A perfect storm: Lessons from two decades of field trials with GE trees

IUFRO Tree Biotechnology

Concepcion, Chile

Steve Strauss

Oregon State University / USA

A purple haze: Lessons from two decades of field trials with GE trees

IUFRO Tree Biotechnology
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Summary of this 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 conduct
beginning in 1995 and continuing to this day. Under USDA APHIS
latory notifications and permits, we have planted nearly 20,000 trees
approximately 100 different constructs in more than two dozen fiel
The large majority of the trials were in *Populus* and included hybrid

Lessons in relation to….

1. Abnormalities due to transformation / in vitro regeneration
2. Trait stability: RNAi-induced gene suppression and other traits
3. GMO regulation costs and realities
4. Single genes for modifying complex, physiological traits
5. Value of 1st generation transgenic traits
6. Summary perspectives on the meaning of biosafety and beyond

Some background on the nature of our experience

A current ~4 ha trial (summer 2016)

Amy Brunner,
Virginia Tech

How many trees?

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

Oregon State University

How many traits and constructs tested in the field?

- Gene discovery
  - Activation tagging
- Gene expression/suppression stability
  - Reporters, qPCR
- Engineering tools
  - Alcohol / heat / chemical inducibility and stability
- Agronomic / management traits
  - Herbicide resistance, insect resistance
- Form and growth rate through GA modifications
  - Acceleration and semi-dwarfism
- Other physiological modifications
  - Lignin modification (4 CL), isoprene reduction
- Flowering modification / containment
  - Barnase, mutant proteins, repressors, RNAi, gene editing

How and what?

- All *in vitro*, organogenic, Agrobacterium transformation
- Vast majority are poplar
  - Aspen and white poplar relatives
    - Giles Pilate / Lise Jouanin / INRA-France
      - *P. tremula x alba* 717-1B4 (♀) and *P. tremula x tremuloides* 353-53 (♂)
    - Maurizio Sabatti / Univ of Viterbo, Italy
      - Early flowering *P alba* 6K10 (♀)
  - Hybrid cottonwoods
    - Brian Stanton, Reini Stettler, and various industry sources
      - *P. trichocarpa x deltoides, P. deltoides x nigra*
- Sweetgum (*Liquididambar*)
  - Schmidt and Westvaco/Arborgen
- Eucalypts (Urograndis hybrid)
  - Futuragene

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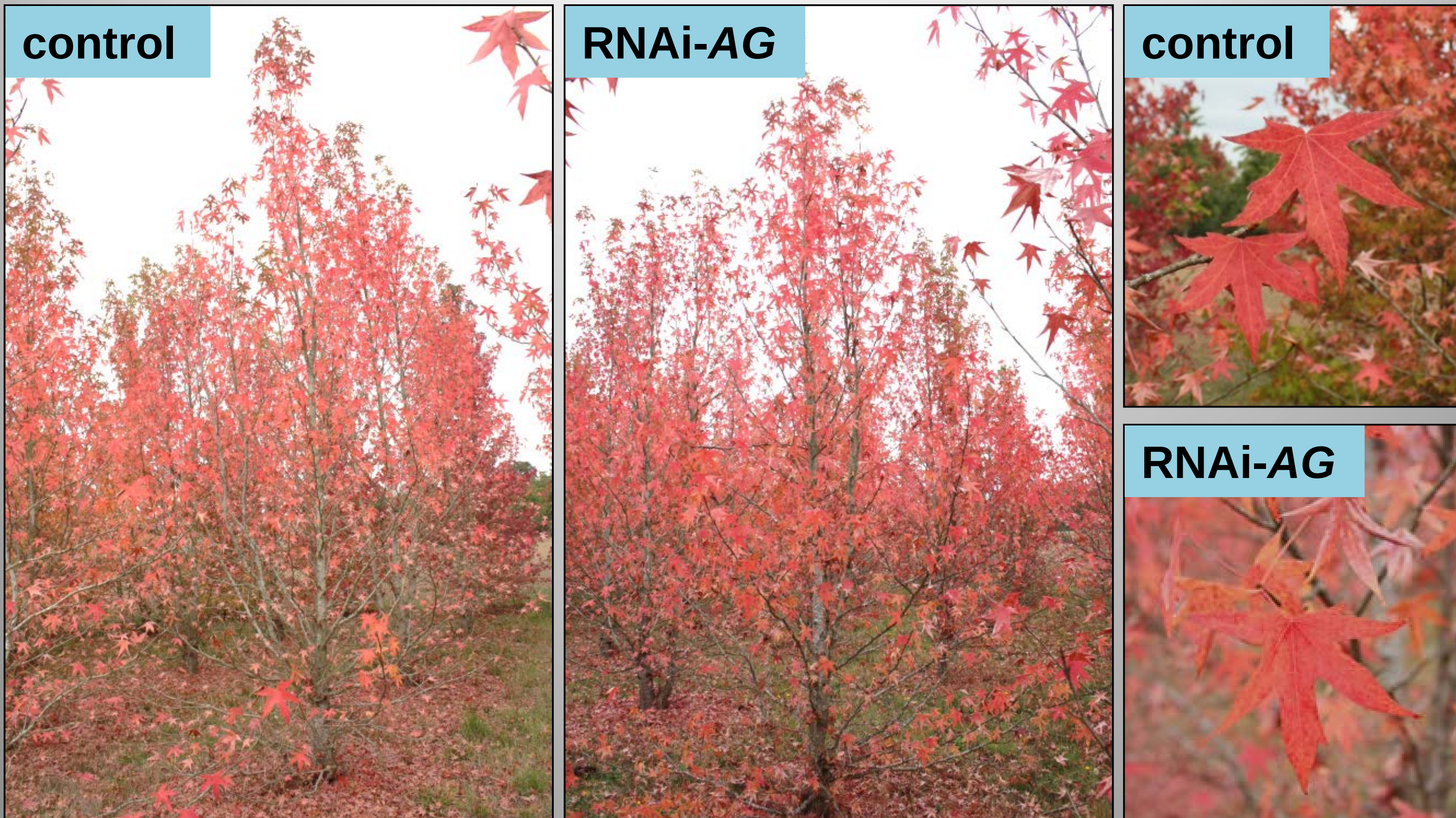
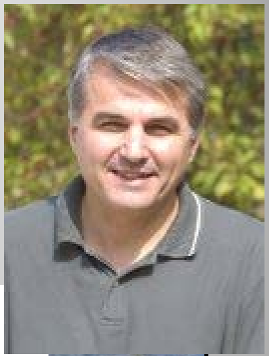
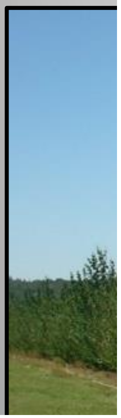
Sweetgum RNAi-*AGAMOUS* plantation (Sept 2016)

RNAi-AG trees had leaves and bright fall foliage like those of wild type ~8 years



Activation tagging facilitated by uniformity of GE transformants





Activation Tagging of a Dominant Gibberellin Catabolism Gene (*GA 2-oxidase*) from Poplar That Regulates Tree Stature[1]

Victor B. Busov, Richard Meilan, David W. Pearce, Caiping Ma, Stewart B. Rood, and Steven H. Strauss*

Department of Forest Science, Oregon State University, Corvallis, Oregon 97331–5752 (V.B.B., R.M., C.M., S.H.S.); and University of Lethbridge, Department of Biological Sciences, Lethbridge, Alberta, Canada T1K 3M4 (D.W.P., S.B.R.)

Plant Physiology, July 2003, Vol. 132, pp. 1283–1291, www.plantphysiol.org

Proceedings of the National Academy of Sciences of the United States of America

PNAS

EARLY BUD-BREAK 1 (*EBB1*) is a regulator of release from seasonal dormancy in poplar trees

Yordan S. Yordanov[a], Cathleen Ma[b], Steven H. Strauss[b], and Victor B. Busov[a,1]

[a]School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931; and

[b]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331-5752

Edited by Ronald R. Sederoff, North Carolina State University, Raleigh, NC, and approved May 16, 2014 (received for review March 27, 2014)

This Issue

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Don't Miss

PNAS Full-Text iOS

Unexpected phenotypes are rare but often showed up after dormancy in field

Mottled color and unusual leaf shapes

Dwarfed transgenic event

In general GE poplars were healthy and grow well – 99%ish of them

Lessons in relation to….

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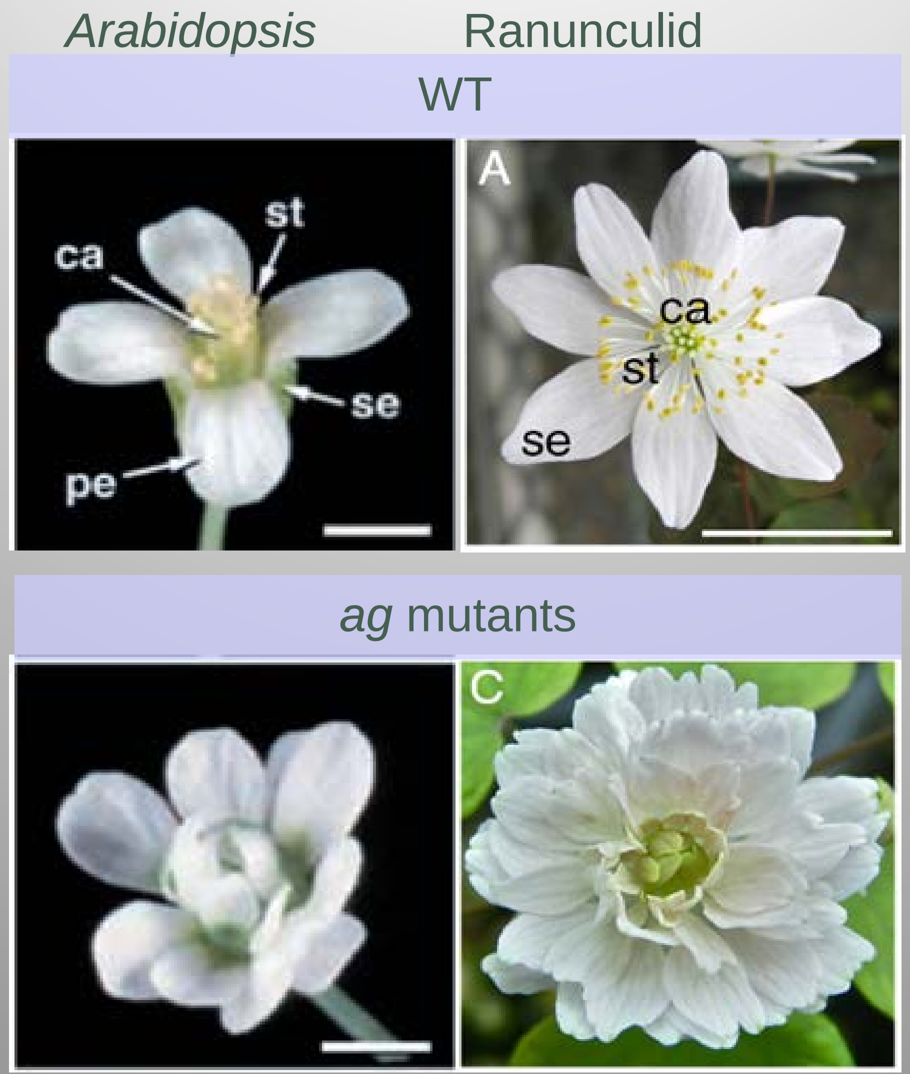
Strong *lfy* mutants appear to have no flowers

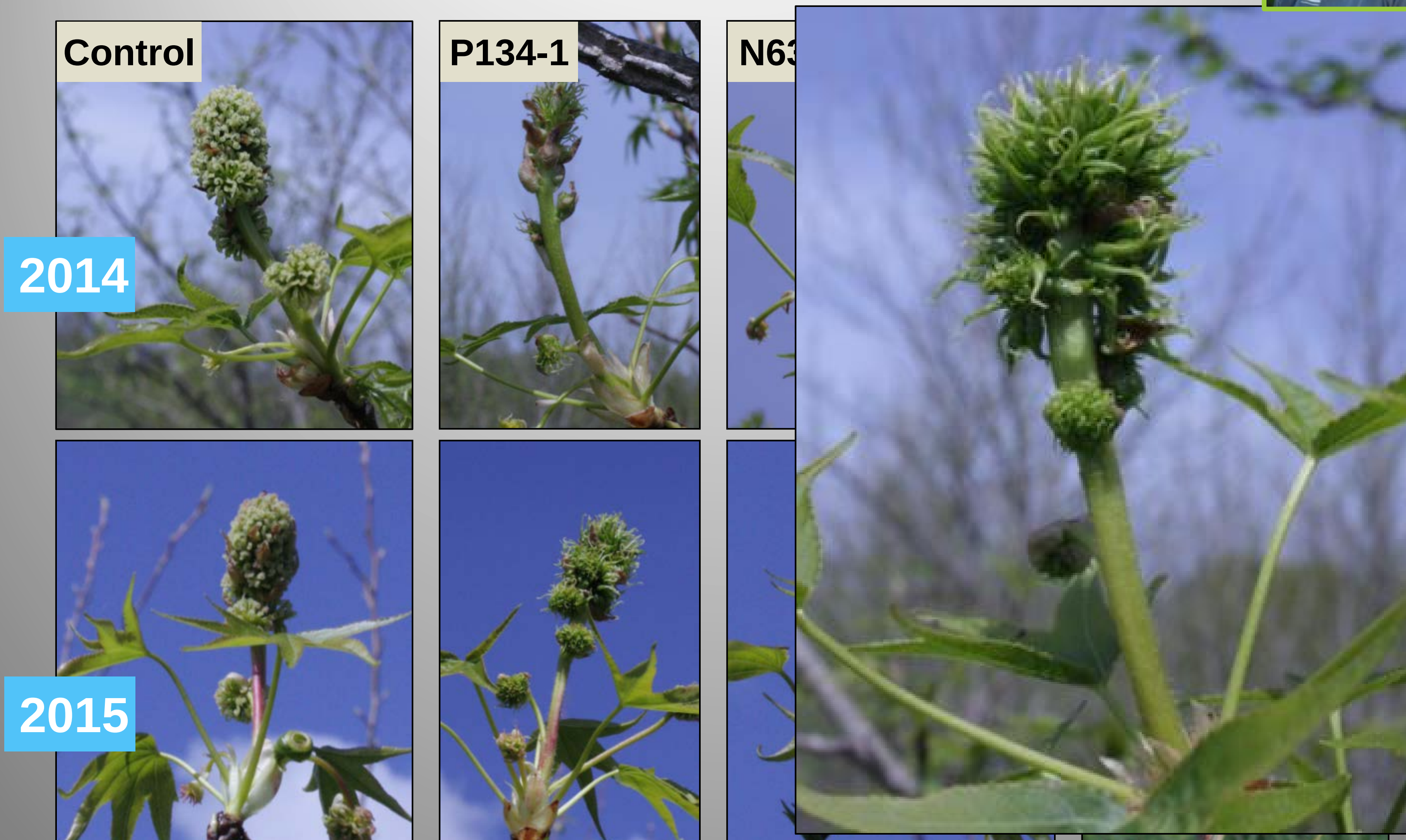
Parcy et al. 2002; Moyroud et al. 2010

Flowers in strong *ag* mutants are missing both stamens and carpels

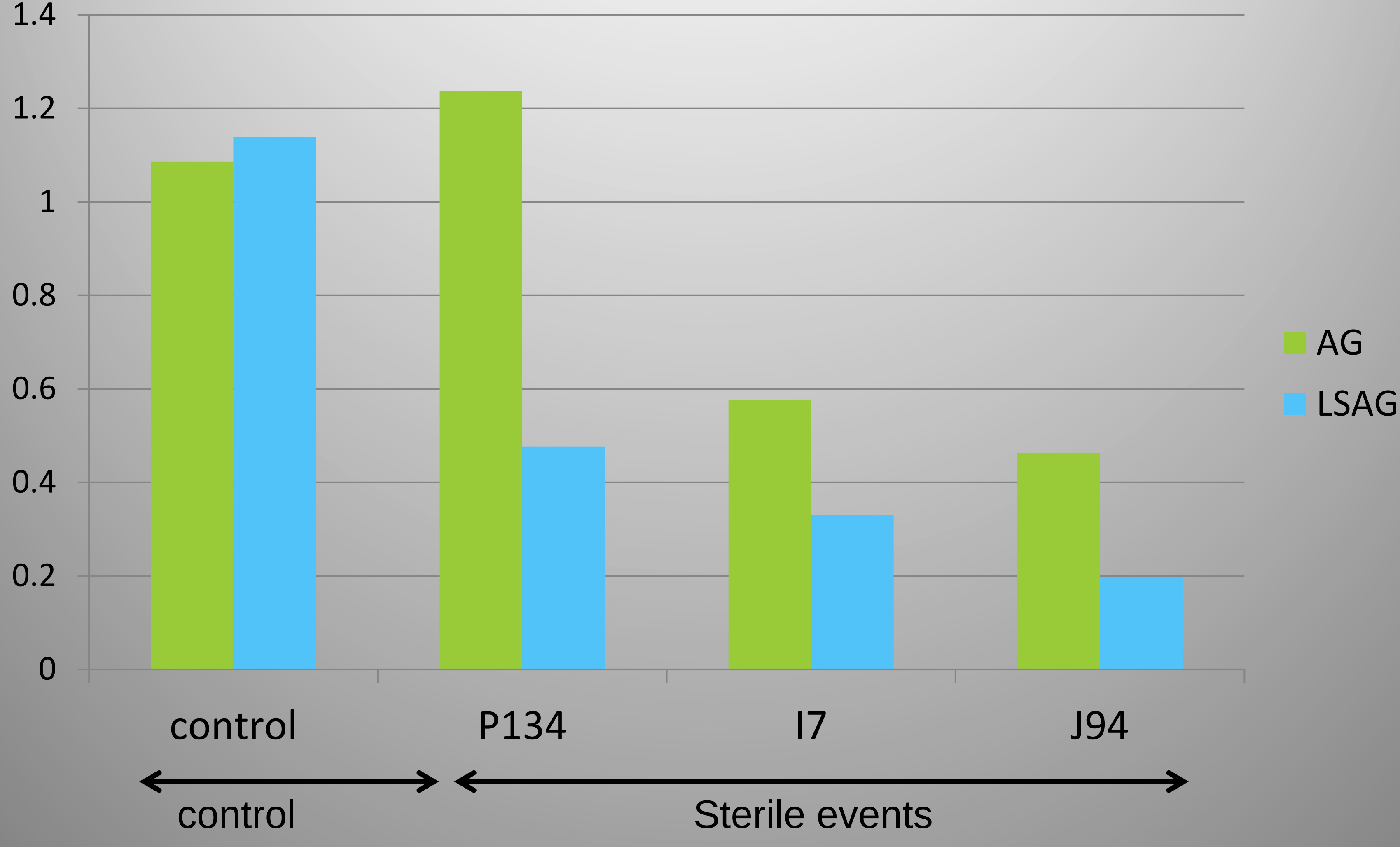


Parcy et al. 2002; Galimba et al. 2012

Altered phenotypes of RNAi-AG sweetgum were stable over 3 years



qPCR shows sterile events have strong suppression of one or both AG-like genes



Relative expression in floral buds, 2 biological and 3 technical replicates

Sterility, normal growth of *LEAFY*-RNAi poplars over four growing seasons

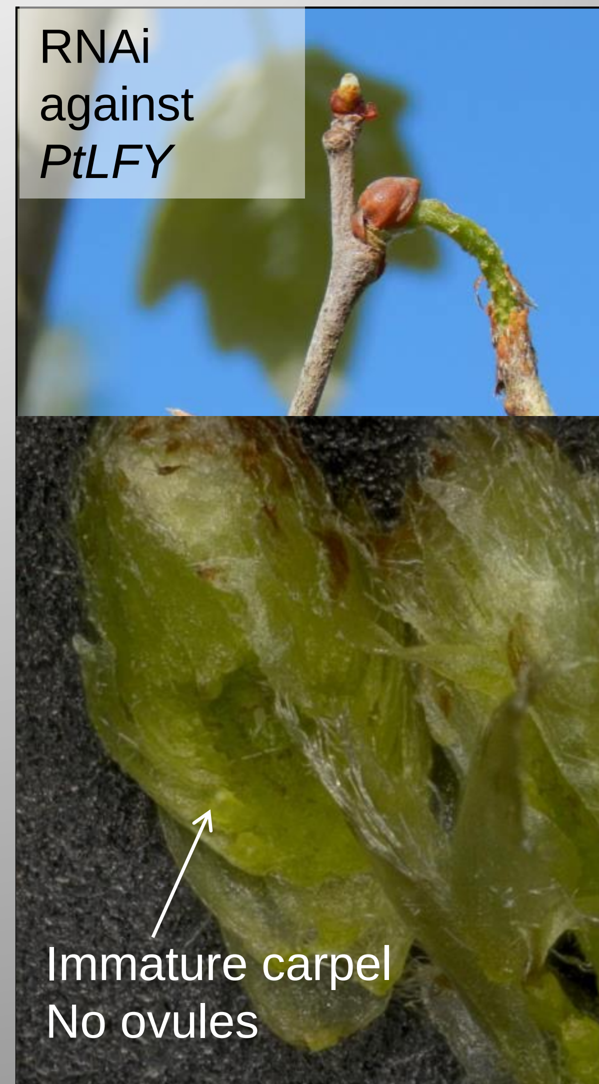
limited, in large part owing to concerns over transgene flow into wild or feral tree populations[1–4]. Unlike other crops, trees are long-lived, weakly domesticated and their propagules can spread over several kilometers[5]. Although male sterility has been engineered in pine, poplar, and eucalyptus trees grown under field conditions by expression of the barnase RNase gene in anther tapetal cells[6,7], barnase can reduce rates of genetic transformation and vegetative growth[6]. Furthermore, barnase expression may not be fully stable[8]. Bisexual sterility would allay concerns over seed dispersal, could be used to control invasive exotic trees, and might increase wood production[9]. We

poplar.

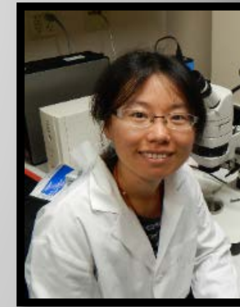
RNAi has been used to reduce gene expression in many plant species[10,11], and the reduction in gene expression that RNAi confers is highly stable in trees under field conditions[12]. *LFY* is required for the early stages of male and female floral organ formation in plants, and encodes a transcription factor that promotes floral meristem identity[13,14]. In *Arabidopsis thaliana*, loss of *LFY* function results in the formation of vegetative structures instead of floral meristems, whereas reduction of *LFY* expression decreases floral abundance and results in partial conversion of floral organs to leaf-like structures[13,14]. We selected *LFY*

Klocko et al. 2016, *Nature Biotechnology*

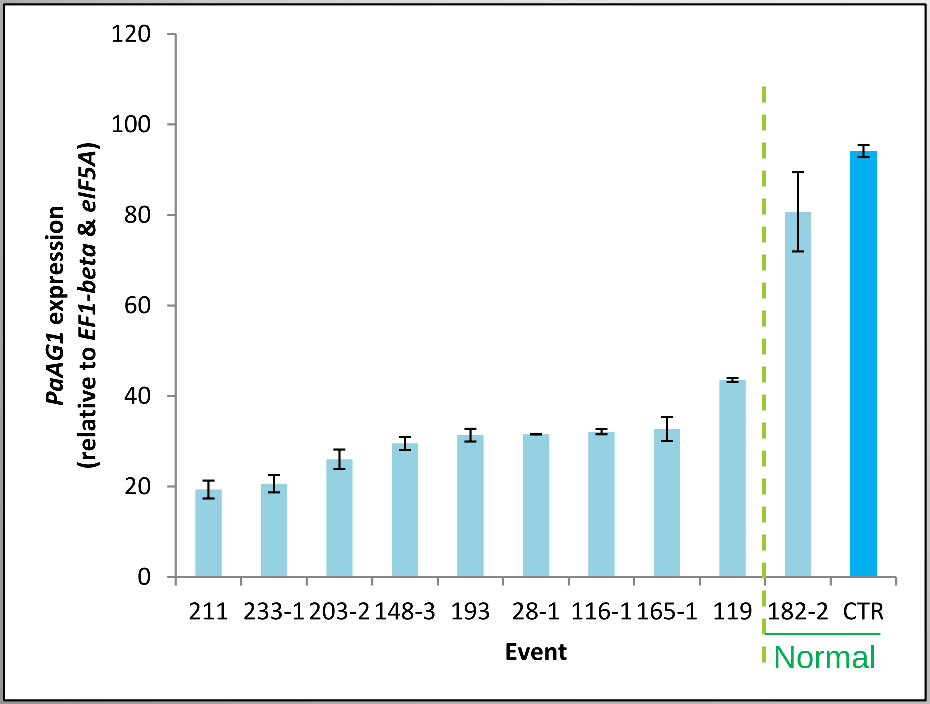
The tiny catkins have no reproductive organs

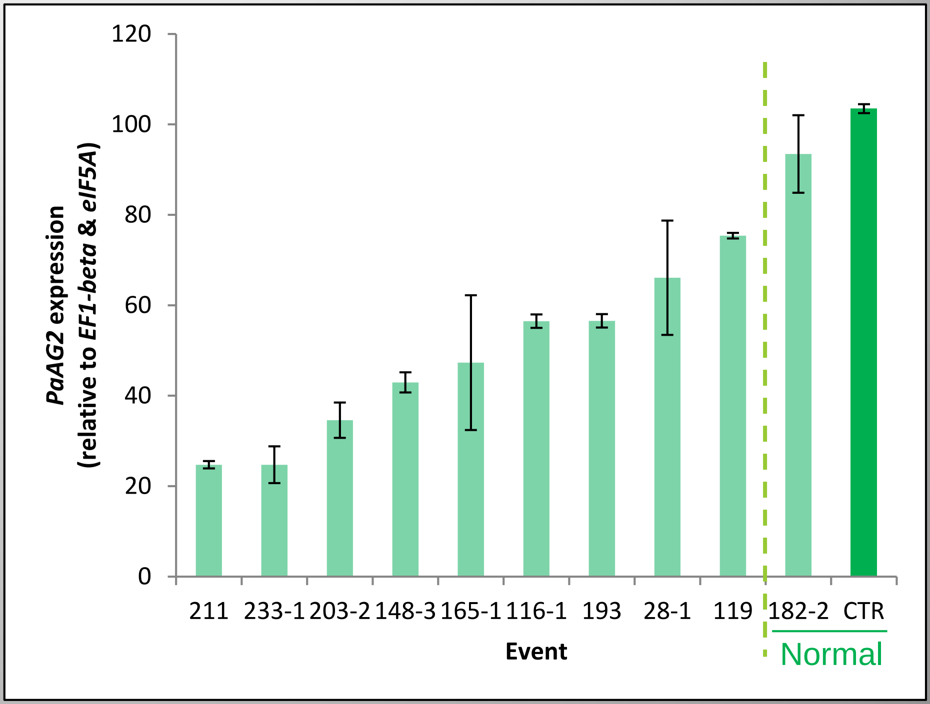


Strong poplar *AG-RNAi* events in the field with mutant flowers stable among/within trees and over 3 years

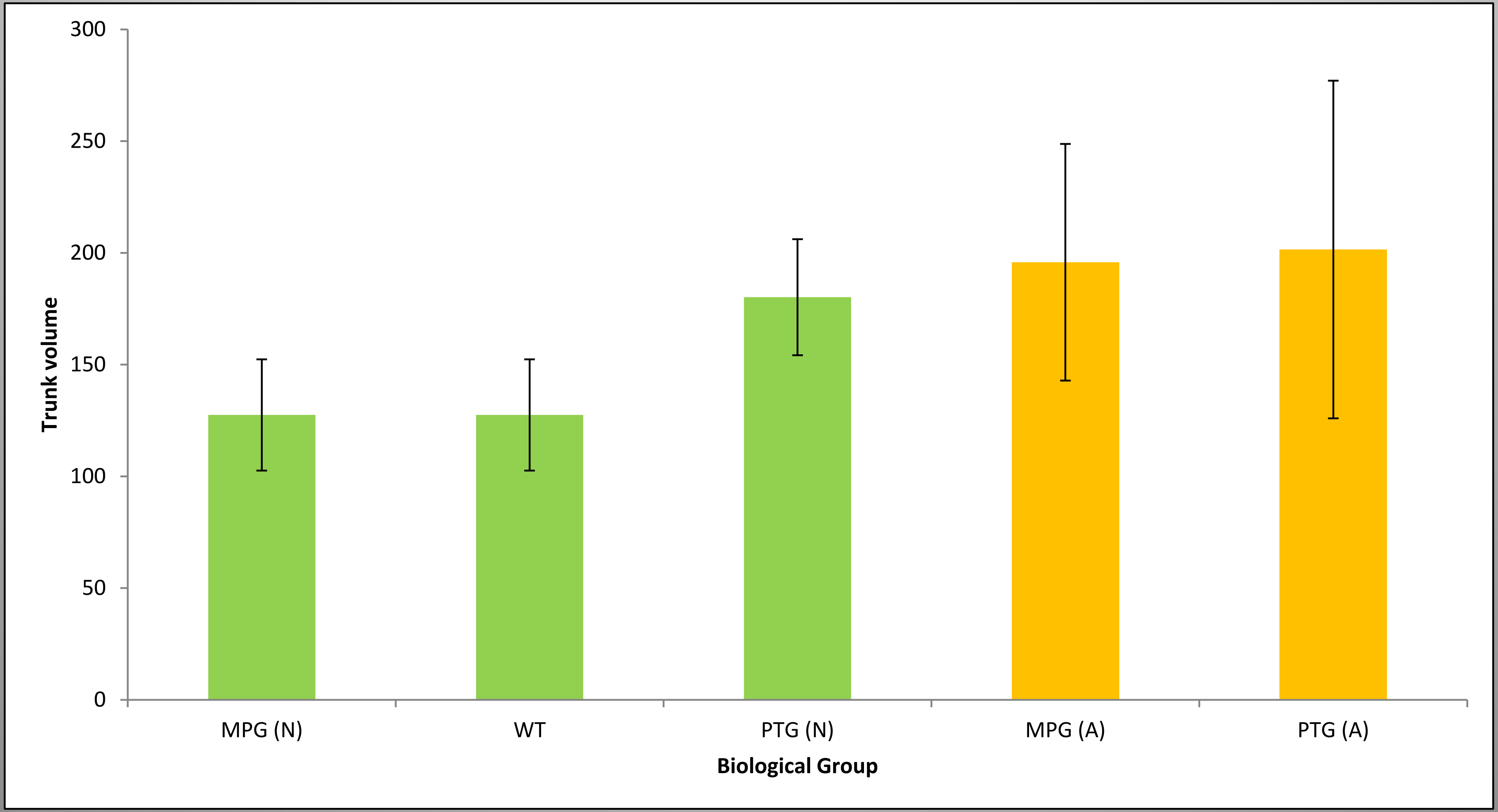


“Mild” suppression of *AG* gave strong sterility phenotypes





Strong *AG*-RNAi trees showed normal vegetative growth as well as sterility



- A= Altered, N=Normal, Bars = SE of the mean

Constructs were designed to delay or prevent floral onset

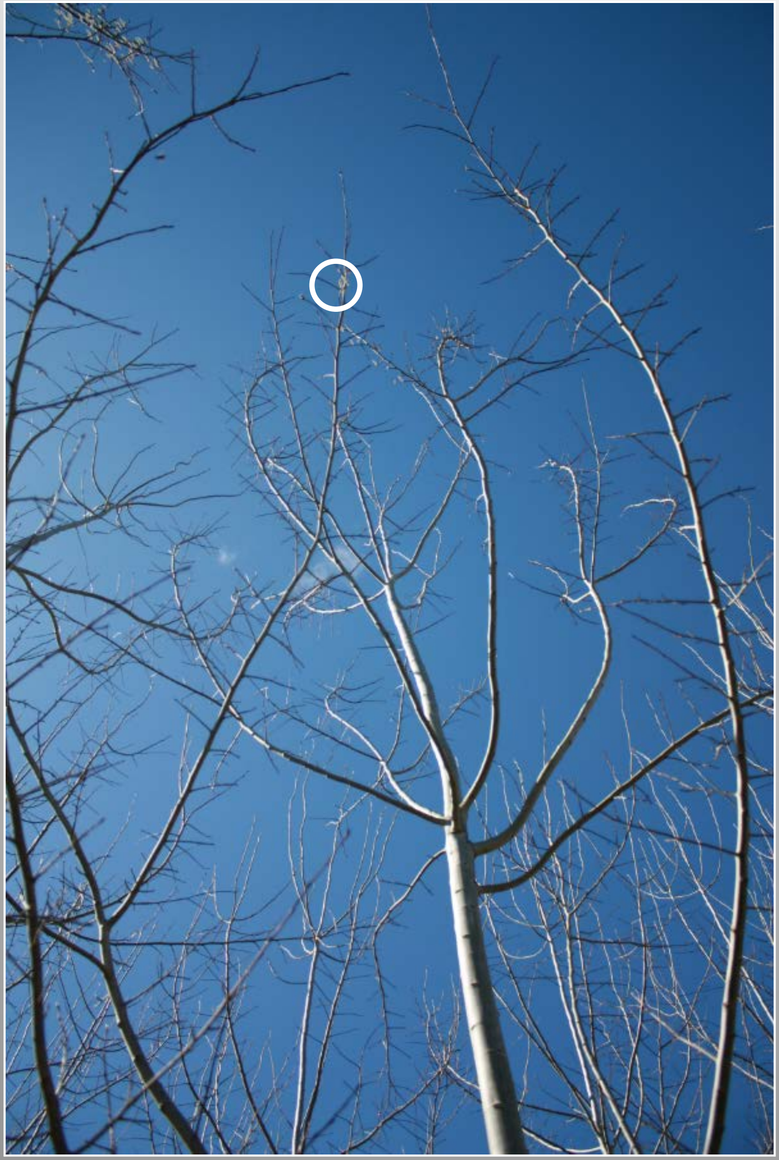
- Short vegetative phase (called *SVP*)
  - Poplar gene *SVP Potri.007G010800*
  - Overexpression of *SVP* should delay floral onset

- Dominant negative *APETALA1* (called "*AP2* and *AP3*")
  - Arabidopsis gene *AP1 AT1G69120*
  - Overexpression of mutated versions should delay onset

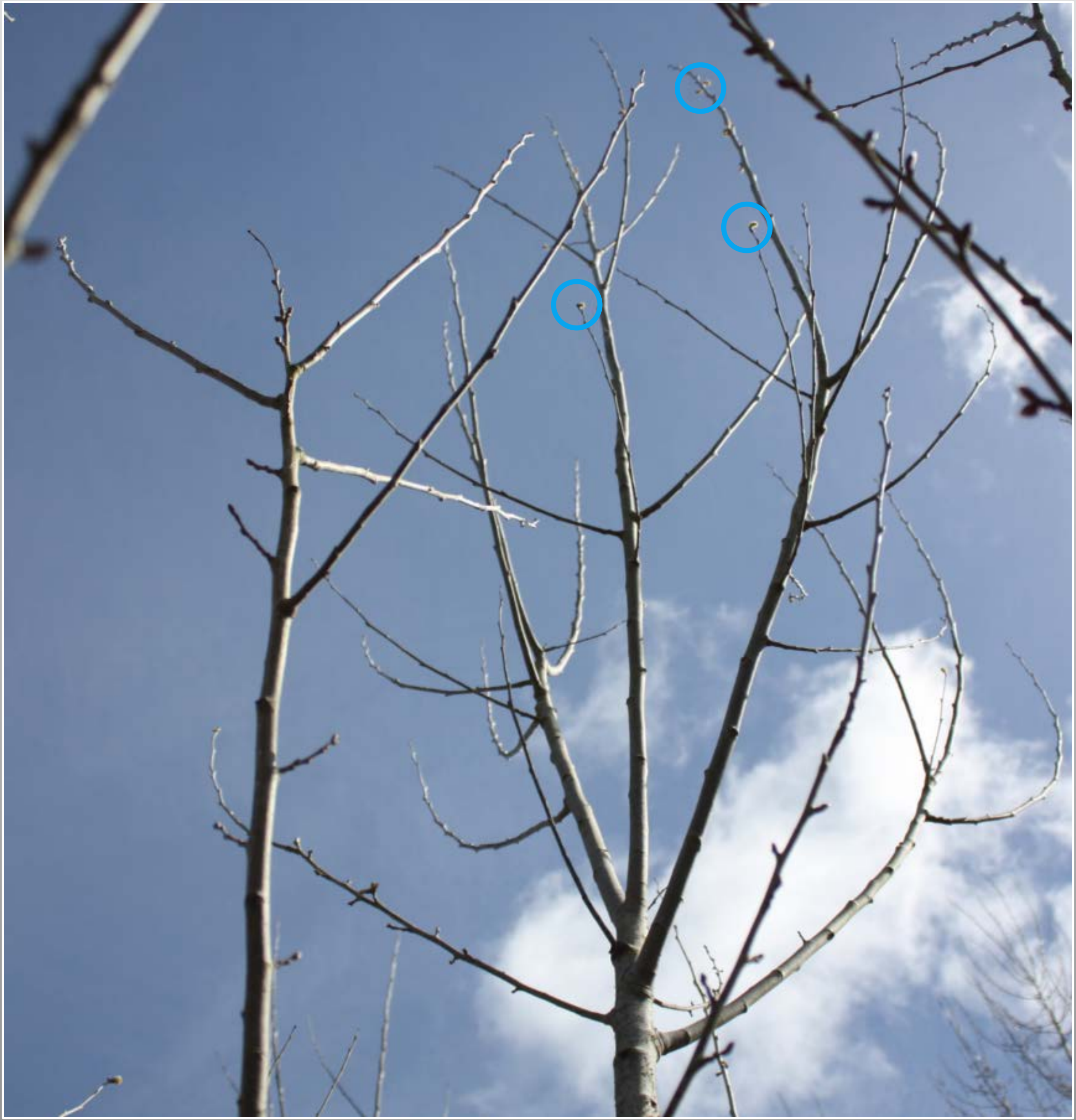
Scored flowering in all trees in ~4 ha trial

Score of 0

Score of 1

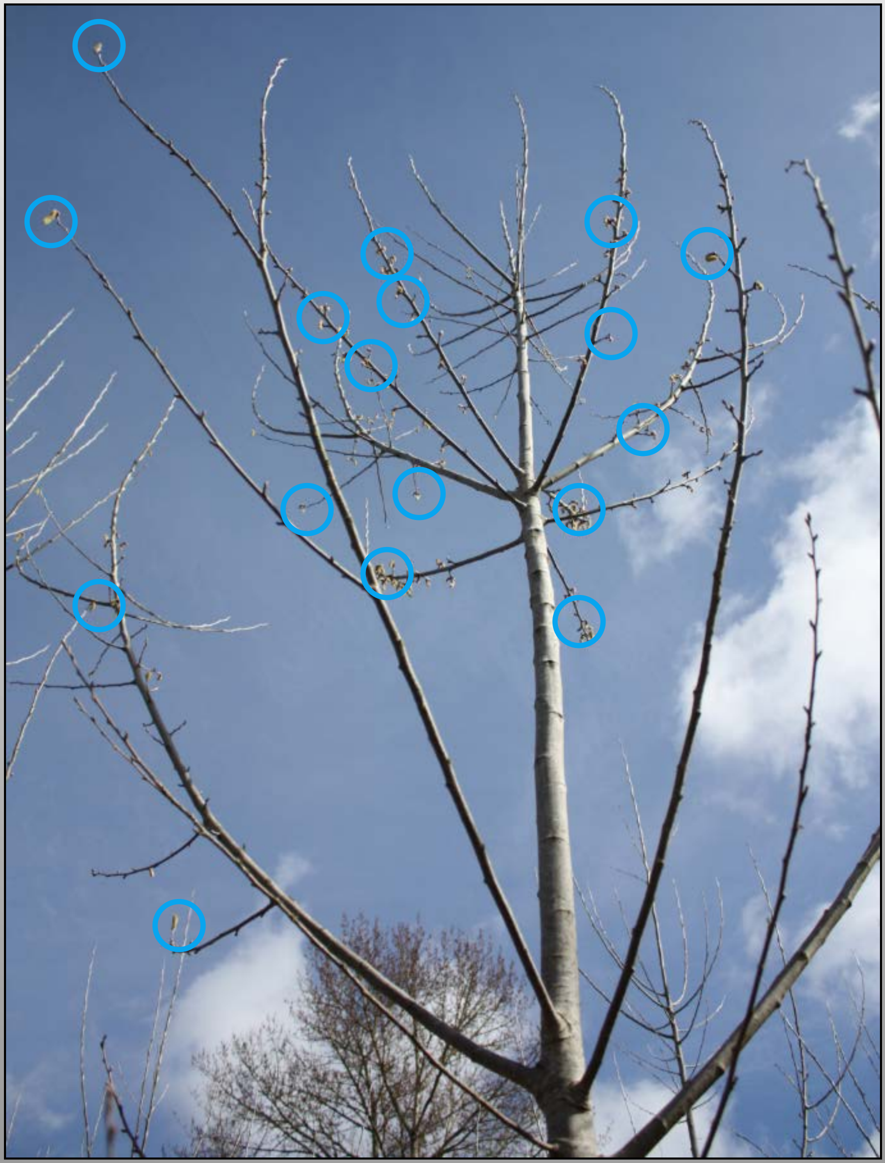


Score of 2



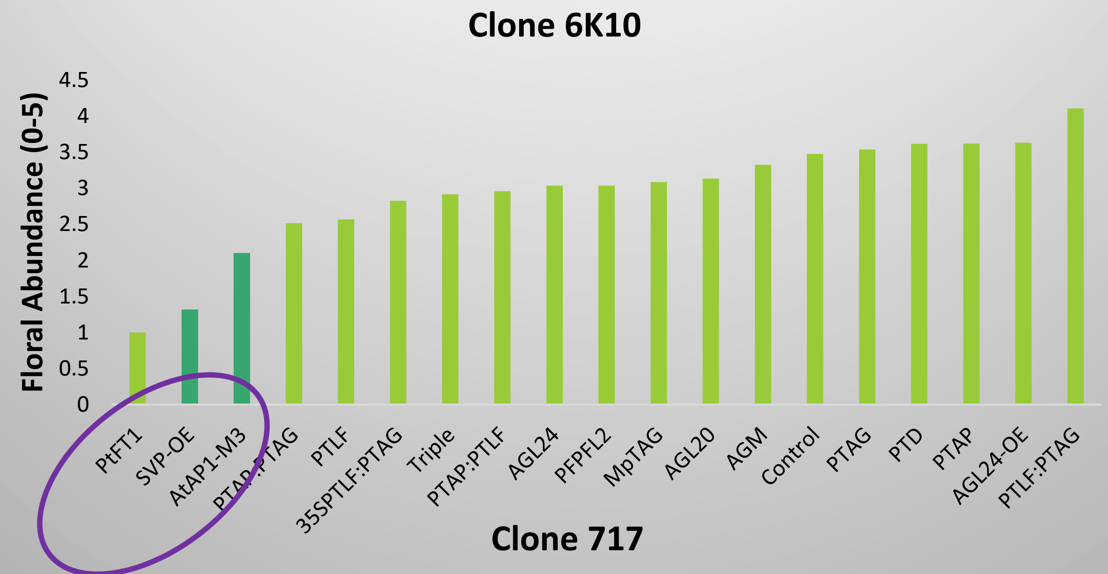
Score of 3

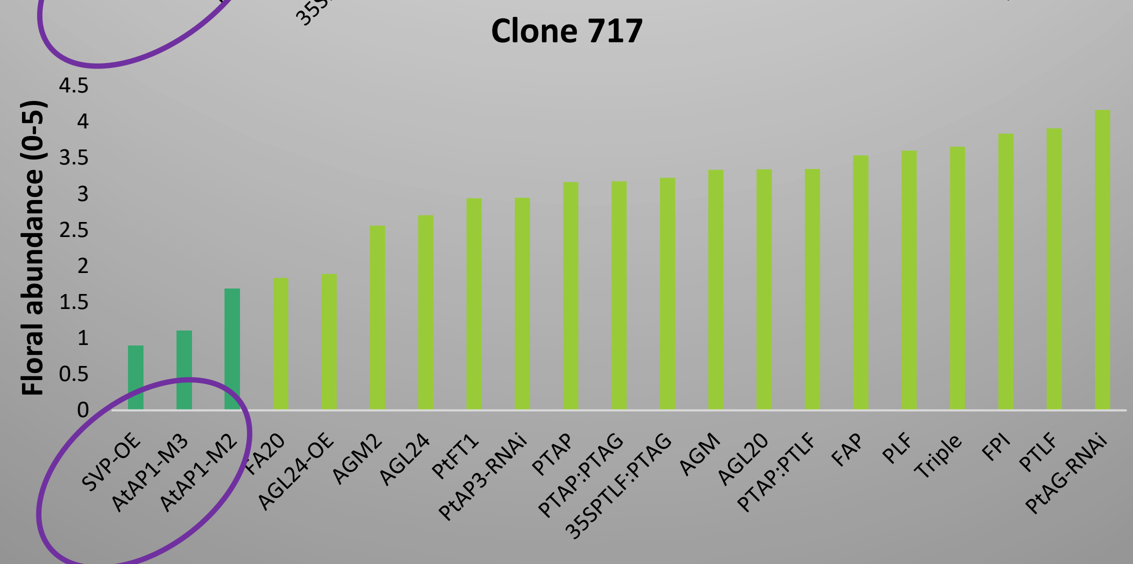
Score of 4



Score of 5

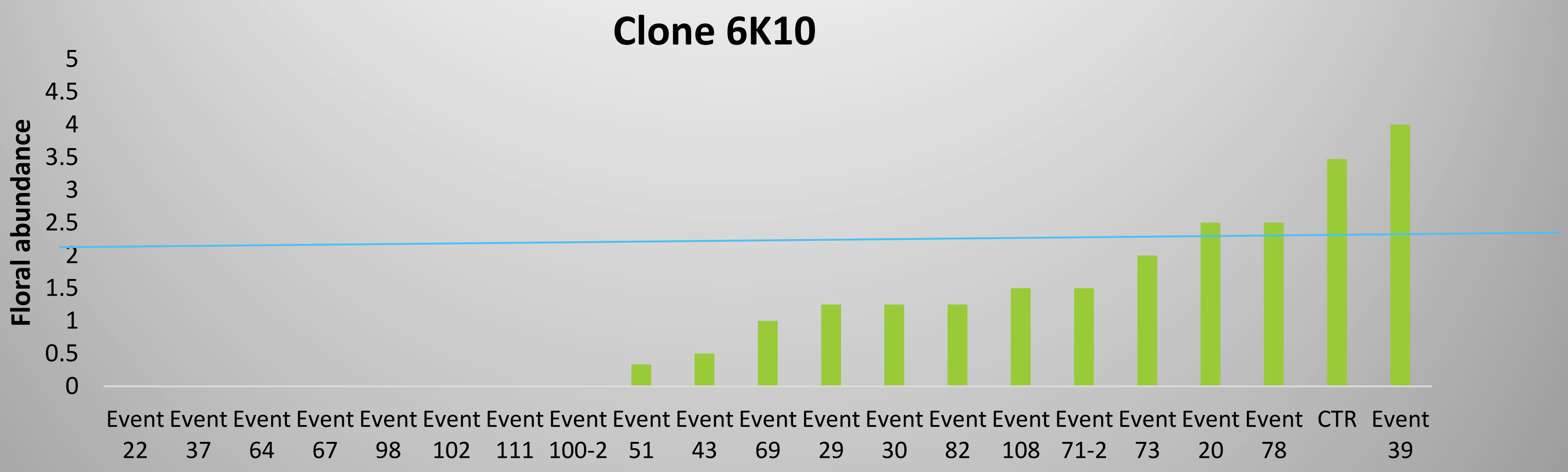
Three constructs resulted in very low floral abundance scores in clones 6K10 and 717

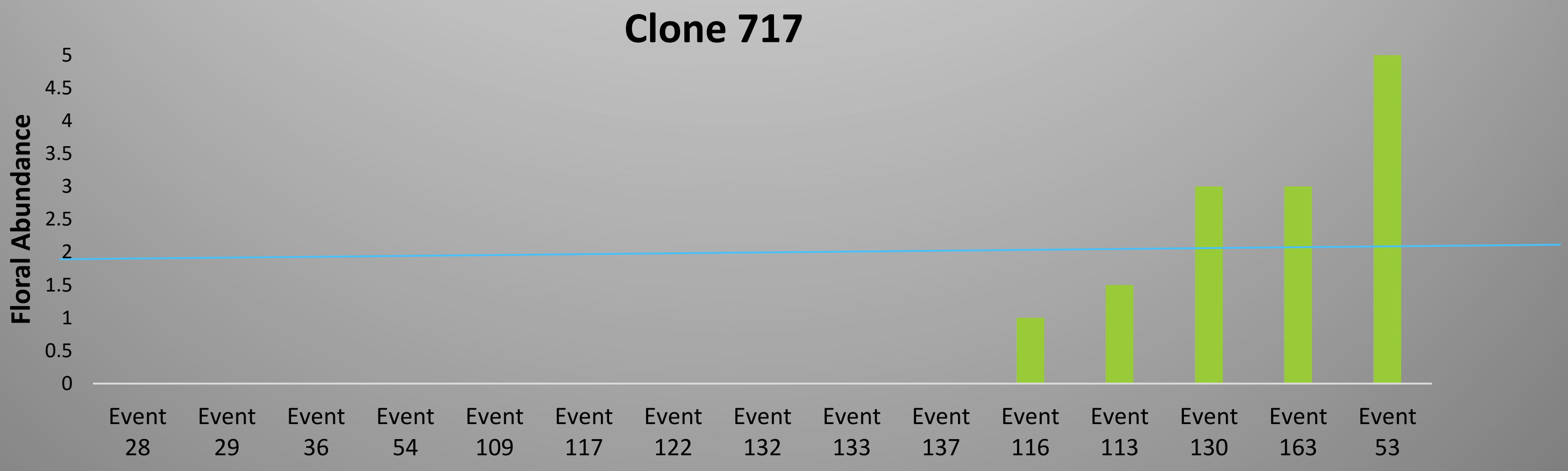




Data from 2016

80% of all SVP-OE events showed floral abundance scores of less than 2 in 2016



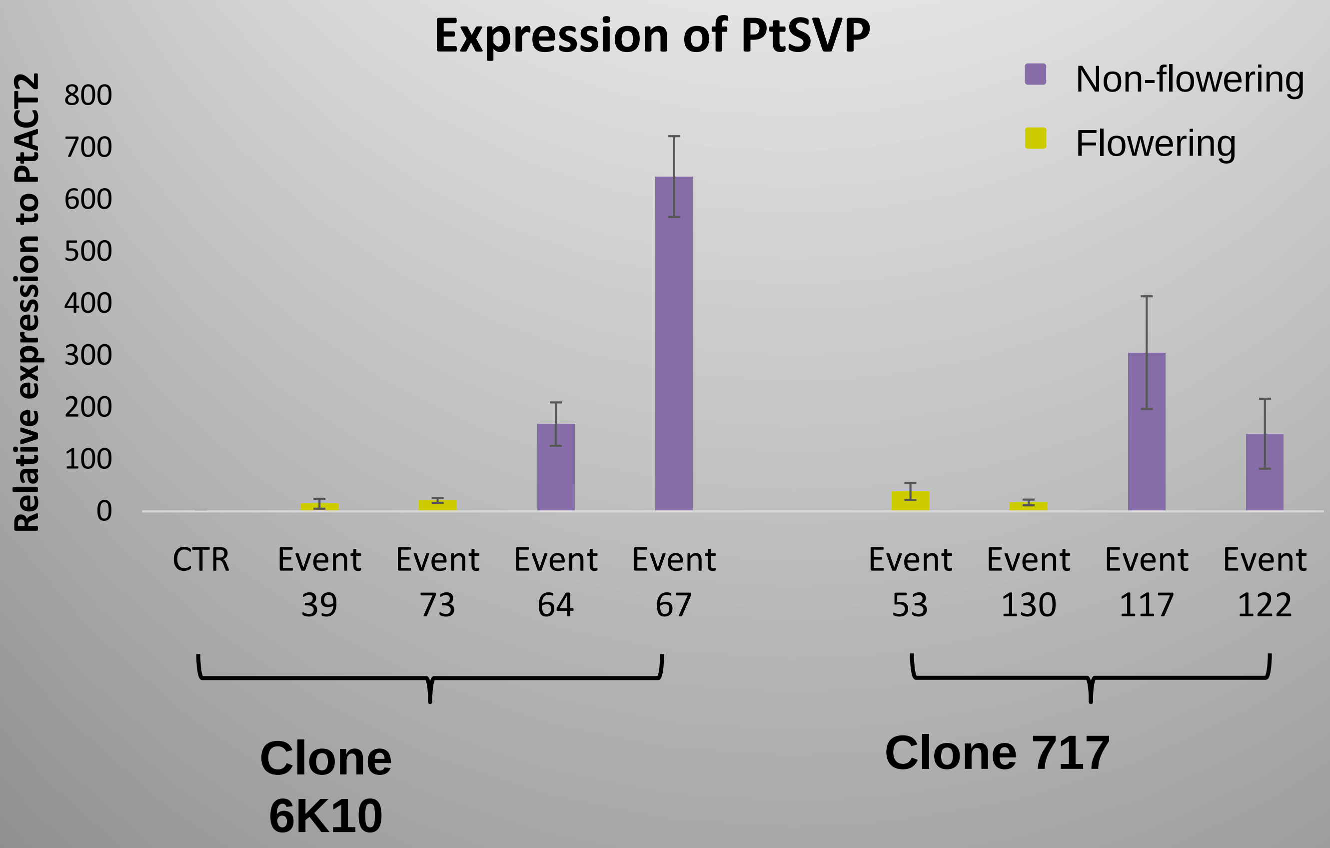


Striking differences among flowering vs. non-flowering adjacent events

717 SVP event 122 no flowers

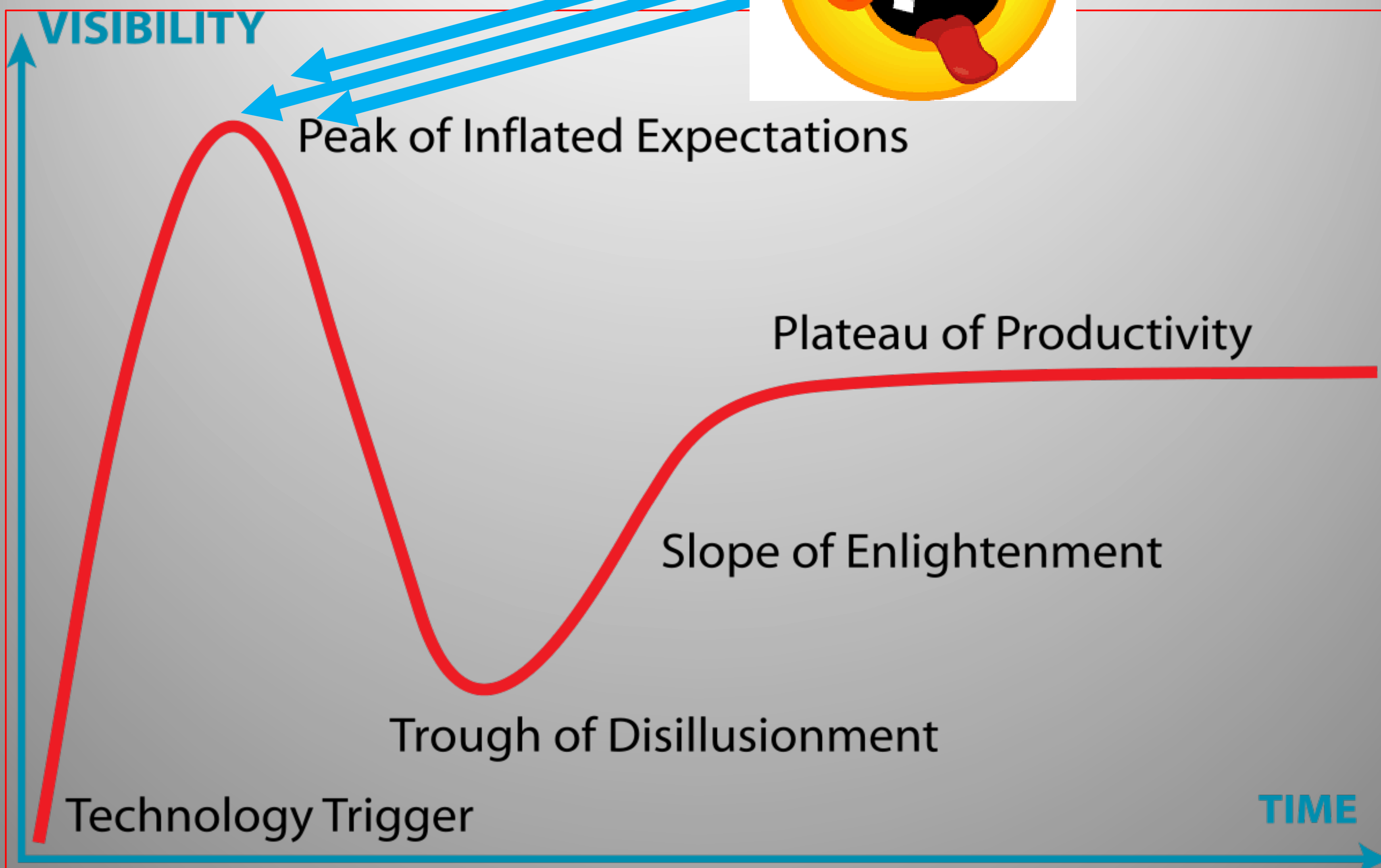
04.10.2017

Non-flowering events had high expression of *PtSVP* in leaves



QPCR by Emily Helliwell

Gene editing knock-ou underway



LFY knock-out in rapid flo background

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A lesson on the meaning of method-based regulation

Typically poplar trees flower in March in Oregon . . . unless they are semi-dwarf GA-modified trees having a good time

Summertime catkins – an unusual, report-mandatory event


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© AP Photo/Rogelio V. Solis

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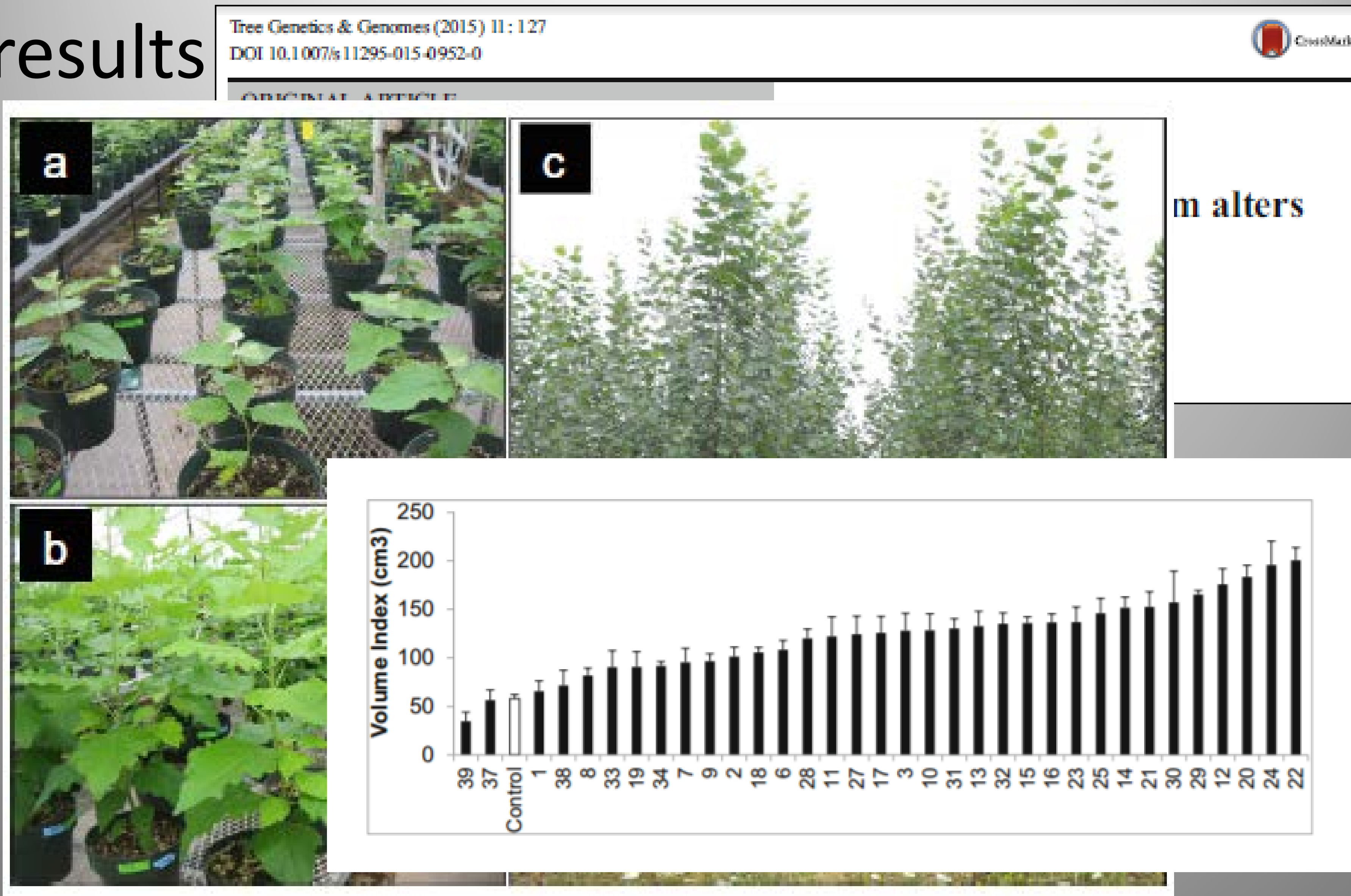
Modification of growth rate using GA-20-oxidase gave wild and inconsistent results



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

m alters

Antisense 4CL poplars gave sick, stiff field grown trees

Plant, Cell & Environment

Plant, Cell and Environment (2011) doi: 10.1111/j.1365-3040.2010.02270.x

Transgenic poplars with reduced lignin show impaired xylem conductivity, growth efficiency and survival

STEVEN L. VOELKER[1], BARBARA LACHENBRUCH[1], FREDERICK C. MEINZER[2], PETER KITIN[3] & STEVEN H. STRAUSS[4]

Antisense Down-Regulation of *4CL* Expression Alters Lignification, Tree Growth, and Saccharification Potential of Field-Grown Poplar[1][W][OA]

Steven L. Voelker, Barbara Lachenbruch, Frederick C. Meinzer, Michael Jourdes, Chanyoung Ki, Ann M. Patten, Laurence B. Davin, Norman G. Lewis, Gerald A. Tuskan, Lee Gunter, Stephen R. Decker, Michael J. Selig, Robert Sykes, Michael E. Himmel, Peter Kitin, Olga Shevchenko, and Steven H. Strauss*

Plant Physiology®, October 2010, Vol. 154, pp. 874–886, www.plantphysiol.org

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Insect resistant *Cry3a* Bt trees with much improved field productivity (~20%)

Cry3a

control

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

Glyphosate tolerance also gave surprising productivity benefits (~20%)

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]

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6. **Purple haze**: Summary perspectives on the meaning of biosafety and beyond

Tree Biotechnology Conference at Oxford in 1999 - Vandalism against lignin modified trees to "welcome" conferees, Euro-press attacks

FRANKENSTEIN'S FOREST

The tree-top protesters, who confounded the Government's road-building programme by camping in the path of bulldozers, are now poised to target the very trees they might once have called home.

Whilst public attention has been focused on the threat of 'Frankenstein Foods', the same corporations who are forcing us to ingest genetically modified (GM) meals have been quietly perpetrating yet another crime

ment. Campaigners fear that GM trees will sap up water, nutrients and light, leaving indigenous trees to die out along with the host of insects, plants and fungi which rely upon them. In turn, birds and animals would lose many of their natural prey. Those surviving creatures would fall victim to herbicide weed-killer, liberally applied once the GM trees become resistant. The result, opponents fear, will be a sanitised, silent forest, cleansed of

1997. The trees, engineered by the University of Derby, to be disease- and insect-resistant were destroyed by removing the bark. A growing spate of raids on food crops caused AstraZeneca to make a statement to the press before a GenetiX Snowball action earlier this year, fearing damage to their GM poplars.

In April, Monsanto teamed up with two of the world's biggest forest and paper corporations, International Paper and Westvaco.

vention, which governs global emissions of greenhouse gases, came into force after the 1997 Kyoto conference, industrialised countries have been forced to clean up. However, the corporations argue that by planting more trees, they should be awarded 'carbon credits', because trees absorb carbon dioxide.

Recently, naturally rich native forests have fallen to the chainsaw, only to be replaced by invasive foreign plantation species such as

Whilst public attention has been focused on the threat of 'Frankenstein Foods', the same corporations who are forcing us to ingest genetically modified (GM) meals have been quietly perpetrating yet another crime against the environment.

Vandalism in Pacific Northwest USA / 2001

Oregon State University,
Corvallis

University of Washington,
Seattle

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

Plantation Certification & Genetic Engineering

FSC's Ban on Research Is Counterproductive

Steven H. Strauss, Malcolm M. Campbell, Simon N. Pryor, Peter Coventry, and Jeff Burley

ABSTRACT

Genetic engineering, also called genetic modification (GM), is the isolation, recombinant modification, and asexual transfer of genes. It has been banned in forest plantations certified by the Forest Stewardship Council (FSC) regardless of the source of genes, traits imparted, or whether for research or commercial use. We review the methods and goals of tree genetic engineering research and argue that FSC's ban on research is counterproductive because it makes it difficult for certified companies to participate in the field research needed to assess the value and biosafety of GM trees. Genetic modification could be important for translating new discoveries about tree genomes into improved growth, quality, sustainability, and pest resistance.

Keywords: biotechnology; entomology and pathology; ethics; genetics; silviculture

Genetic engineering, commonly called genetic modification (GM) in much of the world, is the use of recombinant DNA and asexual gene transfer methods to breed more productive or pest-resistant crops. It has been the subject of considerable controversy, with concerns raised from biological, socioeconomic, political, and ethical perspectives. Some of the issues are similar to those raised by the use of molecular biology and genetic engineering in medicine, which we see in the news headlines daily. However, genetic modification in agriculture and forestry raises environmental issues as well.

GM crops, mainly herbicide- and pest-resistant varieties of soybeans, maize, or cotton, have been vigorously adopted by farmers in North America because they are easy to manage and they improve yields, reduce costs, or reduce pesticide ecotoxicity (Carpenter and Gianessi 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 trees adapt to the stresses and special demands of human-dominated systems. Examples would be trees that are more tolerant of heavy metals or other pollutants, resist urban pests or diseases, grow slower, or do not produce fruits when these create hazards in street environments (Brunner 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 (Romm 1994). Plantations can relieve pressure 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 10 states that plantations should "complement the management of, reduce pressures on, and promote the restoration 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 pine and eucalypt plantations of the Southern Hemisphere. Although many environmental mitigations are built into these certified plantation systems, within the areas dedicated to wood 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 silvicultural management. It is in the context of these biointensive systems that the additional expense of GM trees is likely to be worthwhile.

However, FSC currently prohibits all uses of GM trees, and is the only certification system to have done so

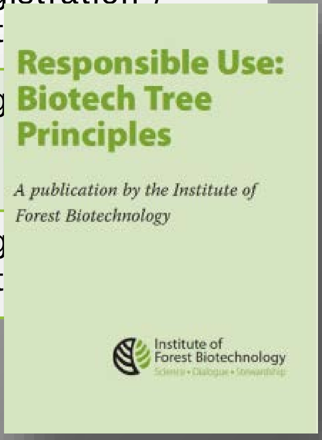
FSC

Forest Stewardship Council

"…genetically modified trees are prohibited…"

All major forest certification systems now ban all GE trees – no research exemptions

System	Region	GM Tree Approach / Reason
PEFC : Programme for Endorsement of Forest Certification	International	**Banned** / Precautionary approach based on lack of data
FSC : Forest Stewardship Council	International	**Banned** / Precautionary approach based on lack of data
CerFlor : Certificação Florestal	Brazil	**Banned** via PEFC registration / No additional rationale
CertFor : Certficación Forestal	Chile	**Banned** via PEFC registration / No additional rationale
SFI : Sustainable Forestry Initiative	North America	**Banned** via PEFC registration / Awaiting risk-benefit data
ATFS : American Tree Farm System	USA	**Banned** via PEFC registration / No additional rat
CSA : Canadian Standards Association	Canada	**Banned** via PEFC reg Allows public to determ
CFCC : China Forest Certification Council	China	**Banned** via PEFC reg No additional rat



Adam Costanza, Institute for Forest Biotechnology

Regulations and certification render GE ineffective as a tool for forest health

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

Forest crises demand regulation and certification reform

By **Steven H. Strauss[1*], Adam Costanza[2], Armand Séguin[3]**

Intensive genetic modification is a long-standing 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

The paradox of “biosafety”

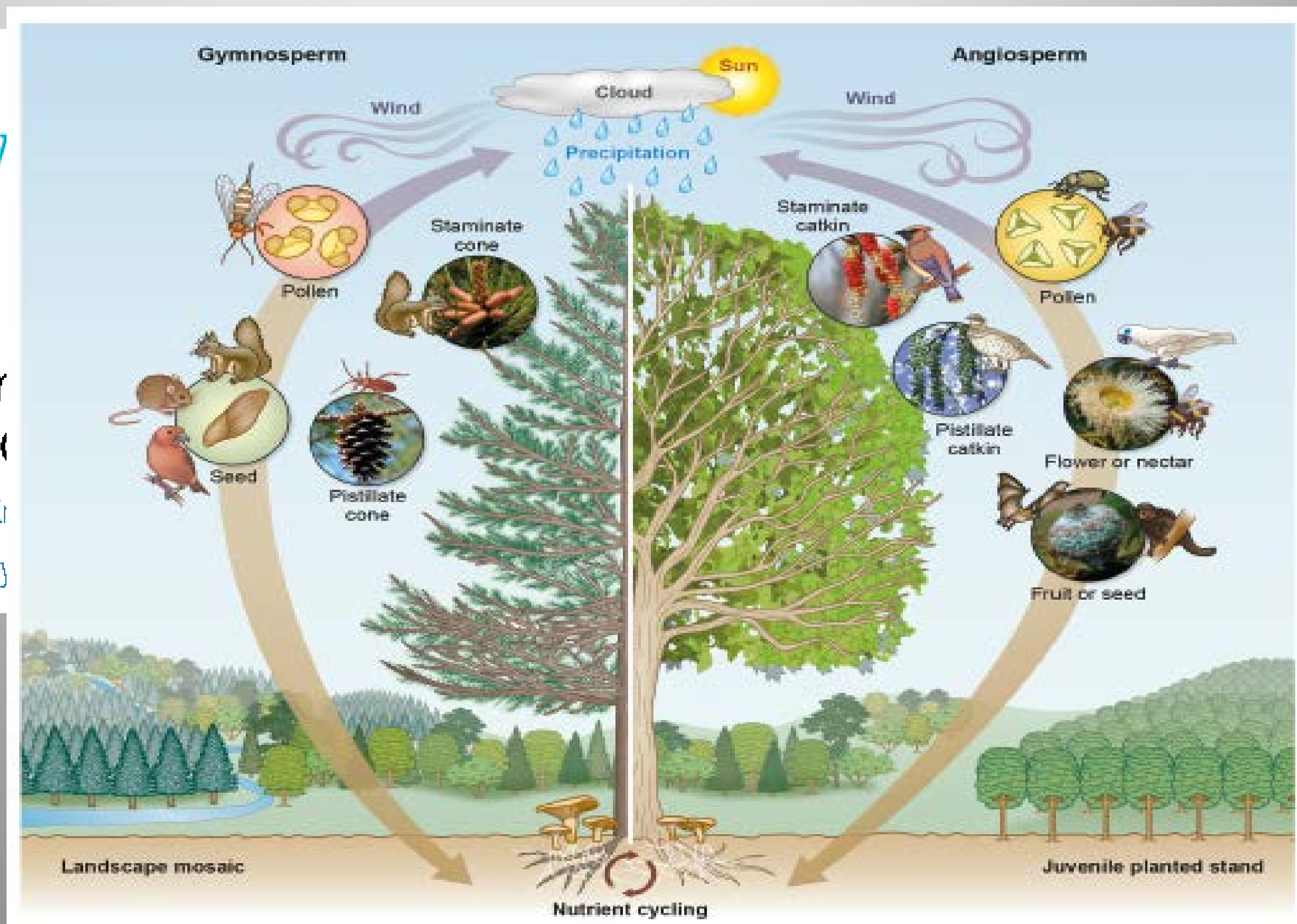
Sometimes essential, often absurd, always enlightening, usually entangling

New

Tansley review

Reproductive m
impacts on bio(

Steven H. Strauss, Kı
Matthew G. Betts, Berry



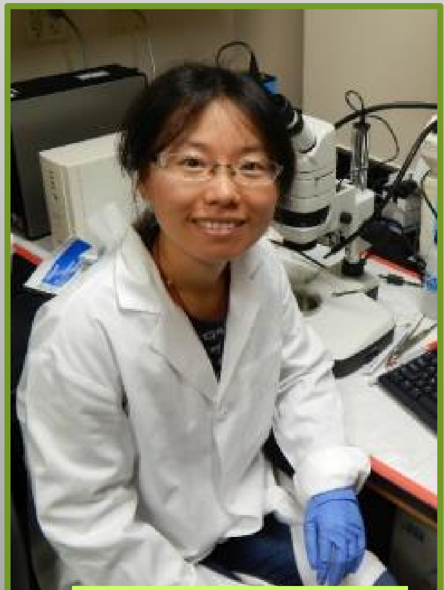
In summary, lessons to date…

1. Abnormalities due to transformation / in vitro regeneration – VERY LITTLE
2. Trait stability: RNAi-induced gene suppression and other traits – VERY HIGH, WE CAN CONTAIN VERY WELL IF WE WANT TO
3. Single genes for modifying complex, physiological traits – SIMPLE GENE MIRACLES NOT ALWAYS SO MIRACULOUS
4. Value of 1st generation transgenic traits – HIGH IF USED SUSTAINABLY, SHOULD NOT BE DISMISSED
5. GMO regulation/market realities – COSTLY, RISKY, IDEOLOGICAL, METHOD VS. RISK/BENEFIT BASED
6. Biosafety plus – KEEP YOUR COOL, KEEP BIG GOALS IN MIND, DO GOOD SCIENCE THAT PUTS IMPACTS IN CONTEXT

Thanks to these key people, and many more over the years

Cathleen Ma

Amy Klocko



Haiwei Lu

Anna Magnuson

Thanks for support

TBGRC

USDA

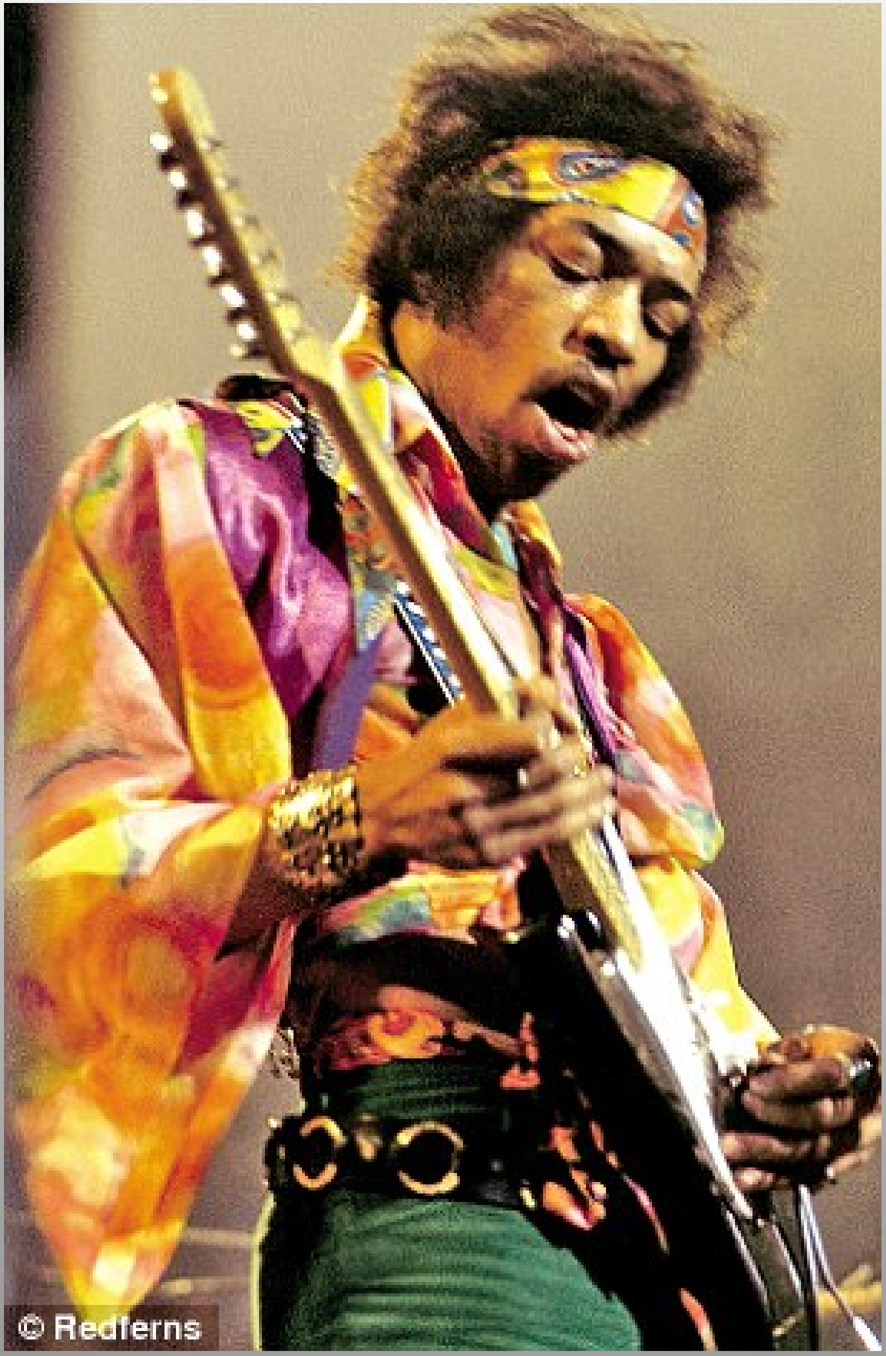
United States Department of Agriculture

National Institute of Food and Agriculture

NSF

The National Science Foundation

Futuragene, SAPPI, SweTree, U. Pretoria, Arborgen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