Tree Biotech
Progress, prospects, and paralysis

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Plan

- The buzz from GMO crops and foods
- What is biotech?
- What is genetic engineering?
- Where might it matter for forestry?
- Some examples of progress
- The current state: Near paralysis
There are many pieces of the GMO controversy

• “It is accurate to say that many of the real ethical issues [of GMOs in agriculture] have little to do with the use of transgenic technologies” (Burkardt et al. 2005, Agricultural Ethics, CAST)
Proponents of various issues frequently distort science to influence perceptions.
Money: Advocacy targeting conventional food & ag, often with GMO/chemical focus, is well funded and growing

More than 500 activist organizations in North America are spending in excess of $2 billion annually engaging in food-related campaigns targeting biotech and many other elements

Jay Byrne, 2012, V-fluence
It is not surprising how much scientists and the public differ in views of GMOs.
88% of AAAS scientists say genetically modified foods are safe to eat; only 37% of the public agrees.
GMO issue with widest split between public and scientists

<table>
<thead>
<tr>
<th>Opinion Differences Between Public and Scientists</th>
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<tr>
<td>% of U.S. adults and AAAS scientists saying each of the following</td>
</tr>
<tr>
<td><strong>Biomedical sciences</strong></td>
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<tr>
<td>Safe to eat genetically modified foods</td>
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<tr>
<td>Favor use of animals in research</td>
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<tr>
<td>Safe to eat foods grown with pesticides</td>
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<tr>
<td>Humans have evolved over time</td>
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<tr>
<td>Childhood vaccines such as MMR should be required</td>
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<table>
<thead>
<tr>
<th><strong>Climate, energy, space sciences</strong></th>
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<tbody>
<tr>
<td>Climate change is mostly due to human activity</td>
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<tr>
<td>Growing world population will be a major problem</td>
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<tr>
<td>Favor building more nuclear power plants</td>
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<tr>
<td>Favor more offshore drilling</td>
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<tr>
<td>Astronauts essential for future of U.S. space program</td>
</tr>
<tr>
<td>Favor increased use of bioengineered fuel</td>
</tr>
<tr>
<td>Favor increased use of fracking</td>
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<tr>
<td>Space station has been a good investment for U.S.</td>
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“Don’t believe everything you read on the Internet just because there’s a picture with a quote next to it.”

–Abraham Lincoln

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What is biotech?

- Use of biological technology for any reason
- Usually refers to genetics and genetic engineering (GE)
- But non-GE biotech powerful and non-controversial
  - Genomics, marker selection, genomic selection, etc
Advanced breeding and selection has a great impact on forest productivity.
Genomic Selection (GS) or Genome-Wide Selection

GS is the selection based on thousands of markers covering most of the genome.

- Gene involved in the target traits
- Genetic markers

D. Grattapaglia
Genomic selection of new genotypes – Determine correlation between DNA and traits, then use that information to speed selection of best parents or clones.

- Hybrid mating
- Selection of best families and best trees in hybrid progeny trials (growth, form, pilodyn density and NIRS)
- Production of cutting for expanded clonal trial
- Selection of top clones in expanded clonal trial (growth, density, disease resistance, wood quality)
- Production of cutting for minicutting expansion
- Best trees are felled
- Production of cutting for first clonal trial

HT, low cost genotyping and phenotype prediction

Genomic selection cuts down the time needed to select top genotypes, and reduces testing expenses.
In Brazil, many companies are already implementing genomic selection in *Eucalyptus*
Questions about genomic selection

- Is it cost effective in the PNW?
  - Need to determine tens of thousands of DNA markers in thousands of trees
  - Up front cost, benefits much later
  - Diversity of breeding zones in the western USA

- It can move things faster based on past performance, is this always good?
  - Can it get us to **wrong** place faster given changing climates, pests, markets?
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What is genetic engineering (GE)

- Direct modification of DNA
  - Vs. indirect modification in breeding and genomic selection
- Asexually modified in somatic cells
  - Then regenerated into whole organisms, usually starting in Petri dishes
GMO method (genetic engineering) defined

Traditional plant breeding

Variety A

Variety B

Genetic engineering

Asexual modification or insertion from any gene source
Regeneration of plants after introduction of DNA
Then propagated normally (seeds, cuttings) and tested for health and new qualities, incorporated into breeding programs.
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Short rotation, clonal plantations most obvious place for GE in forestry
Lignin reduced variety of poplar for pulp or biofuels

Courtesy of G. Pilate, INRA)
Lepidopteran-resistant poplars commercially approved in China - Bt cry1

- Trait stable
- Helps to protect non-Bt trees
- Reduced insecticide use
- Improved growth rate
Beetle resistant Bt-cottonwoods in eastern Oregon field trial
Growth benefits (10-20%) despite low insect pressure during large field trial of resistant genotypes.
Glyphosate herbicide resistance in cottonwood

Screen of primary transformants

2 yr-old field trial

Wild type controls
Growth benefit in Roundup-resistance tailored system: ~20% volume at 2 years
Testing genetic containment methods in the field in poplar

August 2015
Complete sterility - Undeveloped catkins due to stable suppression of native “LEAFY” gene in poplar (RNAi)

Klocko et al. 2014, American Soc. For Plant Biology, Portland, Oregon
Better yet, “gene editing” by CRISPRs enable predictable, stable, certain sterility? ~50% biallelic mutation rate for floral genes!
Insect control via RNAi

Creation of a new plant gene that suppresses a critical gene in a pest using its own machinery

Control of coleopteran insect pests through RNA interference

James A Baum, Thierry Bogaert, William Clinton, Gregory R Heck, Pascale Feldmann, Oliver Hagan, Scott Johnson, Geert Platenck, Tichafa Munyikwa, Michael Pleau, Ty Vaughn & James Roberts

Commercial biotechnology solutions for controlling lepidopteran and coleopteran insect pests on crops depend on the expression of Bacillus thuringiensis insecticidal proteins [1,2], most of which permeabilize the membranes of gut epithelial cells of susceptible insects [3]. However, insect control strategies involving a different mode of action would be valuable for managing the emergence of insect resistance. Toward this end, we demonstrate that ingestion of double-stranded (ds)RNAs supplied in an artificial diet triggers RNA interference in several coleopteran species, most notably the western corn rootworm (WCR) Diabrotica virgifera virgifera LeConte. This may result in larval stunting and mortality. Transgenic corn plants engineered to express WCR dsRNAs show a significant reduction in WCR feeding damage in a growth chamber assay, suggesting that the RNAi pathway can be exploited to control insect pests via in planta expression of a dsRNA.

Figure 2: F1 plants expressing a V-ATPase A dsRNA are protected from WCR feeding damage. (a) Map of the expression cassette. (b) Mean root damage ratings for eight F1 populations, the parental inbred line (negative control) and the corn rootworm-protected Cry3Bb event MON863; NIS, nodal injury score (Iowa State ranking system). (c) The plant on the left is a non-transgenic control with average root damage, whereas the plant on the right shows the average root protection seen when the transgene is expressed.
Eucalypt plantation another obvious place for GE applications
Wood modification to promote growth rate – just authorized by Brazilian government for commercial use.
Cold tolerant GE *Eucalyptus*

Proposed for commercial deregulation in USA

Results from first winter in South Carolina

Results from second winter in Alabama

Field results indicate freezing tolerance to ~16°F (-8° to -9°C)
Many eucalypt field trials underway

12 Months

Two years

Three years

Four years

Seven Years

Courtesy of Les Pearson, Arbogen
Male sterile eucalypts and pine - Arborgen

Anther-specific promoter driving expression of a strong RNAse prevents pollen maturation and release.
Overexpression of endogenous flowering genes induce early flowering in several tree species

- Apple
- Orange
- Plum
- Eucalyptus
Wild forest tree protection or restoration another place for GE trees?

American Chestnut restoration with help of GE?
Forest health a global and growing concern
Many exotic diseases have severely impacted US forests

- 1892 - White pine blister rust
- 1904 - Chestnut blight
- 1923 - Port-Orford-cedar root disease
- 1920s - Beech scale complex
- 1930 - Dutch elm disease
- 1967 - Butternut canker
- 1976 - Dogwood anthracnose
- 2000s - Sudden oak death

American elm
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 Blozan snuck behind a small church here 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 bide 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
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The case for paralysis
(August 2015, Science)

Genetically engineered trees: Paralysis from good intentions
Forest crises demand regulation and certification reform

By Steven H. Strauss¹, Adam Costanza², Armand Séguin³

Recent innovations in genetic modification have the potential to transform agriculture and forestry. However, concerns about the environmental and social impacts of genetically engineered (GE) trees are growing. The development of a clear regulatory framework for GE trees is essential to ensure that they are safely introduced into the environment.

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.

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.
Regulatory problems fundamental

- Presumption that all GE is harmful to environment regardless of gene, problem
  - Very hard to go beyond boutique research without very costly regulatory approval (millions of dollars)
  - Public sector, small companies cannot afford
  - USDA Forest Service hesitant to invest, engage
- Essentially impossible to do field research in many countries due to costs, politicized nature
  - Vandalism a major issue in Europe still
Market barriers large

“Green” certification of forests create severe barriers to field research, markets

Forest Stewardship Council

“...genetically modified trees are prohibited...”
Forest certification systems universally ban all GM trees – no exemptions

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

• Trees often rich in diversity due to early state of domestication
  • GE often not essential, other options can be found

• Genetic engineering methods often very difficult and highly genotype-specific
  • Very limited advances outside of a few intensively studied species, public research ~halted

• Gene flow extensive, wild or feral relatives
  • Ethical questions, regulatory questions, science challenges
  • Political opponents active, powerful

• No consensus on what precaution means in relation to genetic engineering
Forest health a major and growing concern
No-analog scientific thinking should dominate today

Novel climates, no-analog communities, and ecological surprises

John W Williams* and Stephen T Jackson²

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. The well documented no-analog plant communities of late-glacial North America are closely linked to “novel” climates also lacking modern analogs, characterized by high seasonality of temperature. In climate simulations for the Intergovernmental Panel on Climate Change A2 and B1 emission scenarios, novel climates arise by 2100 AD, primarily in tropical and subtropical regions. These future novel climates are warmer than any present climates globally, with spatially variable shifts in precipitation, and increase the risk of species reshuffling into future no-analog communities and other ecological surprises. Most ecological models are at least partially parameterized from modern observations and so may fail to accurately predict ecological responses to these novel climates. There is an urgent need to test the robustness of ecological models to climate conditions outside modern experience.

How do you study an ecosystem no ecologist has ever seen? This is a problem for both paleoecologists and global change ecologists, who seek to understand ecosystem past or future, is heavily conditioned by our current observations and personal experience. The further our explorations carry us from the present...

“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.”
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?
Was Voltaire talking about biotech regulations?

“The perfect is the enemy of the good”
In summary

• Many examples of progress with GE trees with a wide variety of traits, in the field
  • Mostly poplar, some eucalypts
• Extraordinary barriers based on GMO regulation, certification, and tree biology
  • Makes implementation of GE tools on a scale and speed relevant to need and benefit ~unworkable
• Growing number of forest stresses where silviculture, conventional breeding, inadequate
  • Much more expected with global travel, climate shifts
  • Is avoidance of GE precautionary or the opposite?
• Need for fundamental regulatory and market change?
  • USA reconsidering many regulations now
  • But fundamental change not likely (in my lifetime)