Transgenic trees

Remarkable progress, extraordinary constraints

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Clarifying terms

- Transgenic, genetically modified, genetically engineered
  - GMO, GEO, GE, GM
- Can modify gene structure or expression that are already present in breeders’ gene pools
  - Cisgenics, intragenics, edited genes
- Can insert genes not within breeders’ gene pools
- GE / GM used interchangeably today to mean direct, asexual, heritable modification of DNA
  - A method not a product
Goals for today

• Value of GMO methods for trees and other woody perennials
  • Horticulture and forestry

• Overview of advanced GMO varieties in production or developmental pipelines

• Regulatory problems needing high level policy solutions
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Constraints to breeding with trees are great – GMO methods offer very significant additional tools

**Constraints include**

- Long breeding cycle
- Difficulty to inbreed / introgress new genes from hybrids
- Hard to identify dominant, major genes
- Common use of asexually propagated varieties of high value
GE of diverse value for trees
All demonstrated in the field

- Improved fruit quality/durability
- Resistance to insects and diseases
- Tolerance to salinity, cold, drought, and high temperature stresses
- Phytoremediation of environmental toxins
- Modified properties to improve processing of wood for biofuels, paper, or solid wood
- Tolerance to herbicides to reduce the environmental impacts, improve efficiency, or reduce costs of weed control treatments
GE of diverse value for trees
All demonstrated in the field

- Accelerated flowering for faster breeding and research
- Fertility control for reduced spread and improved growth rate
- Improved growth and yield
- Synthesis of new, renewable bioproducts such as plastics, enzymes, and fragrances
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Virus-resistant papaya

“Immunization” via by implanting a viral gene in the papaya genome – RNAi (RNA interference)

Courtesy of Denis Gonsalves, formerly of Cornell University

GMO, virus-resistant trees
HoneySweet plum with GE resistance to plum pox virus

Ralph Scorza  USDA-ARS
Non-browning “Arctic Apple”
Suppression of native polyphenol oxidase gene expression

Courtesy of Jennifer Armen, Okanagan Specialty Fruits, Canada
Native grape genes used to produce fruit rot resistance

**Grape** VvAlb gene

Non-Engineered | Engineered
---|---
‘Syrah’ Powdery Mildew Resistance

**Grape** VvTL-1 gene

Non-Engineered | Engineered
---|---
‘Thompson Seedless’ Rot Resistance

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

[http://mrec.ifas.ufl.edu/grapes/genetics](http://mrec.ifas.ufl.edu/grapes/genetics)
Native grape genes imparts black rot resistance in field trials

‘Thompson Seedless’ Control

‘Thompson Seedless’ containing VvTL-1

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

http://mrec.ifas.ufl.edu/grapes/genetics
GMO-based resistance transgenes promising in citrus
Rapid spread throughout Florida and of great concern in other citrus growing areas

32 counties

Courtesy of Eric Mirkov, Texas A & M
Defensin-like proteins from spinach promising

Courtesy of Eric Mirkov, Texas A & M
Insertion of a transgene that elevates natural systemic acquired resistance also promising.
Overexpression of endogenous flowering genes induce early flowering in several tree species

Apple

Orange

Plum

Poplar
Rapid flowering of plum in the field to speed virus resistance breeding

Courtesy of Ralph Scorza, USDA ARS
Early flowering effective in eucalypts too...
And flowering accelerated in poplar by suppression of native genes
Insect resistant poplars commercially approved in China - Bt *cry1*

- Trait stable
- Helps to protect non-Bt trees
- Reduced insecticide use
- Improved growth rate
Currently creating new hybrids by crossing *Bt* poplar with other poplars

Courtesy of Menghu-Zhu Lu, Chinese Academy of Forestry
Growth rate benefits substantial for other Bt-poplars (cry3a) – 10-20%
Growth benefits despite low insect pressure during large field trial of resistant genotypes
Lignin-modified trees – much improved ethanol or pulp yields
Freeze-tolerant *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)

Provided by Arborgen
Many eucalypt field trials underway

12 Months

Two years

Three years

Four years

Seven years

Courtesy of Les Pearson, Arborgen
Field grown male-sterile eucalypts and pine - Arborgen

Complete and stable male-sterility over several years in the field
Similar results for poplar
Brunner et al. 2007, Elorriaga et al. 2014
Tree Genetics and Genomes
Complete sterility - Undeveloped catkins by suppression of native \textit{LEAFY} gene in poplar (RNAi)

Klocko et al. 2014, American Soc. For Plant Biology, Portland, Oregon
Sterility a valuable tool for battling invasive, exotic forest trees: “Wilding” in New Zealand
GE appears to be a useful tool for battling the many exotic diseases that have ravaged North American forests.

**Examples**
- 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 Chestnut most advanced case

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

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—
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There is hardly a trickle of GMO tree products compared to its scientific potential – why?

Social / market and regulatory barriers are great
Genetically modified arboriculture

Down in the forest, something stirs
Transgenic trees are controversial

Critical report from anti-GMO Center for Food Safety in USA – Released Nov 2013
Major environmental groups promoting wild forests dislike GE trees

“The possibility that the new genes spliced into GE trees will interfere with natural forests isn't a hypothetical risk but a certainty. …genetic engineering may do as much damage to forests and wildlife habitat as chain saws and sprawl.” (11/10/13)
“Green” certification of forests create severe barriers to field research, markets

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**Abstract**

Genetic engineering, commonly called genetic modification (GM), is the inclusion of genetic modification and is a method of ensuring that GM crops found in forests and their surrounding areas remain free from contamination that may affect the genetic makeup of natural forests.

GM crops, mainly herbicide- and pest-resistant varieties of soybeans, maize, or cotton, have been vigorously adopted by farmers in South America because they are easy to manage and they improve yields, reduce costs, or reduce pesticide contamination (Carpenter and Gieserl 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 trees are used in forestry 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 meet the pressures and special demands of human-dominated systems. Examples would be trees that are tolerant of heavy metals or other pollutants, soil-adapted pests or diseases, or drought, or do not produce fruits when these create hazards in street environments (Braun 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 (Koorn et al. 1984). Plantations can release species on natural forests for exploitation and can be of great social value by supplying community 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 18 states that plantations should “complement the management of, reduce pressures on, and preserve the regeneration and conservation of natural forests” (FSC 2001).

FSC has certified some of the most intensively managed plantations in the world, including poplar plantations and the intensive and export-oriented plantation of the Southern Hemisphere. Although many environmental initiatives are built into these certified plantation systems, within the areas dedicated to woody production, they function as tree farms. Such intensive plantation systems often use highly bred genotypes, possibly including exotic species, hybrids, and clones, as well as many other forms of intensive agro-cultural management. It is in the context of these highly intensive systems that the additional expense of GM trees is unlikely to be worthwhile.

However, FSC currently prohibits all use of GM trees and is the only certification system to have done so...
Regulatory problems with GMO trees also severe

Event-specific decisions and costs

- Slowness/difficulty of introgression
- Need diverse genotypes transformed (varieties)
- Much smaller economic benefits to pay back regulatory costs from single events
Regulatory problems with GMO trees also severe

Presumption of harm from GE method during research and breeding

• All gene flow must be prevented during research
  • Some movement will occur due to incomplete domestication, wild and feral relatives, wide pollen and often seed movement

• Impedes or prevents stress resistance and other complex trait development
  • Require extensive field trials to test many concepts and insertion events

• No longer makes sense in era of precision breeding, cisgenics, intragenics
A serious regulatory problem under USA system

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
An international regulatory 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 entered the CBD
Millions of dollars of regulatory costs to use a gene we eat daily in spinach.
Lignin-modified trees
Concept proven, but much refinement needed

Type of gene, promoters, extent of modification, environment, stand age, genotype modified
Cold tolerant *Eucalyptus*

Concept proven, much refinement needed

Type of gene, promoters, extent of modification, environment, stand age, genotype modified

Provided by Arborgen
Pest epidemics increasing with travel and climate change
Need rapid use of all available tools, including GE – regulations make impossible

Examples
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
Gene targeting, cisgenics, intragenics coming along fast in genomic age = increased precision, safer than breeding!

PLANT BIOTECHNOLOGY

Zinc fingers on target

Matthew H. Porteus

The existing methods of creating genetically modified plants are inefficient and imprecise. Zinc-finger technology offers the prospect of opening up a swifter and more exact route for crop improvement.

CRISPRs

TALENs
In summary

• Many examples show great progress on a wide variety of fronts
  • Despite very large social barriers and disinvestment over the last decade plus

• Extraordinary regulatory barriers based on the process rather than the product
  • Makes implementation of GE tools on a scale and speed relevant to need and benefit unworkable

• Need for fundamental regulatory change
  • A start: Adventitious presence allowances during research and development for cisgenes, intragenes, and edited genes with known, safe marker systems