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

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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

Tree species	Trait	Reference
American Chestnut *Castanea dentata*	Fungal blight resistance	Maynard *et al.* (2009); Zhang *et al.* (2013)
American Elm *Ulmus americana*	Dutch elm disease resistance	Newhouse *et al.* (2007); Sherif *et al.* (2016)
Silver Birch *Betula pendula*	Fungal rust resistance	Pasonen *et al.* (2004)
Poplar		
Populus tremula × *alba*	Biomass allocation	Lu *et al.* (2015)
P. tremula × *alba*	Tree size	Elias *et al.* (2012)
P. tremula × *alba*	Improved pulpabillity	Pilate *et al.* (2002); Coleman *et al.* (2012); Mansfield *et al.* (2012)
P. tremula × *alba*	Decreased lignin	Franke *et al.* (2000); Pilate *et al.* (2002)
P. tremula × *alba*	Specialty chemical production	Costa *et al.* (2013)
P. × *canescens*	Reduced isoprene emissions	Behnke *et al.* (2012)
P. tremula × *alba*	Nitrogen assimilation	Jing *et al.* (2004)
P. trichocarpa × *deltoides*, *P. tremula* × *alba*, *P. tremula* × *tremuloides*, *P. trichocarpa* × *nigra*	Herbicide tolerance	Meilan *et al.* (2002); Ault *et al.* (2016)
P. nigra, *P. deltoides* × *nigra*, *P. trichocarpa* × *deltoides*	Insect resistance	Hu *et al.* (2001); Klocko *et al.* (2014)
P. davidiana × *bolleana*	Salt tolerance	Yang *et al.* (2015)
P. alba	Flowering control	Klocko *et al.* (2016b)

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 *cryI-Bt* poplars in China

Lignin-modified poplars in Belgium

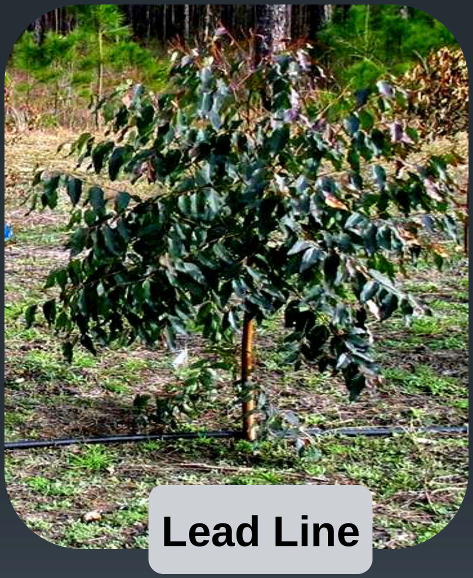
Courtesy of W. Boerjan

Improved ethanol yield (~50%) but reduced growth rate

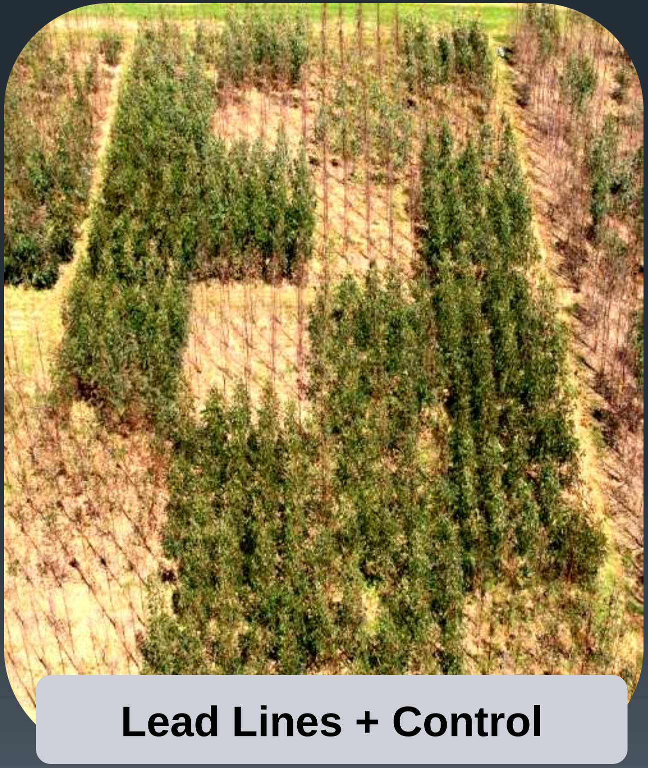
Freeze tolerant, male-sterile transgenic *Eucalyptus – Arborgen*

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 i
beginning in 1995 and continuing to this day. Under USDA APHIS fed
latory notifications and permits, we have planted nearly 20,000 trees de
approximately 100 different constructs in more than two dozen field ex
The large majority of the trials were in *Populus* and included hybrid whi

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 <u>every single catkin by hand from over 100 trees</u>

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

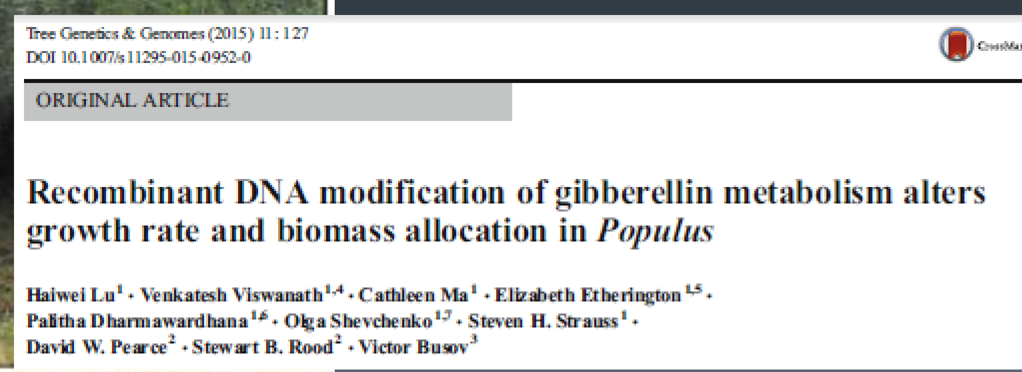
Dwarfed transgenic event

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


Tree Genetics & Genomes (2015) 11 : 127
DOI 10.1007/s11295-015-0952-0

ORIGINAL ARTICLE

Recombinant DNA modification of gibberellin metabolism alters growth rate and biomass allocation in *Populus*

Haiwei Lu[1] · Venkatesh Viswanath[1,4] · Cathleen Ma[1] · Elizabeth Etherington[1,5] · Palitha Dharmawardhana[1,6] · Olga Shevchenko[1,7] · Steven H. Strauss[1] · David W. Pearce[2] · Stewart B. Rood[2] · Victor Busov[3]

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)

New Forests (2016) 47:653–667
DOI 10.1007/s11056-016-9536-6

CrossMark

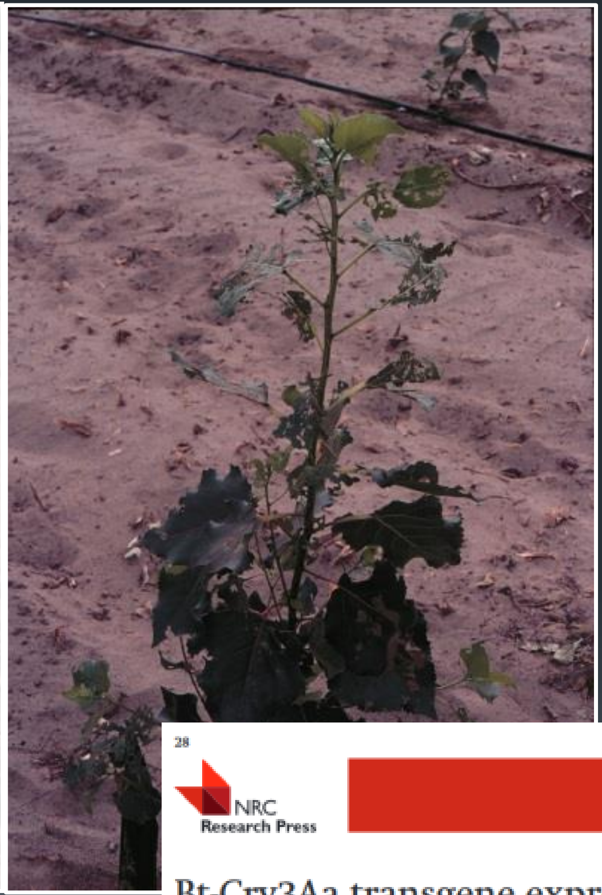
Improved growth and weed control of glyphosate-tolerant poplars

Kori Ault[1] · Venkatesh Viswanath[1,4] · Judith Jayawickrama[1] · Cathleen Ma[1] · Jake Eaton[2] · Rick Meilan[1,5] · Grant Beauchamp[2,6] · William Hohenschuh[3] · Ganti Murthy[3] · Steven H. Strauss[1]

Insect resistant *Cry3a* Bt trees with improved productivity

Cry3a

control



Stable across trials and growing seasons

28

NRC Research Press

ARTICLE

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

http://e11e-k.deviantart.com/art/The-Walking-tree-277837080

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

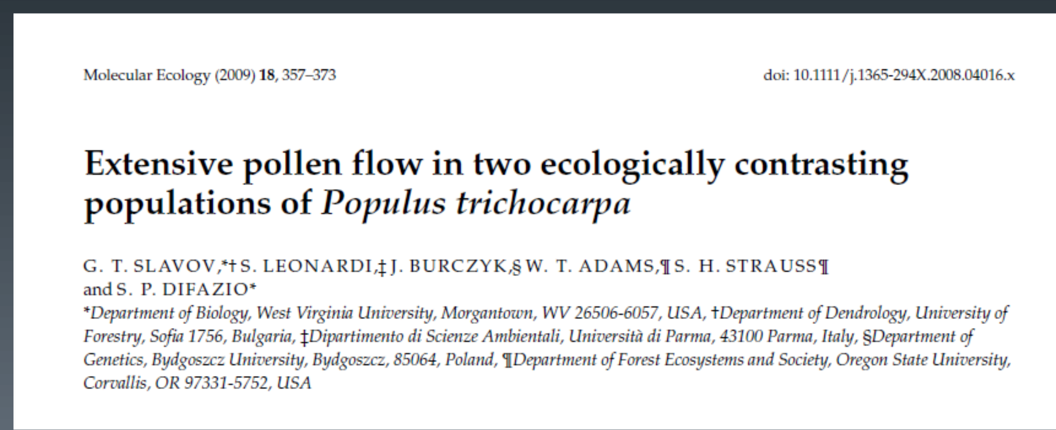
Red – perimeter fence
Yellow – zone of monitoring

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



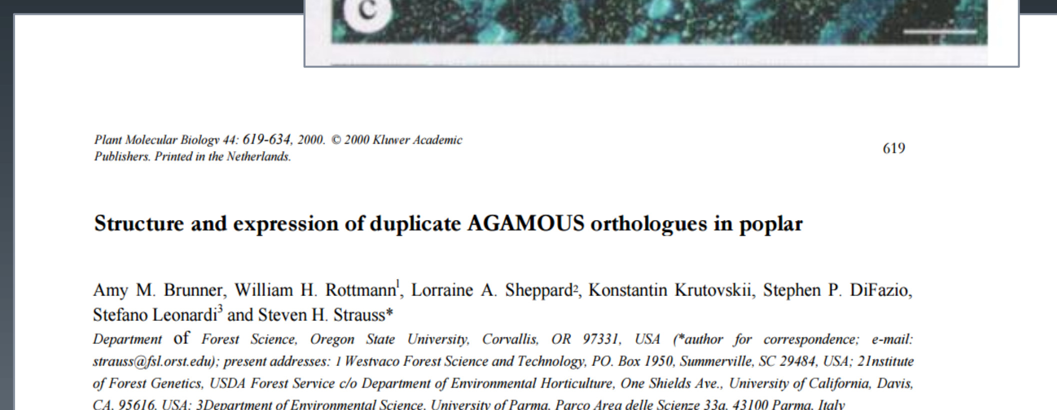
Molecular Ecology (2009) 18, 357–373
doi: 10.1111/j.1365-294X.2008.04016.x

Extensive pollen flow in two ecologically contrasting populations of *Populus trichocarpa*

G. T. SLAVOV,*† S. LEONARDI,‡ J. BURCZYK,§ W. T. ADAMS,¶ S. H. STRAUSS¶ and S. P. DIFAZIO*
**Department of Biology, West Virginia University, Morgantown, WV 26506-6057, USA, †Department of Dendrology, University of Forestry, Sofia 1756, Bulgaria, ‡Dipartimento di Scienze Ambientali, Università di Parma, 43100 Parma, Italy, §Department of Genetics, Bydgoszcz University, Bydgoszcz, 85064, Poland, ¶Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331-5752, USA*

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



Plant Molecular Biology 44: 619-634, 2000. © 2000 Kluwer Academic Publishers. Printed in the Netherlands. 619

Structure and expression of duplicate AGAMOUS orthologues in poplar

Amy M. Brunner, William H. Rottmann[1], Lorraine A. Sheppard[2], Konstantin Krutovskii, Stephen P. DiFazio, Stefano Leonardi[3] and Steven H. Strauss*

*Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA (*author for correspondence; e-mail: strauss@fsl.orst.edu); present addresses: 1 Westvaco Forest Science and Technology, PO. Box 1950, Summerville, SC 29484, USA; 2Institute of Forest Genetics, USDA Forest Service c/o Department of Environmental Horticulture, One Shields Ave., University of California, Davis, CA, 95616, USA; 3Department of Environmental Science, University of Parma, Parco Area delle Scienze 33a, 43100 Parma, Italy*

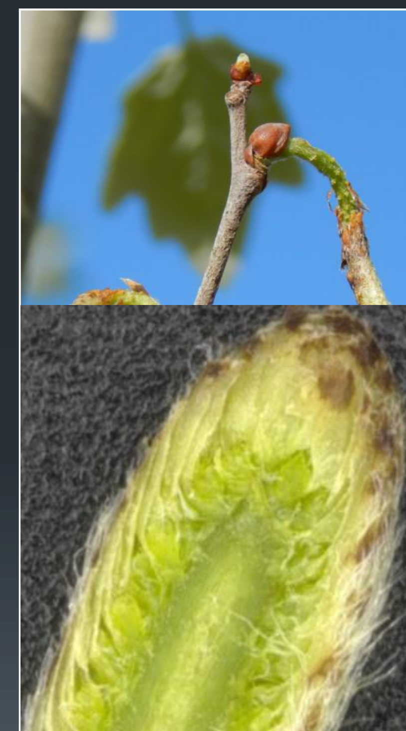
Most sterility trial trees grow well

Suppression of floral development genes leads to stable female sterility

control

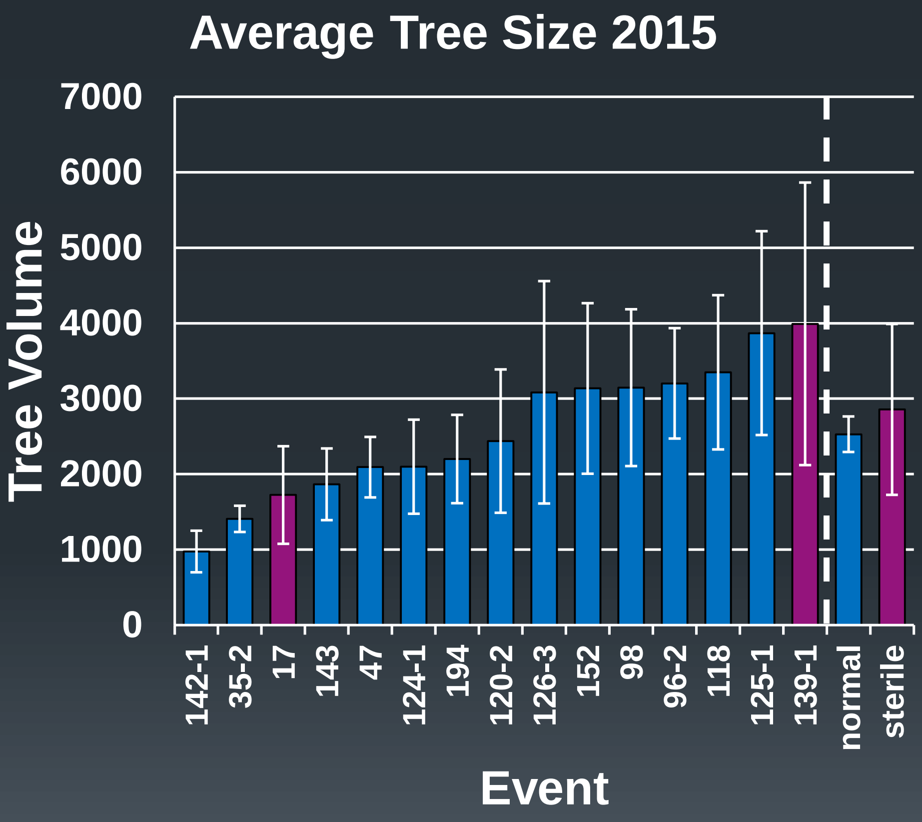
AG suppression (two constructs)
11 of 12 events (91.7%)
6 of 22 events (27.3%)

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



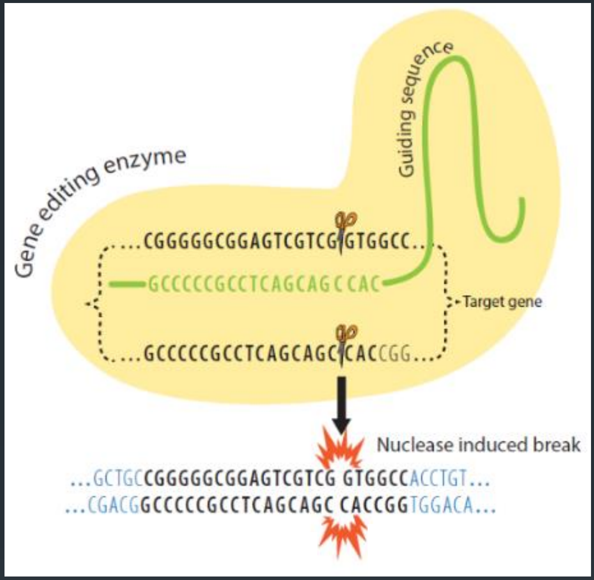
Data were spatially adjusted for variation in soil quality over field

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)
- <u>Permanent changes to genes of interest</u>
- Inexpensive and easy to create the vector
- High rate of gene targeting in plants (up to 91%)
- Known to work very well in poplar

<u>Synthetic nuclease system</u>

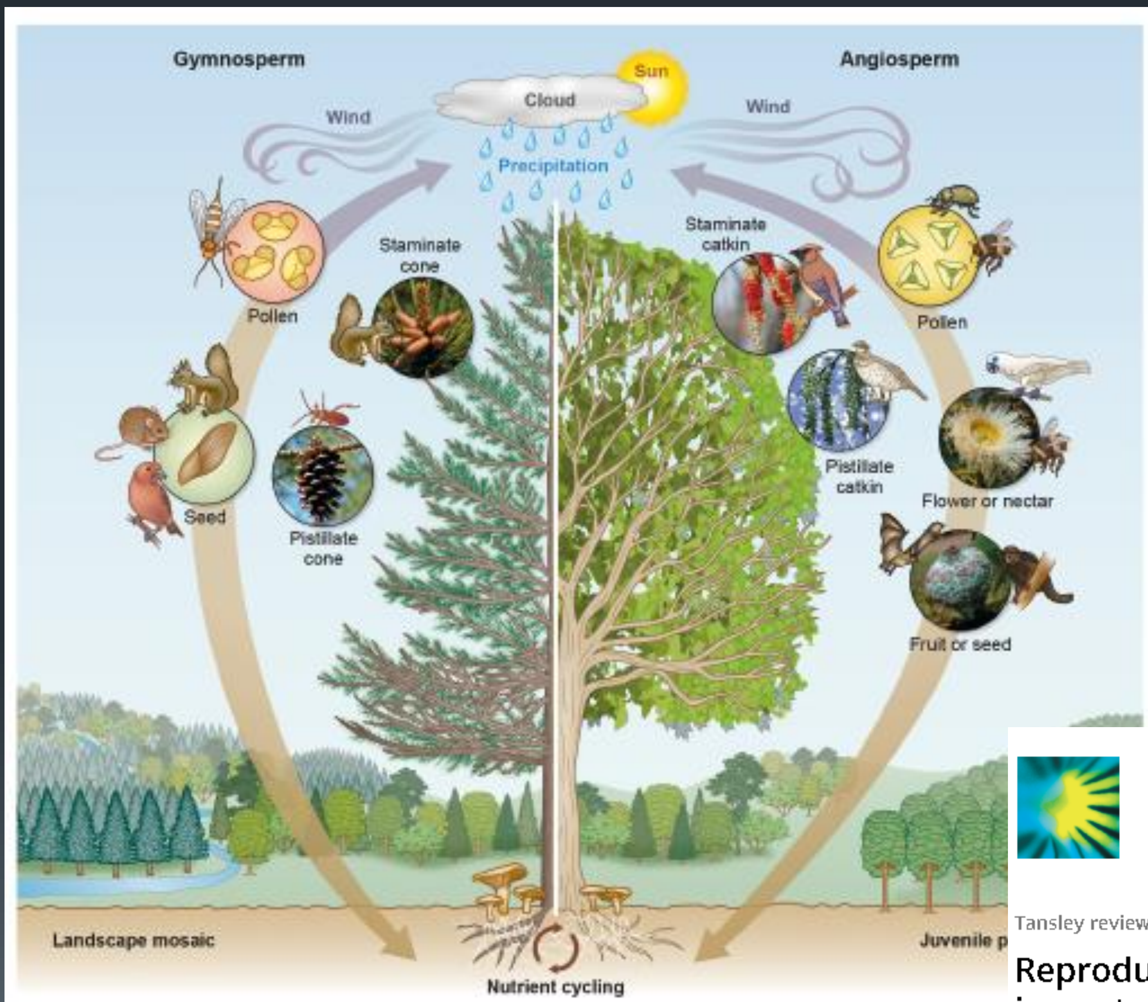


Current work is testing CRISPR-based methods for disrupting target genes to obtain sterility

- 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



New Phytologist

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Tansley review

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. Brosi, Robert J. Fletcher Jr, Mark D. Needham

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.

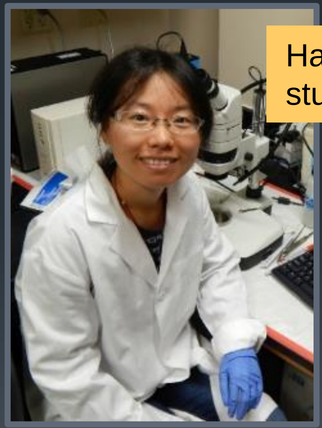
Acknowledgments

Cathleen Ma, transformation & greenhouse experiments

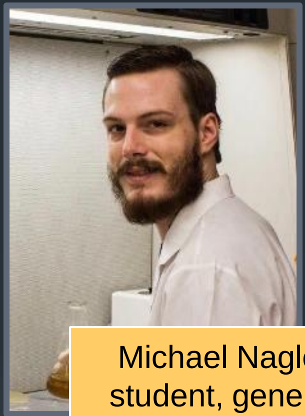
Steve Strauss, Director TBGRC, professor

Anna Magnuson, program & field manager

Estefania Elorriaga, grad student, CRISPRs



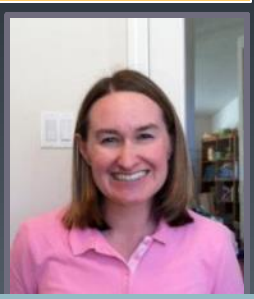
Haiwei Lu, grad student, ZFNs



Michael Nagle, grad student, gene targets

Amy Klocko, Post-doc, gene cloning, gene expression, flowering

Emily Helliwell, Post-doc, genomics and bioinformatics



Sarah Robertson, Technician, floral analysis

Funding sources

USDA

United States Department of Agriculture

National Institute of Food and Agriculture

TBGRC

NSF

The National Science Foundation

Advanced **Hardwood Biofuels** Northwest