Why Biotech Solutions are Needed to Address Forest Health

Steve Strauss
Oregon State University / USA
Why advocate for recDNA tech?

• **Science** – rDNA starts from nature
• **Innovation** – Builds on nature to enhance values
• **Trees** – Can enhance forests, wild and planted
• **Urgency** – Tools for growing forest health crises
• **Controversy** – Enjoy battles of ideas, interests
Key messages

• rDNA methods are powerful tools to supplement breeding in the right niches

• Serious technical and social obstacles prevent their significant use, or even research, for forest health

• In the face of forest health crises, we have an ethical obligation to create technological capacity and social conditions to enable wider use
Agenda

- Basics
- Rationales
- Constraints
- Solutions
What is rDNA biotech?

- Equivalent to genetic engineering (GE), genetic modification (GM), and including gene editing like CRISPR
- Direct modification of DNA
  - Vs. indirect modification in breeding and genomic selection
- Asexually modified, usually in somatic cells
  - Then regenerated into whole organisms, most often starting in Petri dishes
Dawn of the gene-editing age

EVErywhere

CONSERVATION
A WORLD OF TWO HALVES
E. O. Wilson’s vision for an Earth shared with nature
PAGE 170

PLANT BIOLOGY
FLOWER ARRANGEMENT
An attractant/receptor pair driving pollen-tube growth
PAGE 178, 241 & 245

GROUP DYNAMICS
THE RIGHT SIZE FOR A LAB
The skills mix and head count needed for success
PAGE 263
High CRISPR mutation rates observed in poplar and eucalypts – Strauss laboratory

- Cas-only control events and off-target sites
  - Several dozens of gene insertions studied
  - No mutations

- CRISPR-Cas events
  - Hundreds of gene insertions studied
  - 80-100% with mutations
  - 50-95% biallelic knock-outs
Agenda

• Basics
• **Rationales**
• Constraints
• Solutions
GMO methods of special value for trees due to breeding constraints

- Long breeding cycle
- Difficulty to inbreed and introgress new genes (genetic load)
- Hard to identify and use dominant, major genes
- Asexually propagated varieties of high value
- A powerful addition to breeding repertoire?
  - Access Mendelian genes and breeding tools
GE of special value for forest health

• Can design biotic resistance genes based on knowledge of gene function
  • General and host-specific toxins
  • Host induced gene silencing (HIGS)
  • Effector targets
  • Induced programmed cell death

• Pyramiding diverse resistance genes by recDNA

• Combining into conventionally bred and adapted ~resistant germplasm

• Tantalizing possibilities with abiotic stress tolerance as well – advanced cold and salt tolerance examples
HIGS can be effective for insect and fungal resistance

“…demonstrating that HIGS is a powerful tool, which could revolutionize crop plant protection.”
Domain for HIGS in pest resistance seems to keep expanding.

New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing

Aline Koch* and Karl-Heinz Kogel

Centre for BioSystems, Land Use and Nutrition, Institute of Phytopathology and Applied Zoology, Justus Liebig University, Giessen, Germany

Summary
RNA interference (RNAi) has emerged as a powerful genetic tool for scientific research over the past several years. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine and agriculture. In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content. In addition, the demonstration that agricultural pests, such as insects and nematodes, can be killed by exogenously supplied RNAi targeting their essential genes has raised the possibility that plant predation can be controlled by lethal RNAi signals generated in planta. Indeed, recent evidence argues that this strategy, called host-induced gene silencing (HIGS), is effective against sucking insects and nematodes; it also has been shown to compromise the growth and development of pathogenic fungi, as well as bacteria and viruses, on their plant hosts. Here, we review recent studies that reveal the enormous potential RNAi strategies hold not only for improving the nutritive value and safety of the food supply, but also for providing an environmentally friendly mechanism for plant protection.

Keywords: genetically engineered plants, host-induced gene silencing, RNA interference, plant protection, resistance.

RNA interference: discovery of a novel mechanism for gene regulation
RNA interference (RNAi) is a conserved and integral aspect of (Hammond et al., 2001a). This latter phenomenon was termed co-suppression in plants and quelling in fungi. PTGS also could be induced in plants by cytoplasmically replicating viruses (Hammond et al., 2001a). Given the similar phenotypes associated with PTGS
Though presumed guilty, the rDNA method appears to be innocent.
Many field applications in literature

- A great diversity of traits, and economic and/or environmental values, have been demonstrated in field trials of trees

- After initial event sorting, stability, tree health, and trait efficacy high

- Examples of traits successfully studied in the field
  - Herbicide tolerance
  - Biotic, abiotic stresses
  - Wood or fruit quality
  - Form/stature and growth rate
  - Containment
  - Accelerated flowering
  - Bioremediation
  - Novel bioproducts

New Phytologist

Reproductive modification in forest plantations: impacts on biodiversity and society

Steven H. Strauss, Kristin N. Jones, Haiwei Lu, Joshua D. Petit, Amy L. Klocko, Matthew G. Betts, Berry J. Broso, Robert J. Fether Jr, Mark D. Needham
Existing 4 ha rDNA poplar trial in Oregon (2016)
Lepidopteran-resistant poplars approved in China - Bt *cry1*

- Trait stable
- Helps to protect non-Bt trees
- Reduced insecticide use
- Improved growth rate
Large growth benefits (10-20%) despite little insect pressure during field trial of resistant genotypes
Agenda

• Basics
• Rationales
• **Constraints**
• Solutions
Constraints are large

- Trees often rich in diversity due to early state of domestication
  - GE often not needed
  - Advanced phenotyping, molecular markers, genomic selection often more potent and rapid approach

- Genetic transformation methods often very difficult and highly genotype-specific
  - Very limited advances outside of a few intensively studied species – often mostly proprietary
  - Very challenging to apply to non-timber species, diverse genotypes in population
  - Training of practitioners diminishing
Constraints - 2

• Resistance genes controlling traits poorly known, and preferably polygenic
  • Sustainable solutions generally require polygenic resistance traits
  • Combine rDNA with conventional resistance breeding

• Economics of intensive genetics often marginal
  • Long life spans, low value products
  • GE science and technology costly
  • Patent and regulatory licenses costly or impossible
  • Unclear social license undermines public sector investment
  • Social restrictions create large risks for private investment

• Regulatory and market barriers extreme
Regulatory and market barriers

- Presumption of harm from rDNA method
- Each insertion is the subject of regulation, yet many needed for forest trees and value unclear until extensively tested in field
- Long distance gene flow during research and breeding the rule – “contamination” can have large legal and economic consequences
- Long periods of adaptive management blur research, breeding, and commercial phases
- “Green certification” exclusions of nearly all rDNA trees make field research impossible or very costly
“Green” certification of forests create severe barriers to field research, markets

...genetically modified trees are prohibited...
All major forest certification systems now ban all GE trees – no research exemptions

<table>
<thead>
<tr>
<th>System</th>
<th>Region</th>
<th>GM Tree Approach / Reason</th>
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<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>
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Adam Costanza, Institute for Forest Biotechnology
Regulations and certification render GE ineffective as a tool for forest health

**BIOTECHNOLOGY**

**Genetically engineered trees: Paralysis from good intentions**

Forest crises demand regulation and certification reform

By Steven H. Strauss¹, Adam Costanza², Armand Séguin³

Intensive genetic modification is a longstanding 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.
A lesson on the risks from method-based federal regulation
Agenda

• Basics
• Rationales
• Constraints
• Solutions
Regulatory reform essential

Regulatory analyses and proposals for change published in many places

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>FSC, detailed data</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</td>
<td>Petition for exemption?</td>
<td>FSC, basic data</td>
<td></td>
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</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 determination of confinement mechanisms.
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-technology-based research, and risk-analysis frameworks can also help.

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 stopped³. 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
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 transgenic trees. To move forward, improve-

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, a protocol was adopted...
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
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
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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
Ending event-based regulation of GMO crops

To the Editor:
Getting regulation of agricultural biotechnologies right is no simple task. Stringent regulations for genetically modified organisms (GMOs) in the European Union (EU: Brussels) have nearly stifled the use of biotech crops on farms or in derived foods there, and in the United States the diversified ‘Coordinated Framework’ has produced a strange patchwork of rules, exceptions and lengthy delays. As the Editorial in the December issue highlights, the US Executive Branch has launched a process to reform its regulatory structure, calling for an integrated system that recognizes and balances safety, environment, innovation and economic growth. On the heels of the release of a White House memo, the US House of Representatives passed the Safe and Accurate Food Labeling Act of 2015, which is on its way to the Senate for consideration. Contrary to current regulations, this legislation would explicitly preempt state-by-state labeling and require the US Food and Drug Administration (FDA) to conduct a safety review for all GMOs entering commerce. This recent activity by both the executive and legislative branches provides a welcome opportunity to take a fresh look at
The biological constraints of forest trees collide with method- and annual crop-oriented regulatory systems and markets.

Regulatory reform essential, including risk/benefit based exemptions, tolerance of gene flow during research and breeding.
Technical solutions

• Public research to develop improved and less genotype-dependent transformation and gene editing systems
  – Science and mechanism focused?
  – NSF Plant Genome Program with new focus here

• Accelerated identification and testing of resistance genes – HIGS and beyond

• But most grant based, applied research programs avoid GE methods and solutions
  – Focus is on risks vs. innovations/solutions (USDA Biotechnology Risk Assessment Grants - BRAG)
Ethics-based campaigns needed

- Education on degree of forest health problems and their consequences for biodiversity and public welfare
- Demonstrations of need and capacity
The American Chestnut's Genetic Rebirth

A foreign fungus nearly wiped out North America's once vast chestnut forests. Genetic engineering can revive them

By William Powell

In 1876 Samuel B. Parsons received a shipment of chestnut seeds from Japan and decided to grow and sell the trees to orchards. Unbeknownst to him, his shipment likely harbored a stowaway that caused one of the greatest ecological disasters ever to befall eastern North America. The trees probably concealed spores of a pathogenic fungus, Cryphonectria parasitica, to which Asian chestnut trees—but not their American cousins—had evolved resistance. C. parasitica effectively strangles trees, killing them within three years.
Hemlock in USA under siege today

BATTLING A GIANT KILLER

The iconic eastern hemlock is under siege from a tiny invasive insect

By Gabriel Popkin in Highlands, North Carolina; photography by Katherine Taylor

On a frigid morning this past March, arborist Will Blazek snuck behind a small church and headed down into a gorge thick with rhododendron. He crashed through the shrubs until he spotted the gorge’s treasure: the world’s largest park, “are in intensive care.” Like the family of a gravely ill patient, ecologists are also preparing for the possibility that these efforts will fail, and the eastern forest will lose one of its defining species.

TSUGA CANADENSIS is one of eastern branches, creating a thick canopy that blocks up to 99% of sunlight. Few plants grow in the gloom, but a hemlock seedling can bid its time for decades or more, waiting for a sunlit opening. Hundreds of species of insects, mites, and spiders appear to live primarily or exclusively in hemlock forests, and some

A creeping conflict

The hemlock woolly adelgid now infests about half of the eastern hemlock’s range, and has been spreading by about 15 kilometers per year.
Emerald Ash Borer killing ~all ashes in USA – costing billions

The emerald ash borer was first detected in North America in 2002. Native to Asia, the beetle has proven to be highly destructive in its new range. Since its arrival, it has killed tens of millions of ash trees and continues to spread into new areas.

Photo credits - Trees: Daniel A. Herms, The Ohio State University - Borer larva: Dr. Robert Lavallée, Natural Resources Canada
Swiss Needle Cast in Oregon
Douglas-fir – breeding ineffective
Science advocacy needed?

• To demand a full suite of tools, including rDNA, for coping with forest health crises
• Targets are method-based regulation and market obstacles
• Social media, legal action, the main tools?
• Who will promote and fund?
  – Foundations? Science organizations like AAAS and ASPB? USDA?
Key messages

• rDNA methods are powerful tools to supplement breeding in the right niches
• Serious technical and social obstacles prevent their significant use, or even research, for forest health
• In the face of forest health crises, we have an ethical obligation to create technological capacity and social conditions to enable wider use