Transgenic trees

Presentation to National Research Council Committee on GMO Crops

Steve Strauss
Distinguished Professor
Oregon State University
Steve.Strauss@OregonState.Edu
Plan

• A bit about me
• Five key contentions
• Thoughts on eight NRC questions
Who am I

- Many years of research in molecular diversity, gene flow, phylogenetics, and basic genomics (epi-, transcript-)
  - Focus on conifers, *Populus, Eucalyptus*
- Many years of research in transgenic tree biotechnology
  - Emphasis on *Populus and Eucalyptus*
  - Industry consortium for >20 years, also DOE and NSF supported
  - Dozens of UDA-APHIS regulated field trials, ongoing
- Director of OSU program on Outreach in Biotechnology with emphasis on agriculture, 8 yrs
  - Public lectures online (40)
Key contentions
1. Trees are not distinct biological categories

There are many kinds of tree systems, including many kinds of “forest” tree systems, thus “GMO tree generalities” are not very useful.

Many variations in forest systems

Great overlap with other crops, esp grasses and woody perennials

Gene flow, perenniality, outcrossing, keystone characters, incomplete domestication

Perception of trees as special mainly in public eye
Poplar plantations an example of ag-like forestry
Eucalypts in Brazil another example of ag-like forestry
Wild forest tree protection or restoration the other extreme

Old growth American Chestnut
2. GMO methods of special value for trees

GMO methods for trees and other woody perennials of particular value due to breeding constraints

- Long breeding cycle
- Difficulty to inbreed and introgress new genes (gen. load)
- Hard to identify and use dominant, major genes
- Asexually propagated varieties of high value
- Powerful means to access Mendelian genes and breeding methods?
Overexpression of endogenous flowering genes induce early flowering in several tree species

Apple

Orange

Plum

Poplar
FT transgene effective for stimulating early flowering in eucalypts
Lignin-modification of elite variety in France
(Courtesy of G. Pilate, INRA)
Native genes for disease resistance in elite grape varieties

**Grape VvAlb gene**

- Non-Engineered
- Engineered

**Grape VvTL-1 gene**

- Non-Engineered
- Engineered

- ‘Syrah’
  - Powdery Mildew Resistance

- ‘Thompson Seedless’
  - Rot Resistance

Courtesy of Denis Gray, UF/IFAS Mid-Florida Research & Education Center

http://mrec.ifas.ufl.edu/grapes/genetics
3. Technology diverse and effective

A great diversity of traits, and economic and/or environmental values, have been demonstrated in field trials of trees. **GMO tree thinking**

After initial event sorting, stability, efficacy, and trait diversity high

- Herbicide tolerance
- Biotic, abiotic stresses
- Wood or fruit quality
- Form/stature and growth rate
- Containment
- Accelerated flowering
- Bioremediation
- Novel bioproducts
Insect resistant poplars commercially approved in China - Bt cry1

- Trait stable
- Helps to protect non-Bt trees
- Reduced insecticide use
- Improved growth rate
Growth benefits (10-20%) despite low insect pressure during large field trial of resistant genotypes
A tapetal ablation transgene induces stable male sterility and slows field growth in *Populus*

Estefania Elorriaga · Richard Meilan · Cathleen Ma · Jeffrey S. Skinner ·
Elizabeth Etherington · Amy Brunner · Steven H. Strauss

Received: 20 March 2014 / Revised: 18 July 2014 / Accepted: 18 July 2014 / Published online: 13 August 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract The field performance of genetic containment technologies—considered important for certain uses of transgenic trees in forestry—is poorly known. We tested the efficiency of a barnase gene driven by the TA29 tapetum-dominant promoter for influencing growth rate and inducing male sterility in a field trial of transgenic hybrid poplar (*Populus tremula* × *Populus tremuloides*). When the growth of 18 transgenic transgenic event grew significantly more slowly than the control. In contrast, when we compared the growth of transgenic trees containing four kinds of β-glucuronidase (GUS) reporter gene constructs to non-transgenic trees—all of which had been produced using the same transformation method and poplar clone and grown at the same field site—there were no statistically significant differences in growth after three grow-
Complete sterility - Undeveloped catkins, stable suppression of native LEAFY gene in poplar (RNAi)

Klocko et al. 2014, American Soc. For Plant Biology, Portland, Oregon

CRISPR studies in progress
4. Market obstacles are formidable

Market constraints are global and near universal, with no research exemptions

- Forest “green” certification
- FSC led, now all systems by mutual affiliation
- No research / emergency exemptions
- Greatly constrain research
- Promotes disinvestment
“Green” certification of forests create severe barriers to field research, markets...

Plantation Certification & Genetic Engineering

FSC’s Ban on Research Is Counterproductive

Genetic engineering, also called genetic modification (GM), in all aspects of the world, is the use of recombinant DNA and natural gene transfer methods to breed more productive or pest-resistant crops. It has been the subject of considerable controversy, with concerns raised from biological, socioeconomic, political, and ethical perspectives. Some of these issues are similar to those raised by the use of molecular biology and genetic engineering in medicine, which we are seeing in the news headlines daily; however, genetic modification in agriculture and forestry raises environmental issues as well.

GM crops, mainly herbicide- and pest-resistant varieties of soybeans, maize, or cotton, have been vigorously adopted by farmers in North America because they are easy to manage and they improve yields, reduce costs, or reduce pesticide contamination (Carpenter and Gimmler 2001). However, the controversy, primarily embodied in regulatory barriers to trade of GM crops with Europe and Japan, has slowed their adoption considerably in recent years. If GM crops are to be grown in forests in the near future, they are likely to occur primarily in intensively managed environments, such as urban forests or plantations. In urban forestry, genetic modification is expected to help trees adapt to the stresses and special demands of human-dominated systems. Examples would be trees that are tolerant of heavy metals or other pollutants, more adapted to climate change, or do not produce fruits when trees create hazards in street environments (Flannery et al. 1998). Plantations, although very different from natural forests in structure and function, are considered part of the spectrum of methods in sustainable forest management (Kimmins 1994).

Plants can release poison on natural forests for exploration and can be of great social value by supplying composites and industrial wood needs and fueling economic development. The environmental role of plantations is recognized by the Forest Stewardship Council (FSC), an international body for certification of sustainably managed forests. FSC Principle 10 states that plantations should “complement the management of, reduce pressure on, and preserve the recreation and conservation of natural forests” (FSC 2011).

FSC has certified some of the most intensively managed plantations in the world, including poplar plantations and the extensive pine and eucalyptus plantations of the Southern Hemisphere. Although many environmental considerations are built into these certified plantation systems, within the areas dedicated to wood production, they function as tree farms. Such intensive plantation systems often use highly bred genotypes, possibly including alien species, hybrids, and clones, as well as many other forms of intensive silvicultural management. It is in the content of these intensive systems that the additional expense of GM trees is likely to be worthwhile.

However, FSC currently prohibits all uses of GM trees, and is the only certification system to have done so...
Forest certification systems universally ban all GM trees – no exemptions

<table>
<thead>
<tr>
<th>System</th>
<th>Region</th>
<th>GM Tree Approach / Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEFC : Programme for Endorsement of Forest Certification</td>
<td>International</td>
<td><strong>Banned</strong> / Precautionary approach based on lack of data</td>
</tr>
<tr>
<td>FSC : Forest Stewardship Council</td>
<td>International</td>
<td><strong>Banned</strong> / Precautionary approach based on lack of data</td>
</tr>
<tr>
<td>CerFlor : Certificação Florestal</td>
<td>Brazil</td>
<td><strong>Banned</strong> via PEFC registration / No additional rationale</td>
</tr>
<tr>
<td>CertFor : Certificación Forestal</td>
<td>Chile</td>
<td><strong>Banned</strong> via PEFC registration / No additional rationale</td>
</tr>
<tr>
<td>SFI : Sustainable Forestry Initiative</td>
<td>North America</td>
<td><strong>Banned</strong> via PEFC registration / Awaiting risk-benefit data</td>
</tr>
<tr>
<td>ATFS : American Tree Farm System</td>
<td>USA</td>
<td><strong>Banned</strong> via PEFC registration / No additional rationale</td>
</tr>
<tr>
<td>CSA : Canadian Standards Association</td>
<td>Canada</td>
<td><strong>Banned</strong> via PEFC registration / Allows public to determine</td>
</tr>
<tr>
<td>CFCC : China Forest Certification Council</td>
<td>China</td>
<td><strong>Banned</strong> via PEFC registration / No additional rationale</td>
</tr>
</tbody>
</table>

Adam Costanza, Institute for Forest Biotechnology
5. Regulatory obstacles are ~disabling

Need fundamental reform for GMO trees to make a significant contribution

- Zero-tolerance during research and breeding unworkable at start

- Similar issues likely with commercial expansion

- Do they make sense given demonstrated values?

- In a world witnessing pest proliferation and rapid climate change?

  Much more to come in one to a few tree generations…
No-analog scientific thinking should dominate today

“No-analog communities (communities that are compositionally unlike any found today) occurred frequently in the past and will develop in the greenhouse world of the future.”
Suggested method exemptions - 1

- Approved, familiar markers and gene transfer systems based on approvals in other crops
- Mutagenesis of transformation system
- Cisgenic (or functionally cisgenic) transfers from similar or closely related species (e.g., congeneric gene sources)
- Modification of expression of native genes and pathways (intragenic)
- Genome editing or mutagenesis
- Individual insertion events, after consideration of gene/protein function and expression
Suggested method exemptions - 2

- Well understood products, or with significant ecological or humanitarian value, and non-toxic
  - Early consult with USDA/FDA re. low level admixture?
- Gene dispersal into the environment and associated AP/LLP during research and breeding, or when crop-appropriate mitigation methods are employed
  - Similar to conventional breeding
  - Presumption: Extensive dilution, limited movement
  - Best management practices (BMPs) not zero- nor strict (e.g., 0.9%) legal tolerances
Exemptions and lower tiers of regulation do *not* mean that GMO traits will be unregulated

- Other, function-based regulations are in place at FDA, EPA, USDA (but need modification/interpretation)
  - Especially at EPA so focus is on novel chemicals as intended by FIFRA
  - At USDA to avoid loopholes
- Companies can choose regulatory reviews where desired, where they believe there is sufficient novelty or risk due to science or trade/economics
- Can enable agencies to challenge based on trait categories, functional novelty, and scientific literature
- Key is presumptive value of genetic innovation and method safety, vs. presumption of harm due to method
  - Comparator is conventional breeding and plant domestication practices
What a regulation-rational world could look like: Lignin-modified trees

Concept proven, but customized refinement needed

Type of gene, promoters, extent of modification, environment, stand age, tree genotype
Detailed discussion of how regulations impede R & D

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
Also an international issue given Cartagena Protocol and trade

Strangled at birth? Forest biotech and the Convention on Biological Diversity

Steven H Strauss, Huimin Tan, Wout Boerjan & Roger Sedjo

Against the Cartagena Protocol and widespread scientific support for a case-by-case approach to regulation, the Convention on Biological Diversity has become a platform for imposing broad restrictions on research and development of all types of transgenic trees.

The Convention on Biological Diversity (CBD) has become a major focus of activist groups that wish to ban field research and commercial development of all types of genetically modified (GM) trees. Recent efforts to influence CBD recommendations by such groups has led to the adoption of recommendations for increased regulatory stringency that are inconsistent with the views of most scientists and most of the major environmental organizations. We suggest that the increasingly stringent recommendations adopted by the CBD in recent years are impeding, and in many places may foreclose, much of the field research needed to develop useful and safe applications of

A convention co-opted
Negotiated under the United Nations (UN) Environment Program, CBD was adopted in June 1992 and subsequently entered into force in December 1993. The CBD has been signed by 191 of the 192 members of the UN, making it one of the largest international treaties. The aim of the CBD is to promote the conservation and sustainable use of biodiversity, and the fair and equitable sharing of benefits from the use of genetic resources. Because transgenic organisms have the potential to affect biodiversity, special provisions of the CBD cover the use and trade in living modified organisms (LMOs, also known as genetically modified organisms; GMOs).

In 2000, the Cartagena Protocol on Biosafety was added to the CBD...
Genomics, Genetic Engineering, and Domestication of Crops

Steven H. Strauss

Genomic sequencing projects are rapidly revealing the content and organization of crop genomes (1). By isolating a gene from its background and deliberately modifying its expression, genetic engineering allows the impacts of all genes on their biochemical networks and organismal phenotypes to be discerned, regardless of their level of natural polymorphism. This greatly increases the ability to determine gene function and, thus, to identify new options for crop domestication (2). The organismal functions of the large majority of genes in genomic databases are unknown.

<table>
<thead>
<tr>
<th>Confinement level</th>
<th>Type 1 field trials (exploratory)</th>
<th>Type 2 field trials (precommercial)</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Biological and physical confinement—detailed data</td>
<td></td>
<td>Highly toxic or allergenic pharmaceuticals and proteins</td>
</tr>
<tr>
<td>Medium</td>
<td>FSC, basic data</td>
<td>FSC, detailed data</td>
<td>Novel pest-management genes, low toxicity pharmaceuticals and proteins</td>
</tr>
<tr>
<td>Stress tolerance</td>
<td>FSC, basic data</td>
<td>FSC, detailed data</td>
<td>Genomics-guided transgenes</td>
</tr>
<tr>
<td>Low Domesticating</td>
<td>Petition for exemption?</td>
<td>FSC, basic data</td>
<td></td>
</tr>
</tbody>
</table>

Categories of confinement and monitoring for small- and large-scale transgenic field trials. Biological confinement includes genetic mechanisms to preclude spread and/or reproduction. Physical confinement requires use of geographical isolation or physical barriers. FSC, farm-scale confinement; use of spatial isolation within and between farms and border crops, combined with monitoring. Detailed data include surveys of gene flow away from the site. Basic data include surveys of gene flow away from the site.
Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics

Kent J Bradford¹, Allen Van Deynze¹, Neal Gutterson², Wayne Parrott³ & Steven H Strauss⁴

The costs of meeting regulatory requirements and market restrictions guided by regulatory criteria are substantial impediments to the commercialization of transgenic crops. Although a cautious approach may have been prudent initially, we argue that some regulatory requirements can now be modified to reduce costs and uncertainty without compromising safety. Long-accepted plant breeding methods for incorporating new diversity into crop varieties, experience from two decades of transgenic crop use, and insights from other disciplines that deal with complex systems can help guide regulatory decisions.

Regulatory costs also play a role in the growing disparity between the expanding global adoption of the large-market transgenic maize, soybean, cotton and canola crops¹ and the so-called ‘small-market’ or ‘specialty’ crops, for which field trials and commercial releases of transgenic food crops have all but stopped⁵. In 2003, fruits, vegetables, landscape plants and ornamental crops accounted for more than $50 billion in value in the United States, representing 47% of the total US farm...
Thoughts on eight NRC questions
Based on the wide natural variation, and ability for clonal propagation, in many tree species, is GE even necessary for introduction of many traits?

- Ask the market
  - Industry was very interested in efficiencies, new options GE brings – but of course not much in the current climate of bad PR, market barriers, absence of public research investment, and very high regulatory costs/risks

- GE not an alternative to breeding, but sometimes useful or ~essential for adding specific traits, freeing breeders to focus on other traits, enabling Mendelian options
  - How it might interact with breeding if freed to do so based on biology is unknown, hard to imagine given current restrictions
Are there specific issues with risk assessment that would be different from most other plant species?

- Very difficult to do scaled-up research, operational breeding, with complete prevention of gene flow prior to commercial authorizations
  - Without engineered sterility added and verified up front – which is impossible to do for many genotypes during breeding

- Time frame for ecological risk assessment of many trees are within frame of expected large scale climate change and species shifts – what are the comparators?
How big of a concern is pollen movement in GE trees, especially tall trees that produce a lot of pollen, e.g., pine?

- It is beneficial in that pollen dilution from wild and planted trees is extensive during early research and scale-up – easy to mitigate/isolate

- It is very detrimental in a zero-tolerance world (regulatory or market driven)

- Coexistence problematic without workable tolerances, BMPs (best management practices)
Can you discuss what kind of traits we could expect to find in trees used in forestry in the next 20 years if the regulatory system for GE trees was optimized?

- Optimization seems like a distant dream and likely not nearly enough. Revolution seems to be needed where all GE gene flow is not a crime, and private and public R & D greatly expanded.

- Many traits could be commercialized – depends on need, context, and complementarity with breeding of specific species. See list of trait diversity presented above.

- A key need is improved transformation methods – but application oriented GMO research hardly supported in recent years.
Are there any trait/tree species combinations that you feel could be harmful to the environment?

- I do not see long term harm to wild environment from traits that I am familiar with, or where risk is higher compared to that presented in conventional breeding (exotics, hybrids, clones).

- **Harm often assumed to result from traits that improve fitness, but they could also be beneficial for resilience in our changing world**
  - Fitness improving genes can be mitigated if needed, herbivore counter-evolution and climate change variances within time frame of significant impact.

- Herbicide resistance can be a harm for management of wild areas (control as exotic) and/or forest/ag management if deployed widely and without containment and acceptable alternative herbicide control options.

- Sterility could have negative impacts on biodiversity but expectation is that mitigation is not difficult if needed, when compared to current management (landscape, stand-level).
Do you see the complete lack of regulation on some GE trees as causing the public to be more concerned about GE trees and GE in general?

- Yes, my concern is that the public and interest groups will see that as an important loophole, that could lead to regressive and sweeping method-based regulation as we see in the EU, and possibly trade sanctions.

- It would be best to bring all GE trees (and GE crops) into a system with clear guides as to what is regulated and not, and how stringently, based on genomic and functional familiarity, and importance/impact (ecological, economic) etc.
What do you think would be the best way to govern GE tree commercialization to ensure the most sustainable forestry practices?

- I think it is a mistake to regulate forestry and sustainability based on a breeding method that can produce very diverse traits and modifications. It is against prior NRC findings about the innocence of the method. And we now know there are great costs to any level of regulation, and that discretion rapidly is engulfed by political expedience (USA, EU, and beyond)

- BMPs at small scale research phase, traits and outcomes (e.g., yield, pest control, biodiversity, invasiveness) at commercial stage. With tiers and associated legal criteria based on presumption of value not harm, and tied to new functional traits, not individual events
How great is the risk that GE cold-tolerant trees, e.g., Eucalyptus, will have niche movement and become more invasive in the US?

- This is a question for ecologists, but doubt that it is truly predictable at all given questions of scale, climate change, and extent of cold tolerance.

- The use of a mitigation gene (male-sterility) should be praised as good stewardship given uncertainty – the GMO right way™ to do new plant introductions.

- Given climate and pest uncertainties, a new, distinctive, contained, and woody/perennial domesticated fiber/fuel species a great thing for the Southern USA? (Can control ecological impacts of large scale use by local regulations on water, fire, endangered species if needed in the future?)
Are our regulations and certification systems worrying too much about the deck chairs on the Titanic, rather than providing tools for improved navigation of the ship?
What Voltaire might have said about zero-tolerance and stringent legal thresholds for adventitious presence?

“The perfect is the enemy of the good”