22 years and 22,979 trees later: Lessons from field-testing GM trees in the USA

Amy Klocko
Oregon State University
Amy.Klocko@oregonstate.edu
Presentation overview

- Background – GE traits in trees
  - Examples of the potential GE could have on forest trees
- Field testing of GE trees by the Strauss lab
  - Field management
  - Regulatory compliance
  - Unexpected phenotypes
  - Success stories
  - Genetic containment
Managed plantations provide large yields and reduce demands on native forests

Eucalypts in Brazil

Poplars in NW USA
GE can provide many traits of interest in forest tree species. All traits shown tested under field conditions.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Trait</th>
<th>Reference</th>
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<tbody>
<tr>
<td>American Chestnut</td>
<td>Fungal blight resistance</td>
<td>Maynard et al. (2009); Zhang et al. (2013)</td>
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<td><em>Castanea dentata</em></td>
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<tr>
<td>American Elm</td>
<td>Dutch elm disease resistance</td>
<td>Newhouse et al. (2007); Sherif et al. (2016)</td>
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<td><em>Ulmus americana</em></td>
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<td>Silver Birch</td>
<td>Fungal rust resistance</td>
<td>Pasonen et al. (2004)</td>
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<td><em>Betula pendula</em></td>
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<tr>
<td>Poplar</td>
<td>Biomass allocation</td>
<td>Lu et al. (2015)</td>
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<td><em>Populus tremula</em></td>
<td></td>
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<tr>
<td>× <em>alba</em></td>
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<tr>
<td><em>P. tremula × alba</em></td>
<td>Tree size</td>
<td>Elias et al. (2012)</td>
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<tr>
<td><em>P. tremula × alba</em></td>
<td>Improved pulpability</td>
<td>Pilate et al. (2002); Coleman et al. (2012); Mansfield et al. (2012)</td>
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<tr>
<td><em>P. tremula × alba</em></td>
<td>Decreased lignin</td>
<td>Franke et al. (2000); Pilate et al. (2002)</td>
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<td><em>P. × canescens</em></td>
<td>Specialty chemical production</td>
<td>Costa et al. (2013)</td>
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<td><em>P. tremula × alba</em></td>
<td>Reduced isoprene emissions</td>
<td>Behnke et al. (2012)</td>
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<td><em>P. trichocarpa × deltoides</em></td>
<td>Nitrogen assimilation</td>
<td>Jing et al. (2004)</td>
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<td><em>P. tremula × alba</em>, <em>P. tremula × alba</em>, <em>P. tremula × tremuloides</em>, <em>P. trichocarpa × nigra</em></td>
<td>Herbicide tolerance</td>
<td>Meilan et al. (2002); Ault et al. (2016)</td>
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<tr>
<td><em>P. nigra, P. deltoides × nigra, P. trichocarpa × deltoides</em></td>
<td>Insect resistance</td>
<td>Hu et al. (2001); Klocko et al. (2014)</td>
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<td><em>P. davidiana × bolleana</em></td>
<td>Salt tolerance</td>
<td>Yang et al. (2015)</td>
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<td><em>P. alba</em></td>
<td>Flowering control</td>
<td>Klocko et al. (2016b)</td>
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Strauss et al. New Phytologist 2016
GE agricultural trees are being grown on a small scale in the USA

Virus resistant papaya

Non-browning Arctic™ apple
Plantations of insect resistant *cryl-Bt* poplars in China

1994-2005

2001-2005
Lignin-modified poplars in Belgium
Courtesy of W. Boerjan

Improved ethanol yield (~50%) but reduced growth rate
Freeze tolerant, male-sterile transgenic *Eucalyptus* – *Arborgen*

Proposed for commercial release 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)

Extreme cold winters in the southern USA happen periodically

Promising concept
Strauss lab has field tested a variety of types of traits in trees since 1995

- Flowering modification (sterility, genetic containment)
  - RNAi, overexpression, DNM, ablation,
- Management
  - Herbicide resistance, insect-resistance
- Form and growth rate
  - GA pathway, semi-dwarfism
- Activation tagging
- Tools and stability
  - Alcohol inducible, transgene stability
- Physiological modifications
  - Lignin modification, isoprene reduction

- Nearly all trials were poplar trees
- Current trials are all for genetic containment
Regulatory considerations for field testing of trees in the USA

- Permits are from USDA APHIS
  - United States Department of Agriculture, Animal and Plant Health Inspection Service
- Costs for management (fencing, weeding, irrigation) are substantial and often require external (grant) funding
- Compliance with permit conditions are the responsibility of individuals, not institutions
- Sites are inspected by both scheduled and unscheduled visits

- Flowering (intentional release) is only allowed if the approved permit includes that condition
- Most field trials are of juvenile trees
Genetic containment of trees could be very useful

- Ecologically dominant species
- Large production of pollen and seeds
  - Gene flow to wild forests, plantations
- Concern over potential GE admixture in certified plantations and forests
- Public concern over GE use

- We don’t know the actual long-term impacts of GE trees in the field

- Having sterile trees could help to mitigate the risk of spread and enable additional field research
Experience and lessons summarized in 2016 book chapter

Lessons from Two Decades of Field Trials with Genetically Modified Trees in the USA: Biology and Regulatory Compliance

Steven H. Strauss, Cathleen Ma, Kori Ault and Amy L. Klocko

Abstract We summarize the many field trials that we have conducted in beginning in 1995 and continuing to this day. Under USDA APHIS regulatory notifications and permits, we have planted nearly 20,000 trees derived approximately 100 different constructs in more than two dozen field experiments. The large majority of the trials were in *Populus* and included hybrid white and aspen.
Field management is a lot of work
Field sites are outdoors

Trees and researchers are subjected to conditions that just don’t happen in greenhouses.
Time and costs associated with regulatory compliance are significant

- Paperwork (permits, reports)
- Equipment rental and irrigation system servicing
- Supervising workers
  - Monitoring for seedlings and suckers
  - Fence inspection, mowing, weed control
  - Animal control (deer!)
- Plantation termination

- Estimated quarter time job for a very busy professional

Anna Magnuson, our very busy field manager
Unexpected phenotypes are rare but have important regulatory implications.

Typically poplar trees flower in February in Oregon . . . unless they are semi-dwarf GA-modified trees.
Summertime “catkins”
We would have loved to study these summertime catkins

- We immediately reported our “unexpected occurrence” as required by our permit

- Biologically interesting flower form and timing
- No pollen is present to fertilize these flowers
- Trees are semidwarf

- Our permit did not allow for flowering

- We removed every single catkin by hand from over 100 trees
We removed every catkin that spring too . . . good thing the trees were short
Other unexpected phenotypes are rare but show the importance of field testing.

- Mottled color and unusual leaf shapes
- Dwarfed transgenic event
- Phenotypes only showed up after field planting
- In general most GE events and trees are healthy and grow well
Field and greenhouse results may not be an exact match

- Greenhouse and field evaluation of biomass of GA pathway modified poplars
- Growth in the field did not correlate with growth in the greenhouse
Management related traits perform very well in the field and could be very valuable.

Glyphosate tolerance
Conventional grown tree (left)
Resistant tree after direct spray (right)
Insect resistant *Cry3a* Bt trees with improved productivity

*Cry3a* control

Stable across trials and growing seasons

*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
Activation tagging reveals some phenotypic alterations possible by native gene overexpression.
Many of our trials focused on targets and methods for genetic containment

- Effective and stable means of obtaining non-fertile trees could serve as an enabling technology
  - Allow for field testing of other traits of interest
  - Could increase acceptance of GE trees

- Trees need to be grown to maturity to assess floral fertility
Regulatory compliance to allow for flowering has specific requirements

- Flowering is considered an intentional release
- We grow species and hybrids that are not compatible with wild relatives
- Trials are managed to confine and mitigate spread
  - Monitor for seedlings and vegetative sprouts
  - Check for seed production and seed viability
- Risk of spread is low
We monitor an extensive area for establishment of seedlings and suckers.

Blue – poplar trial
Orange – sweetgum trial
Red – perimeter fence
Yellow – zone of monitoring

We have yet to find seedlings.

Suckers (vegetative sprouts) normally show up after tree removal.
Identify, report, terminate.
Male and female sterility would be desirable for poplar

- Poplar trees are either male or female (in general)
- Trees are wind pollinated – often at great distances (kilometers)
- Seeds are wind-dispersed on cotton-like fluff
Floral development genes are good targets for obtaining bisexual sterility

- **LEAFY** – floral meristem prior to organ differentiation
- **AGAMOUS** – Male and female organ development and floral determinacy
Most sterility trial trees grow well
Suppression of floral development genes leads to stable female sterility

control

AG suppression (two constructs)
11 of 12 events (91.7%)
6 of 22 events (27.3%)

LFY suppression
2 of 15 events (13.3%)

Stable across flowering seasons
We don’t know the copy number of the genetic insertions
Vegetative performance of sterile trees appear to be normal.

Data were spatially adjusted for variation in soil quality over field.
While our trees were growing, so was the field of custom genome editing

- **CRISPR-Cas9**

  - Discovered in 2002
  - Over 1,100 studies (80% published after 2013)
  - Permanent changes to genes of interest
  - Inexpensive and easy to create the vector
  - High rate of gene targeting in plants (up to 91%)
  - Known to work very well in poplar
Current work is testing CRISPR-based methods for disrupting target genes to obtain sterility

- Our trees have the CRISPR components continually present
- Research is needed for questions of scientific and regulatory importance
  - Do we need excision/removal?
    - A generalized system for doing so needed
  - Is there continued mutagenesis of on-target sites?
  - Is there off-target mutagenesis?

- Field planting anticipated fall 2017
Additional research is needed on potential ecological impacts of non-flowering trees.
Major findings

- Compliance with regulatory standards are often costly and challenging, and are a major impediment to use of GE for field research or breeding.

- Field studies often reveal major surprises when compared to laboratory or greenhouse studies. They are essential for understanding the practical and physiological significance of GE modifications.

- When produced by overexpression or RNA interference, traits are highly stable over many years, including genetic containment/sterility traits.
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Sarah Robertson, Technician, floral analysis

Michael Nagle, grad student, gene targets

Anna Magnuson, program & field manager

Starting January 2015

Sarah Robertson, Technician, floral analysis

Emily Helliwell, Post-doc, genomics and bioinformatics

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