded all genetically
or gene-edited (GE) trees from
, including from field research
lands that is essential for
ng local benefits and impacts
ing forest biotechnology
om around the world, with
of more than 1000 globally
atories to a recent detailed
call for all forest certification
romptly examine and modify
s.
ce mounting stresses posed
ests and climate change (*6*).

Engineering, and Medicine recently completed an in-depth study on forest health and biotechnology, concluding that the potential benefits are numerous and rapidly increasing (*12*). Our forests are in dire need of assistance, and GE trees hold tremendous potential as a safe and powerful tool for promoting forest resilience and sustainability.

Steven H. Strauss[1]*, Wout Boerjan[2], Vincent Chiang[3], Adam Costanza[4], Heather Coleman[5], John M. Davis[6], Meng-Zhu Lu[7], Shawn D. Mansfield[8], Scott Merkle[9], Alexander Myburg[10], Ove Nilsson[11], Gilles Pilate[12], William Powell[13], Armand Seguin[14], Sofia Valenzuela[15]

[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA. [2]Department of Plant Biotechnology and Bioinformatics, Ghent University and Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium. [3]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA. [4]Chapel Hill, NC 27517, USA. [5]Department of Biology, Syracuse University, Syracuse, NY 13244, USA. [6]School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA. [7]State Key Laboratory of Subtropical Silviculture, School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou 311300, China. [8]Forest Sciences Centre, University

standard-pefc-st-2002-2013.

Gene-edited and genetically engineered trees, such as these poplars, should be allowed in certified forests.

The result: Helped to initiate a reconsideration by FSC of a small part of their ban -- that of “associated uses” -- whereby a certified company can also grow and sell non-certified GMO materials

A small start, but better than the last ~30 years of zero movement? Status of proposal unclear at present, may be seriously watered down….stay tuned

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Biological priorities … if we wish to make biotech tools broadly available for dealing with the climate emergency…

- Much more efficient transformation, regeneration and editing systems – much promise, but supported by very limited public translational research
- Much better developed and crop-tested recombinant DNA/synthetic biology tools such as insulator, induction, excision, and promoter systems
- Much expanded public field tests in key crop systems of physiological trait modifications – in diverse environments and genotypes and species

Social system changes … if we wish to make biotech tools broadly available for dealing with the climate emergency…

- Extensive pre-field-research regulatory decisions to enable facile integration with breeding – USDA SECURE intentions tested and much expanded
- Modified regulatory emphasis on novel classes of *traits* compared to breeding and management, not based on novel insertion events or expression tweaks
- Elimination of the “green” certification ban on all GMO/GEs from certified companies and forests

The challenge is immense

- The pace of climate change is outpacing the capacity for adaptation in general, and via breeding and biotech as we know them
  - To have a chance to contribute we need extreme efficiency and to embrace, not seek to eliminate, risk of novel research avenues
  - We have perverted the "precautionary principle" ?
- There is no shortage of research demonstrations of heat, drought, salt, flood resistances in crops – but most never go beyond the lab or boutique study – we need much expanded public field tests
- An ambitious, large scale *"translational biotech climate research initiative"* is urgently needed if we wish to have these options at hand in a time frame that might matter