Gene editing in forest trees
Policies and progress to enable innovation

Steve Strauss / Oregon State University / USA

11th National Poplar Symposium, Hebei, China - 2019

Forest health a major and growing concern that breeding alone cannot keep up with

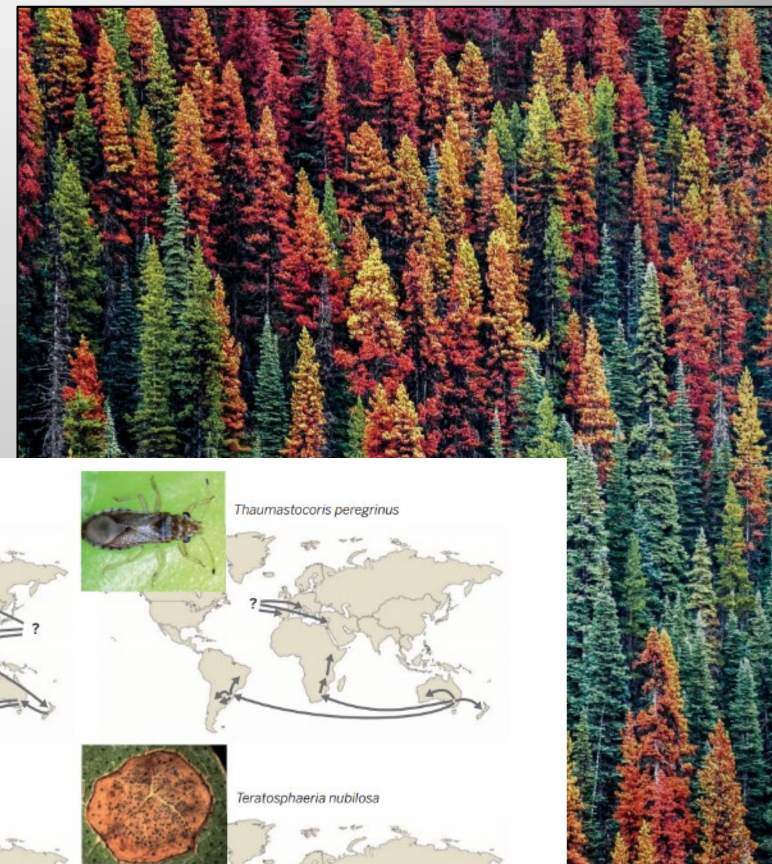

REVIEW

Planted forest health: The need for a global strategy

M. J. Wingfield,[1]* E. G. Brockerhoff,[2] B. D. Wingfield,[1] B. Slippers[1]

ed forests worldwide, and these represent valuable
easingly threatened by insects and microbial
ntally and/or have adapted to new host trees.
ergence, despite a growing awareness of the
costs, and an increased focus on the importance of
ntial of planted forests, innovative solutions and a
eeded. Mitigation strategies that are effective only in
s elsewhere in the world, ultimately leading to global
ns in the future should mainly focus on integrating
than single-country strategies. A global strategy to
nd urgently needed.

r 8, 2015



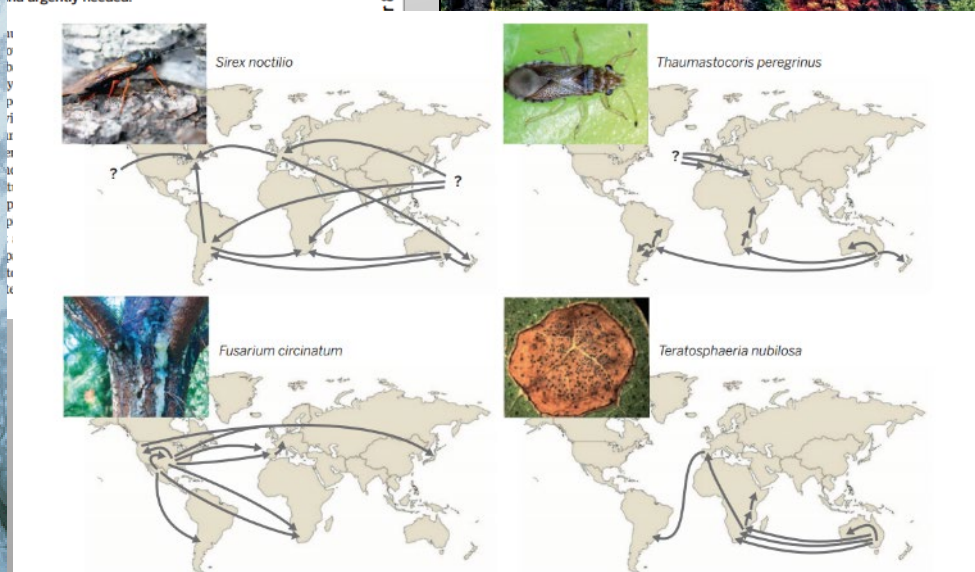


Fig. 2. Examples of invasion routes of pests of planted forests that illustrate an apparently common pattern of complex pathways of spread to new environments, including repeated introductions and with either native or invasive populations serving as source populations (18). Invasion routes of the pine pitch canker pathogen *Fusarium circinatum* (origin in Central America) (39), eucalypt leaf pathogen *Teratosphaeria nubilosa* (origin in southeast Australia) (40), the pine woodwasp *Sirex noctilio* (origin in Eurasia) (23), and the eucalypt bug *Thaumastocoris peregrinus* (origin in southeast Australia) (41) were determined through historical and genetic data. [Photo credits: (top left) Brett Hurley; (top right) Samantha Bush; (bottom left) Jolanda Roux; (bottom right) Guillermo Perez]

834 21 AUGUST 2015 • VOL 349 ISSUE 6250 sciencemag.org SCIENCE

Demands for renewable, sustainable materials and energy—and social standards for safety for stewardship—are also growing

Today I will argue that:

1. The precision of biotech tools, regulated and chosen wisely, are essential to meet these growing expectations
2. But we are far from the smart systems we need for such progress
3. And given climate change, population growth, and ecological decline, we need change urgently

Agenda

- Definition of gene editing (GE) compared to GMO and conventional breeding
- The social environment that makes use of GE and GMO, and integration with breeding, very difficult
  - A recent attempt by scientists to stimulate change
- A GE example – showing precision and stewardship possibilities
  - CRISPR for reproductive modification in Eucalyptus

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What is genetic engineering (GMO) and gene editing (GE)?

- Direct modification of DNA
  - vs. indirect modification in breeding
- Asexually modified, usually in somatic cells
  - Then regenerated into whole organisms, usually starting in Petri dishes
- <u>Specificity</u> of modification, common use of new vs. modified native genes, differentiates GE from GMO

Overview of steps to create a GMO or GE plant

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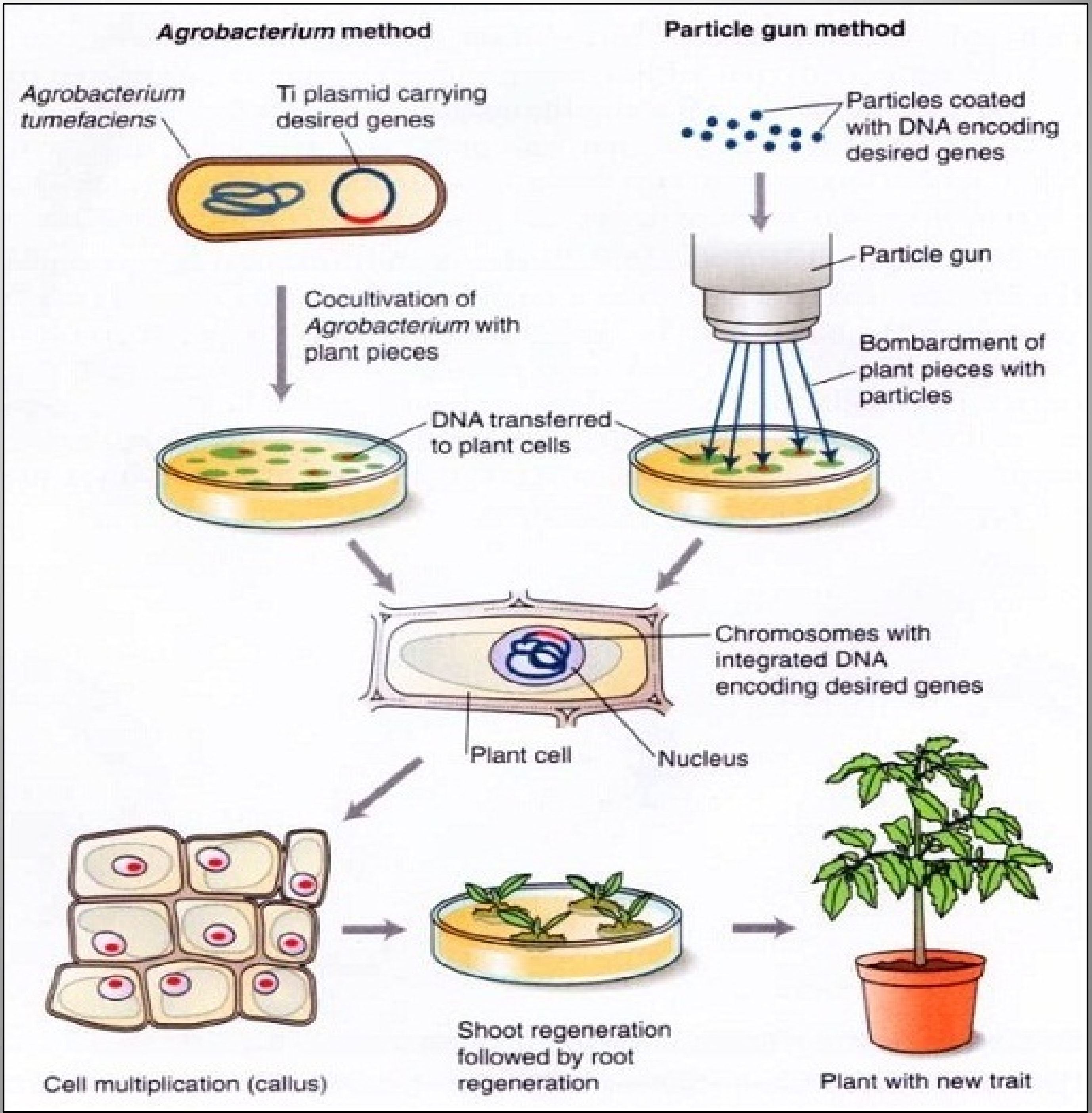
New genes usually removed or deactived after GE



Many forms of GE, but CRISPR gene editing technology considered a major scientific breakthrough

Science magazine names CRISPR 'Breakthrough of the Year'

By Robert Sanders | DECEMBER 18, 2015

1K+

In its year-end issue, the journal *Science* chose the CRISPR genome-editing technology invented at UC Berkeley 2015's Breakthrough of the Year.

A runner-up in 2012 and 2013, the technology now revolutionizing genetic research and gene therapy "broke away from the pack, revealing its true power in a series of spectacular achievements," wrote *Science* correspondent John Travis in the Dec. 18 issue. These included "the creation of a long-sought 'gene drive' that

CRISPR a very general technology – it works well wherever it’s been tested

Overview of CRISPR gene edit machinery

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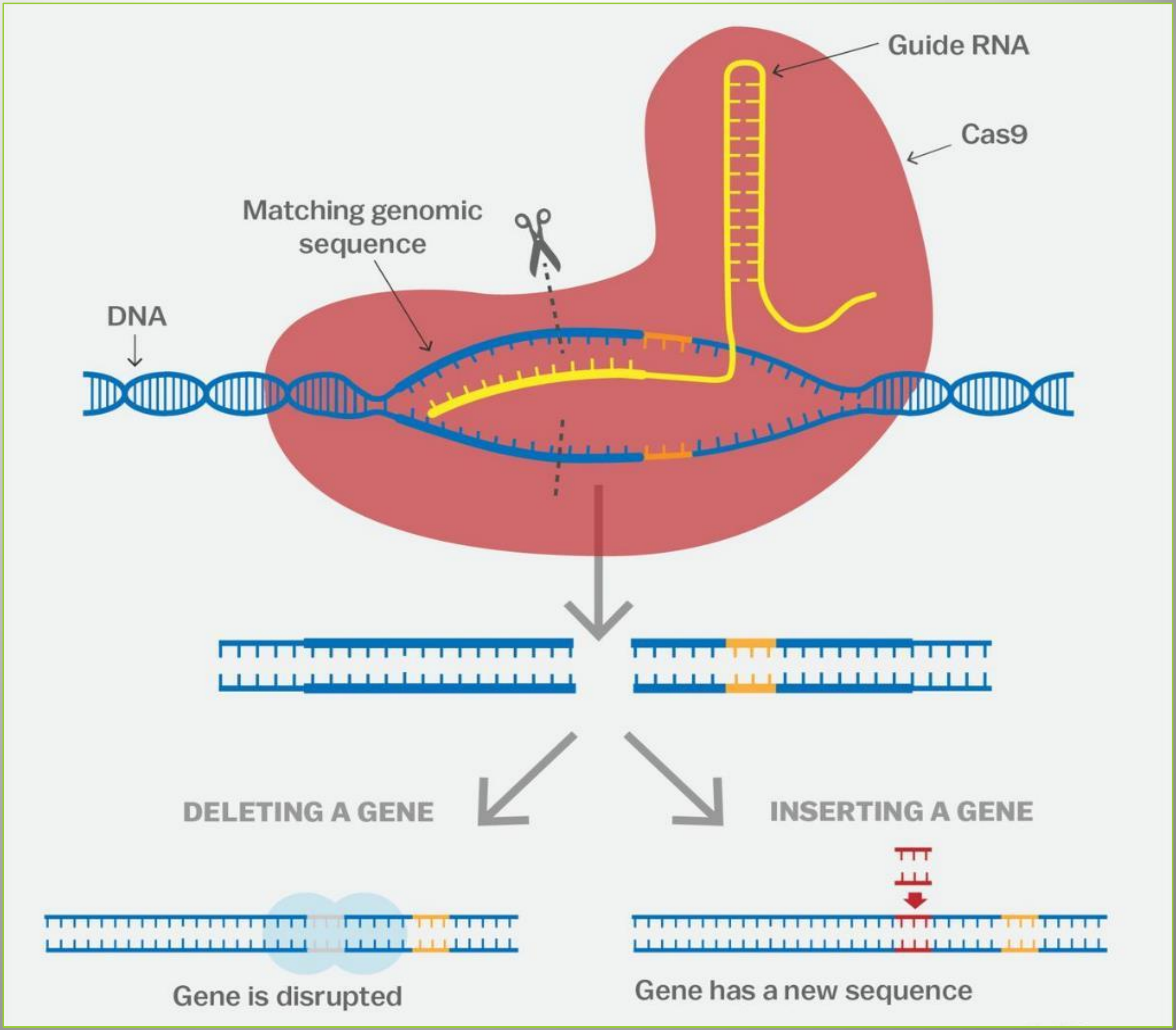
Two parts: Nuclease and guide RNAs to direct it in genome



A big deal for plants and trees?

Ability to modify native genes efficiently makes growing science knowledge of gene trait-relationships actionable

The formerly theoretical becomes practical

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Current Opinion in Biotechnology

Editing plant genomes with CRISPR/Cas9

Khaoula Belhaj[1], Angela Chaparro-Garcia[1], Sophien Kamoun, Nicola J Patron and Vladimir Nekrasov

CrossMark

"CRISPR/Cas9 is a game-changing technology that is poised to revolutionize basic research and plant breeding."

<u>Even more powerful for trees</u>? The long generation time, and inability to inbreed, make specific genome modifications by conventional breeding ~ impossible

Agenda

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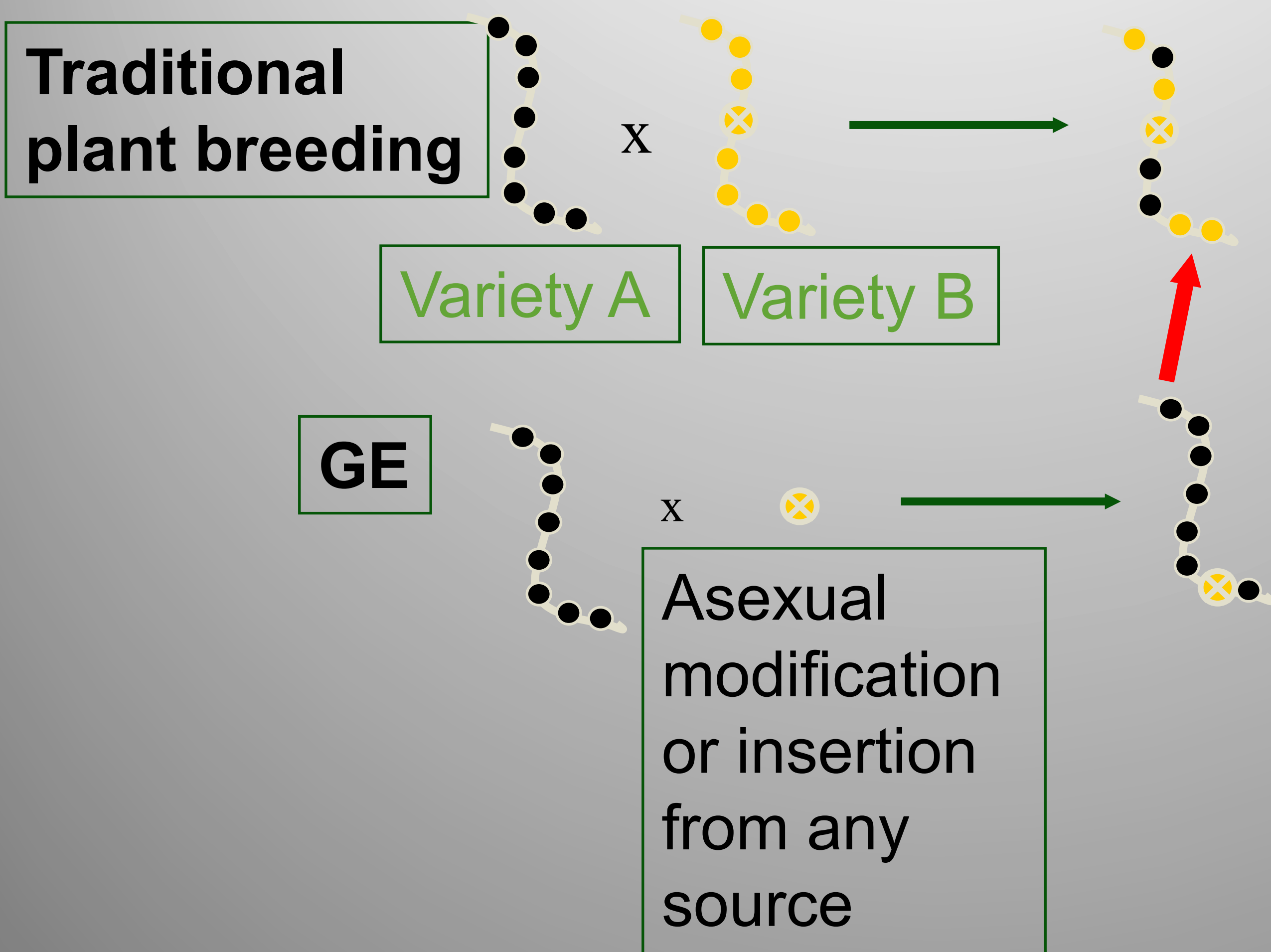
But efficient integration with conventional breeding is critical



Integration with conventional breeding

- In the field
- Many genotypes
- Many local environments
- Many years
- Incomplete containment

But such integration nearly impossible with regulations that presume the method is a hazard until proven innocent

Articles

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

October 2010 / Vol. 60 No. 9 • BioScience 729

Fortunately, some kinds of regulations may be avoided with “clean” GE – but that is difficult in trees and would preclude the many powerful GMO type modifications -- *thus not an answer to the larger problem*

Gene flow regulation and social views a major obstacle for trees

- Wild/feral populations
- Record of invasiveness of many exotic trees/shrubs
- Long distance pollen and/or seed movement
- Limited domestication
- Keystone species / Larger role in providing ecosystem services
- Scientific uncertainty - Introgression experiments costly or impossible to do, models speculative
- Public view of forests as natural or wild: “contamination, impurity”

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

<u>A big deal</u>:
Many of the most highly managed forests and their products are certified

500 million hectares, 13% global forest area

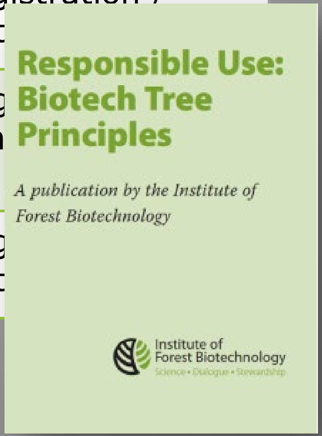
FSC

Started by the Forest Stewardship Council, major principle:

“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

In 2001 and 2015, forest genetic and biotech scientists publicly criticized FSC for their complete ban – no field research on certified lands

…with little effect

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

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

A new strategy in 2019: A petition to certifiers to allow field research

Petition in Support of Forest Biotechnology Research

Petition | Committee of Scientists | Examples of Biotech Trees | Background Literature | FAQ | Pubs-Press

Drone image of an rDNA-modified poplar plantation in the USA

The goal of this petition is to urge forest certification systems to better align their certification criteria with scientific findings in biotechnology.

http://biotechtrees.forestry.oregonstate.edu

Impemented by the Alliance for Science at Cornell University, USA

Endorsed by the largest scientific society of plant biologists in the world

American Society of Plant Biologists

ASPB has studied and endorsed the petition.

members to support a petition to change certification rules for forests to enable field research on biotech (gene edited, genetically engineered) trees. Amazingly, all of the private certification systems have a complete ban in place that extends to research, at a time when forest health is in growing crisis due to expanding pests and climate change. Biotech is not a panacea, but its also too powerful to ignore—and can sometimes provide powerful solutions where other approaches fail.The petition follows the release of a major report on The Potential for Biotechnology to Address Forest Health from the USA National Academy of Sciences that has identified biotechnologies as a key tool for helping to manage forest health and associated pest epidemics.

ASPB has studied and endorsed the petition.

Alerts to tens of thousands of scientists sent by American Association for the Advancement of Science - AAAS (worlds largest scientific organization)

AAAS | Policy Alert

Petition Launched to Change Certification of Biotechnology Forest Research

A committee of forest biotechnologists from around the world, which includes several AAAS honorary fellows, have launched a petition to change certification rules for forests to enable field research on gene-edited and genetically engineered trees. Currently, private certification systems include a ban on research using biotechnology tools in forest research. The petition comes on the heels of a recent report from the National Academies that discusses the importance of biotechnology research to help improve forest health. For additional background, visit the petition website. (**BACK TO THE TOP**)

1,161 signatures, majority PhDs

Support modern forest biotechnology research

May 30 2018 · Cornell Alliance for Science · Closed on Jun 11 2019

1161 Signatures

https://www.gopetition.com/petitions/petition-in-support-of-modern-forest-biotechnology.html

Letter published in Science about it

Certification for gene-edited forests

Forest certification bodies were estab-
lished to provide consumers with
confidence that they are purchasing
sourced wood products. Over
hectares of forests, or about
l forest area, are certified
rgest certification systems
ver, certification bodies have
excluded all genetically
or gene-edited (GE) trees from
, including from field research
lands that is essential for
ng local benefits and impacts
ing forest biotechnology
om around the world, with
of more than 1000 globally
atories to a recent detailed
call for all forest certification
romptly examine and modify
es.
ce mounting stresses posed
pests and climate change (*6*).

Engineering, and Medicine recently completed an in-depth study on forest health and biotechnology, concluding that the potential benefits are numerous and rapidly increasing (*12*). Our forests are in dire need of assistance, and GE trees hold tremendous potential as a safe and powerful tool for promoting forest resilience and sustainability.

Steven H. Strauss[1]*, Wout Boerjan[2], Vincent Chiang[3], Adam Costanza[4], Heather Coleman[5], John M. Davis[6], Meng-Zhu Lu[7], Shawn D. Mansfield[8], Scott Merkle[9], Alexander Myburg[10], Ove Nilsson[11], Gilles Pilate[12], William Powell[13], Armand Seguin[14], Sofia Valenzuela[15]

[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA. [2]Department of Plant Biotechnology and Bioinformatics, Ghent University and Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium. [3]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA. [4]Chapel Hill, NC 27517, USA. [5]Department of Biology, Syracuse University, Syracuse, NY 13244, USA. [6]School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA. [7]State Key Laboratory of Subtropical Silviculture, School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou 311300, China. [8]Forest Sciences Centre, University

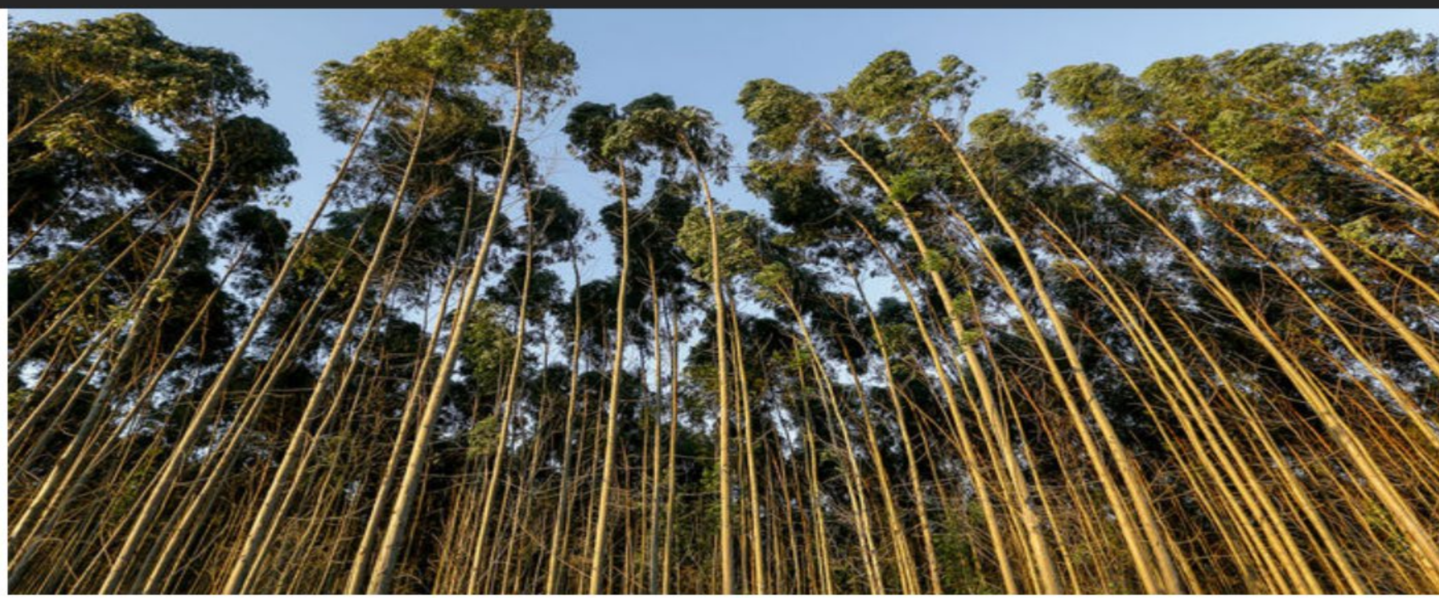
standard-pefc-st-2002-2013.

Gene-edited and genetically engineered trees, such as these poplars, should be allowed in certified forests.

News feature also there

AAAS Become a Member

Science Contents News Careers Journals



Productivity of eucalyptus plantations could be increased with trees genetically modified for faster growth.
CASADAPHOTO/SHUTTERSTOCK.COM

Scientists say sustainable forestry organizations should lift ban on biotech trees

By Erik Stokstad | Aug. 23, 2019 , 5:45 PM

Forest health a major and growing concern – we need biotech tools

REVIEW

Planted forest health: The need for a global strategy

B. D. Wingfield,[1] B. Slippers[1]

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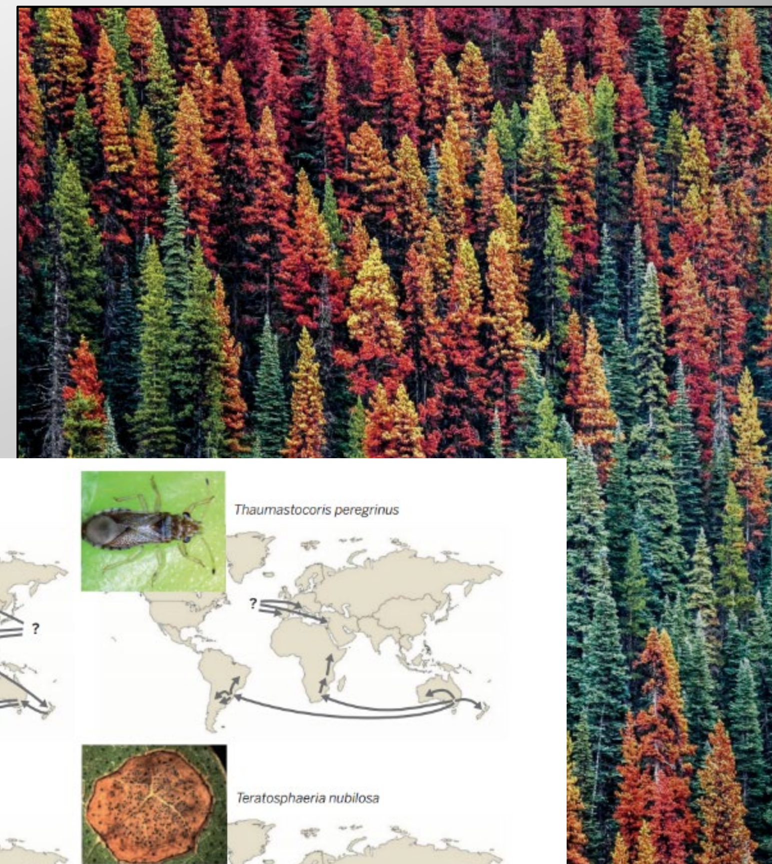

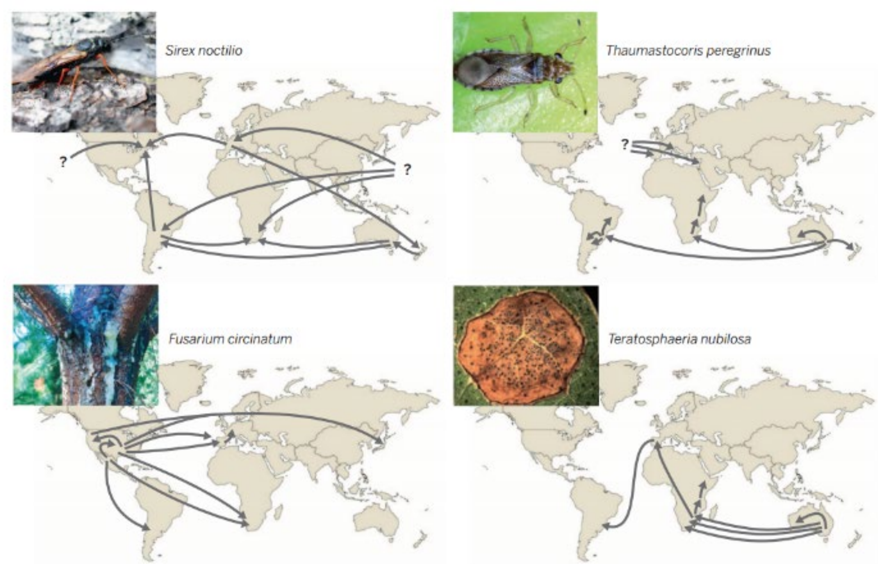

Fig. 2. Examples of invasion routes of pests of planted forests that illustrate an apparently common pattern of complex pathways of spread to new environments, including repeated introductions and with either native or invasive populations serving as source populations (18). Invasion routes of the pine pitch canker pathogen *Fusarium circinatum* (origin in Central America) (39), eucalypt leaf pathogen *Teratosphaeria nubilosa* (origin in southeast Australia) (40), the pine woodwasp *Sirex noctilio* (origin in Eurasia) (23), and the eucalypt bug *Thaumastocoris peregrinus* (origin in southeast Australia) (41) were determined through historical and genetic data. [Photo credits: (top left) Brett Hurley; (top right) Samantha Bush; (bottom left) Jolanda Roux; (bottom right) Guillermo Perez]

GE trees: Reliable in the field

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

Many studies done -- show value and promise for many traits (2018 review)

In Vitro Cellular & Developmental Biology - Plant (2018) 54:341–376
https://doi.org/10.1007/s11627-018-9914-1

INVITED REVIEW

CrossMark

Genetic engineering of trees: progress and new horizons

Shujun Chang[1] · Elizabeth L. Mahon[2] · Heather A. MacKay[2] · William H. Rottmann[3] · Steven H. Strauss[4] · Paula M. Pijut[5] · William A. Powell[6] · Vernon Coffey[6] · Haiwei Lu[4] · Shawn D. Mansfield[2] · Todd J. Jones[1]

Received: 5 February 2018 / Accepted: 20 June 2018 / Editor: Marie-Anne Lelu-Walter / Published online: 5 July 2018

Abstract
Genetic engineering of trees to improve productivity, wood quality, and resistance to biotic and abiotic stresses has been the primary goal of the forest biotechnology community for decades. We review the extensive progress in these areas and their current status with respect to commercial applications. Examples include novel methods for lignin modification, solutions for long-standing problems related to pathogen resistance, modifications to flowering onset and fertility, and drought and freeze tolerance. There have been numerous successful greenhouse and field demonstrations of genetically engineered trees, but commercial application has been severely limited by social and technical considerations. Key social factors are costly and uncertain regulatory hurdles and sweeping market barriers in the form of forest certification systems that disallow genetically modified trees. These factors limit and, in many cases, preclude field research and commercial adoption. Another challenge is the high cost and uncertainty in transformation efficiency that is needed to apply genetic engineering and gene editing methods to most species and genotypes of commercial importance. Recent advances in developmental gene-based transformation systems and gene editing, if combined with regulatory and certification system reform, could provide the foundation for genetic engineering to become a significant tool for coping with the increasing environmental and biological stresses on planted and wild forests.

Key petition arguments

- Forest health crises growing, need biotech tools to help
- Extensive research and field trials show promise and safety for many kinds of traits
- Gene editing of natural genes more precise than conventional breeding
- Local, site specific research as part of breeding programs are needed to understand value, economics
- The ban contradicts scientific opinion that the trait, not the method, is of significance
- Details here: http://biotechtrees.forestry.oregonstate.edu/

What next?

- The petition one part of larger efforts by companies to gain access to biotech while under certification
- The stigma, poor reputation of GMOs to many in public a key barrier
- Is this a good model for scientific advocacy for primacy of science in business and policy decisions ?
- What else or what next?
- What can China and Chinese scientists do?

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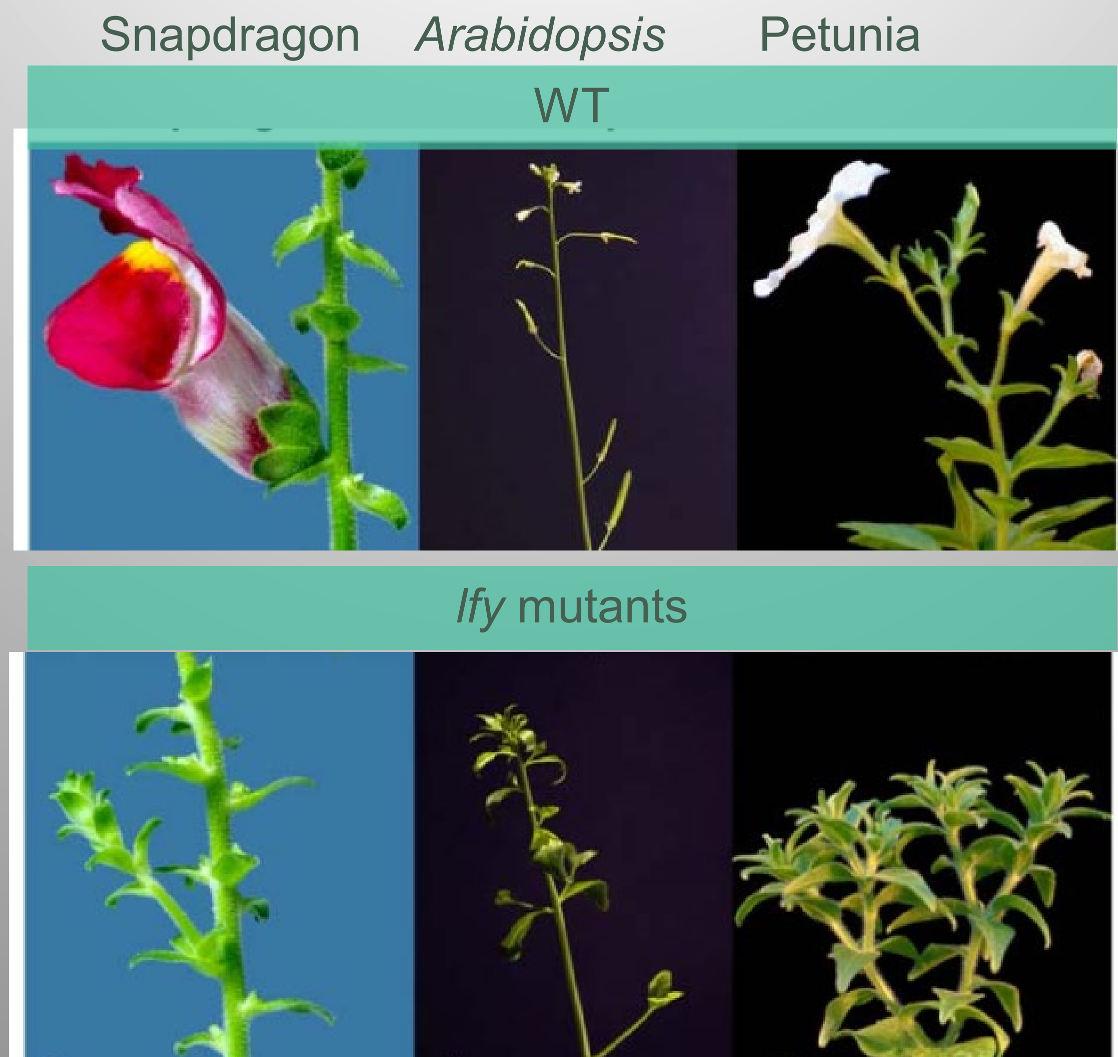
Gene flow: A major reason for strict regulation and market barriers to GMOs

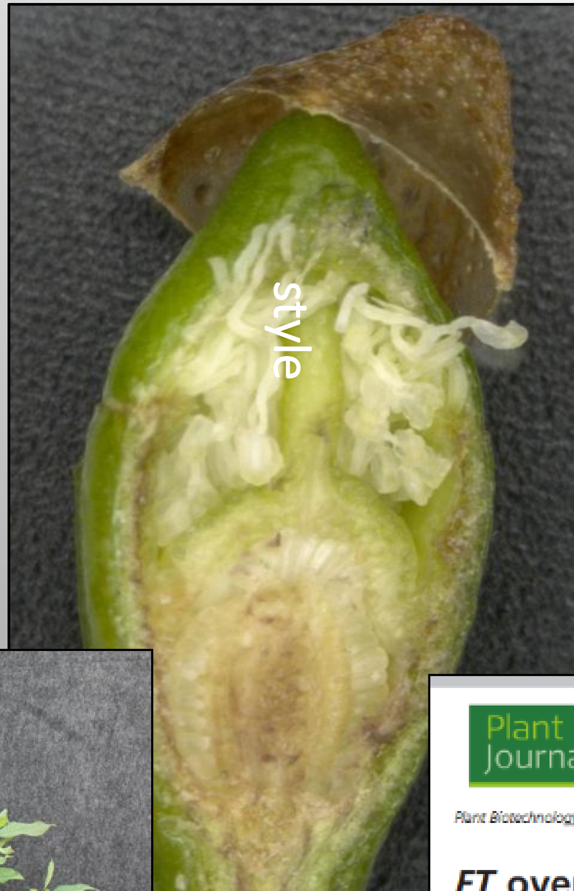
- Bigger for forest trees than most ag crops – for many reasons
  - Wild/feral populations
  - Record of invasiveness of many exotic trees/shrubs
  - Keystone roles in ecosystems
  - Long distance pollen and/or seed movement
  - Limited domestication
  - Larger role in providing ecosystem services
  - Public view of forests as natural or wild
  - Scientific uncertainty - Introgression experiments costly or impossible to do, models speculative
- Gene flow prevention an essential tool, especially for more novel and high impact GMOs?
- A major concern with eucalypts in the USA today

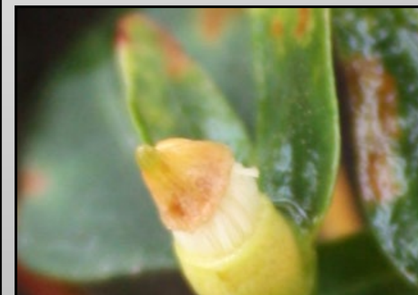
LEAFY gene target for sterility: Strong mutants appear to have no flowers

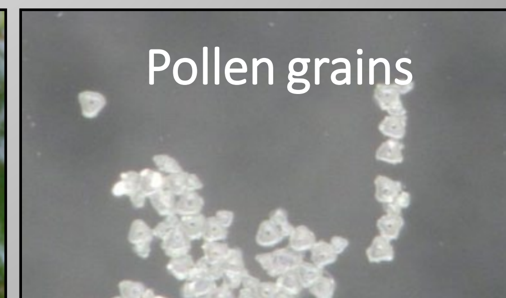


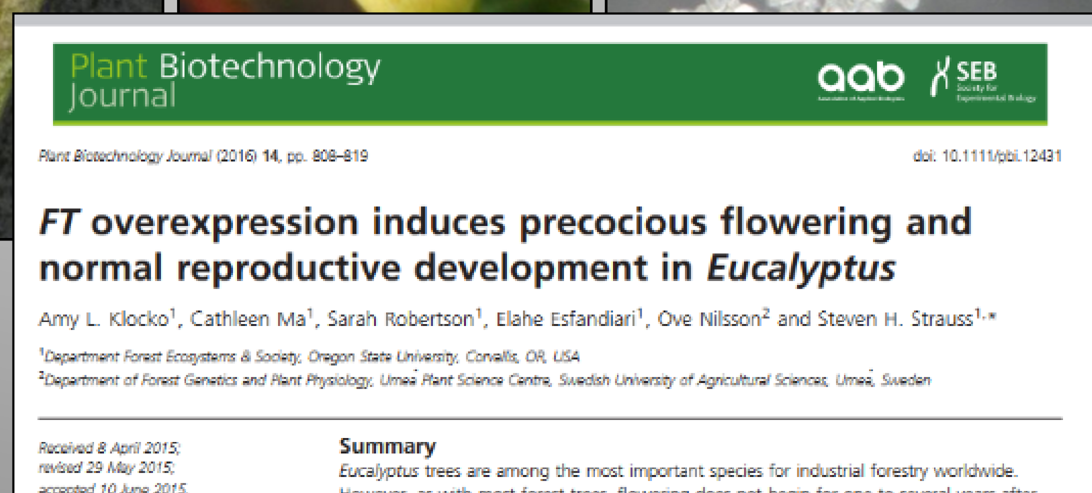
Parcy et al. 2002, Moyroud et al. 2010

Early flowering FT-eucalypts to speed phenotyping








Plant Biotechnology Journal

Plant Biotechnology Journal (2016) 14, pp. 808–819 doi: 10.1111/pbi.12431

FT overexpression induces precocious flowering and normal reproductive development in *Eucalyptus*

Amy L. Klocko[1], Cathleen Ma[1], Sarah Robertson[1], Elahe Esfandiari[1], Ove Nilsson[2] and Steven H. Strauss[1,*]

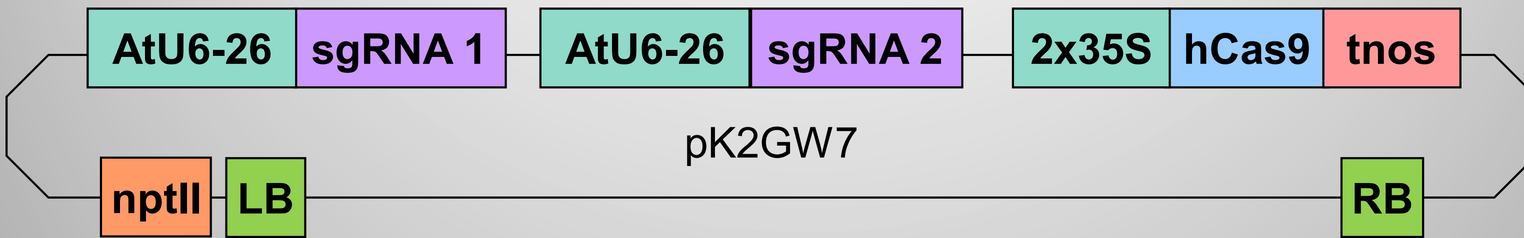
[1]Department Forest Ecosystems & Society, Oregon State University, Corvallis, OR, USA
[2]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden

Received 8 April 2015;
revised 29 May 2015;
accepted 10 June 2015.

Summary
Eucalyptus trees are among the most important species for industrial forestry worldwide. However, as with most forest trees, flowering does not begin for one to several years after

Constructs employed: Two targets in the *LEAFY* gene

CRISPR



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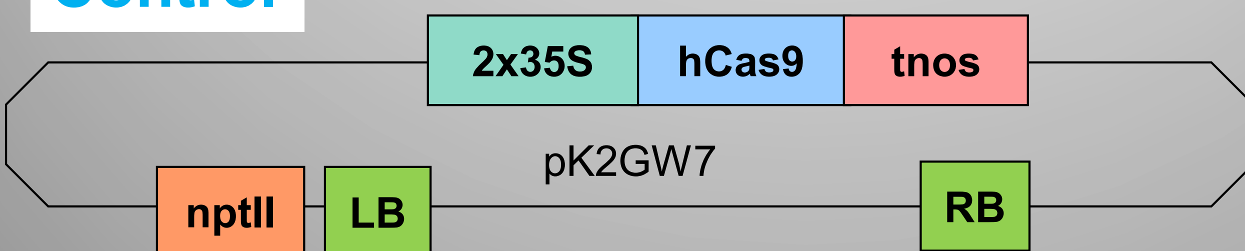
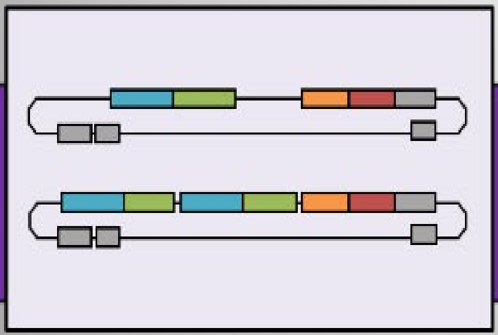
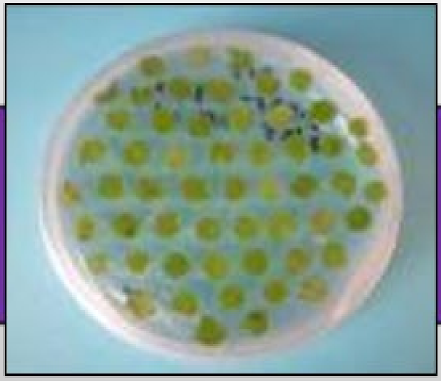


ORIGINAL RESEARCH ARTICLE
Front. Plant Sci., 07 May 2018 | https://doi.org/10.3389/fpls.2018.00594

Variation in Mutation Spectra Among CRISPR/Cas9 Mutagenized Poplars

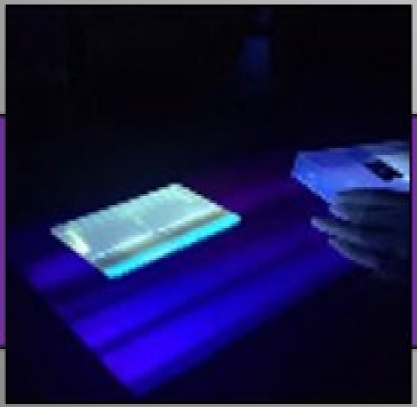
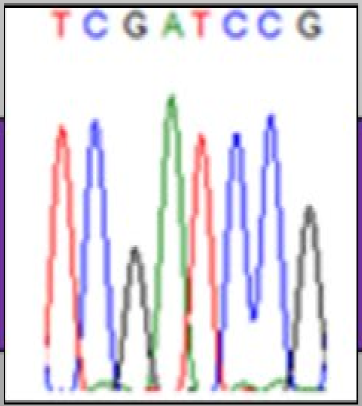
Estefania Elorriaga[1], Amy L. Klocko[2], Cathleen Ma[1] and Steven H. Strauss[1*]
[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR, United States
[2]Department of Biology, University of Colorado Colorado Springs, Colorado Springs, CO, United States

CRISPR pipeline


Construct

Transformation and regeneration

PCR and gel analysis

Sequencing of targets, alignment, and phenotyping

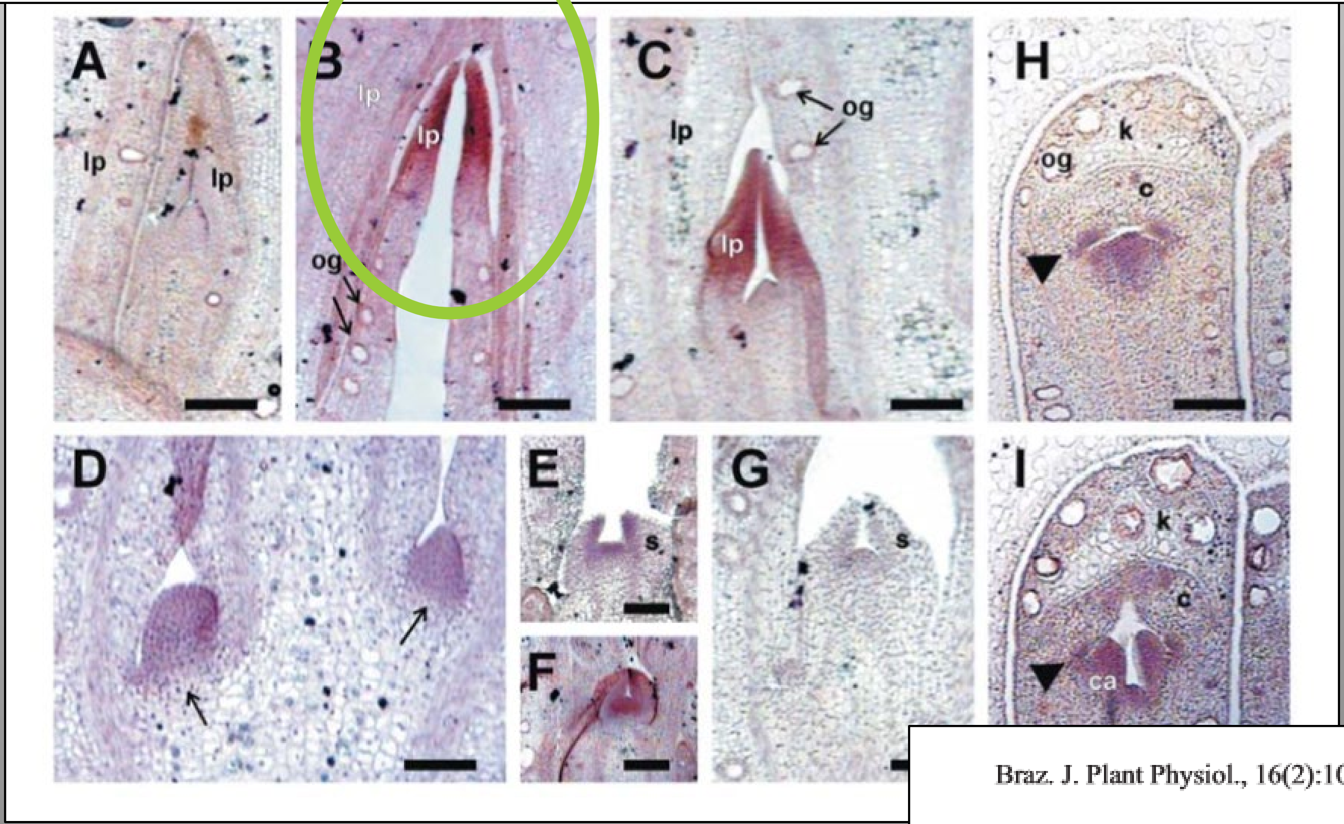
The full roles of *LFY* unknown

- Discovery studies did not have significant analysis of vegetative/productivity effects
  - An absence of studies of gene mutation/knock-out in the field
- No studies in the very divergent floral types of important forest tree taxa
  - Often parts of gene families
- Found to have vegetative as well as floral expression
  - Meristematic vegetative cell expression

Eucalyptus *LFY* vegetative expression

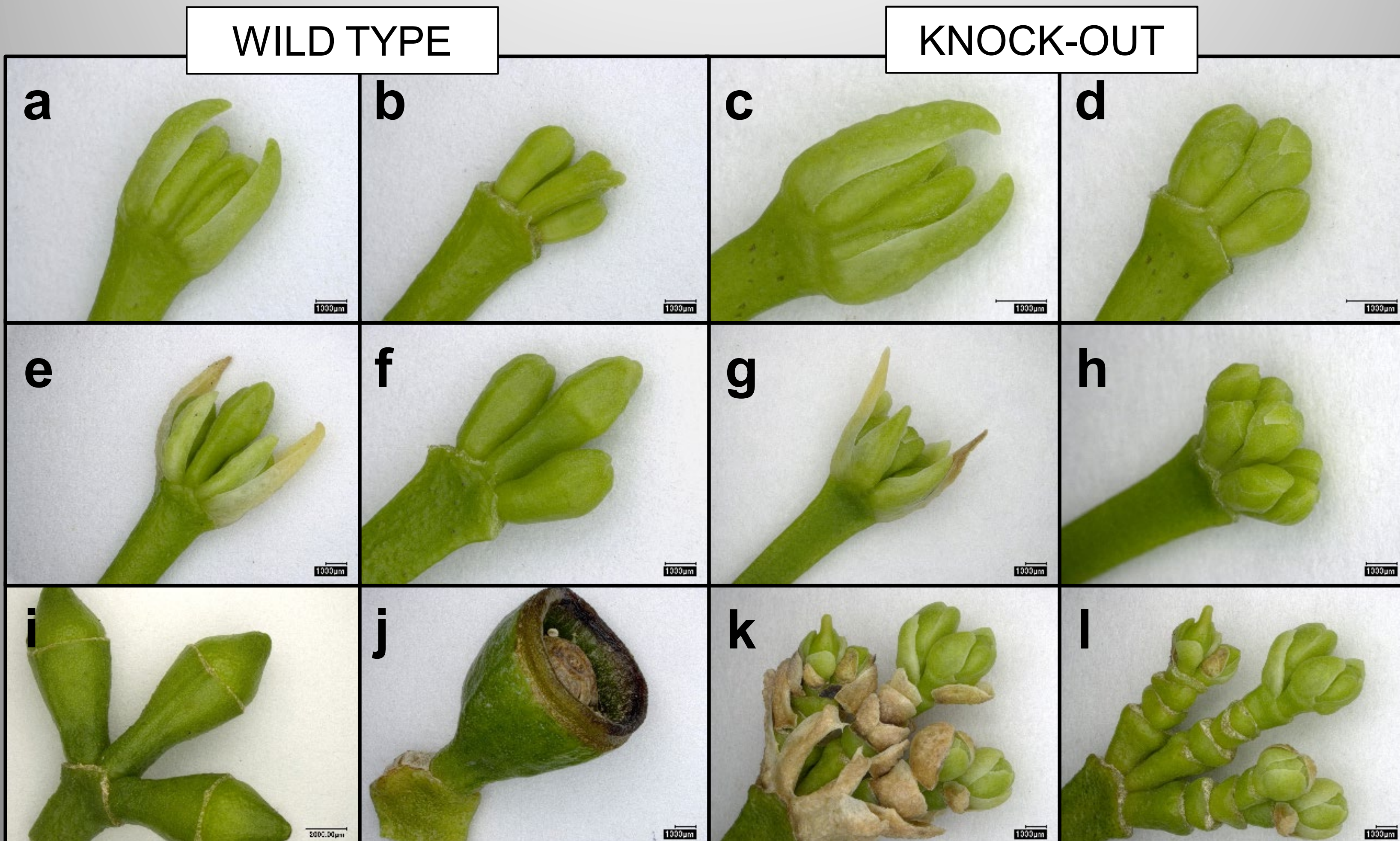
***EgLFY*, the *Eucalyptus grandis* homolog of the *Arabidopsis* gene *LEAFY* is expressed in reproductive and vegetative tissues**

Marcelo Carnier Dornelas[1*], Weber A. Neves do Amaral[2] and Adriana Pinheiro Martinelli Rodriguez[1]

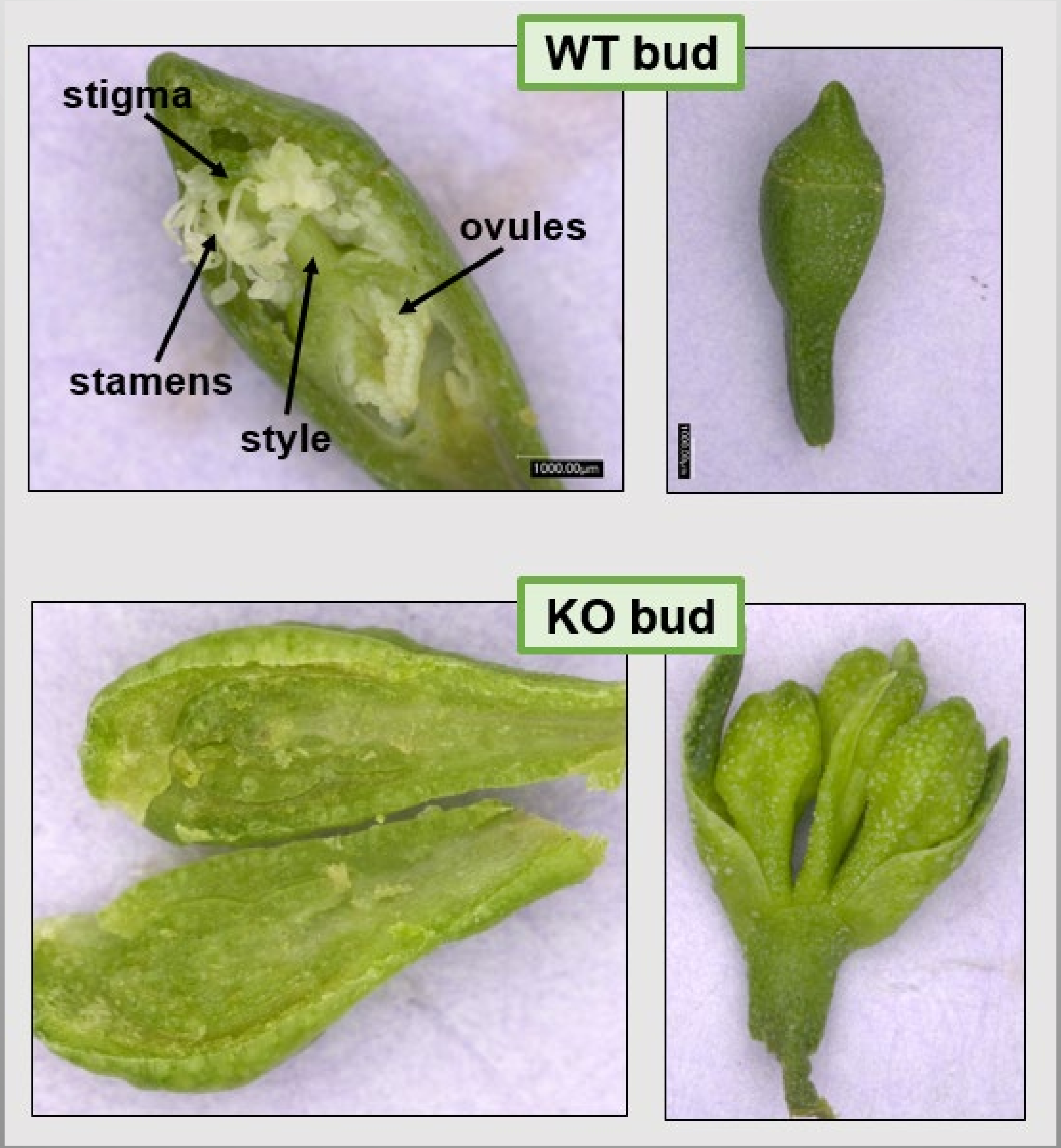


Braz. J. Plant Physiol., 16(2):105-114, 2004

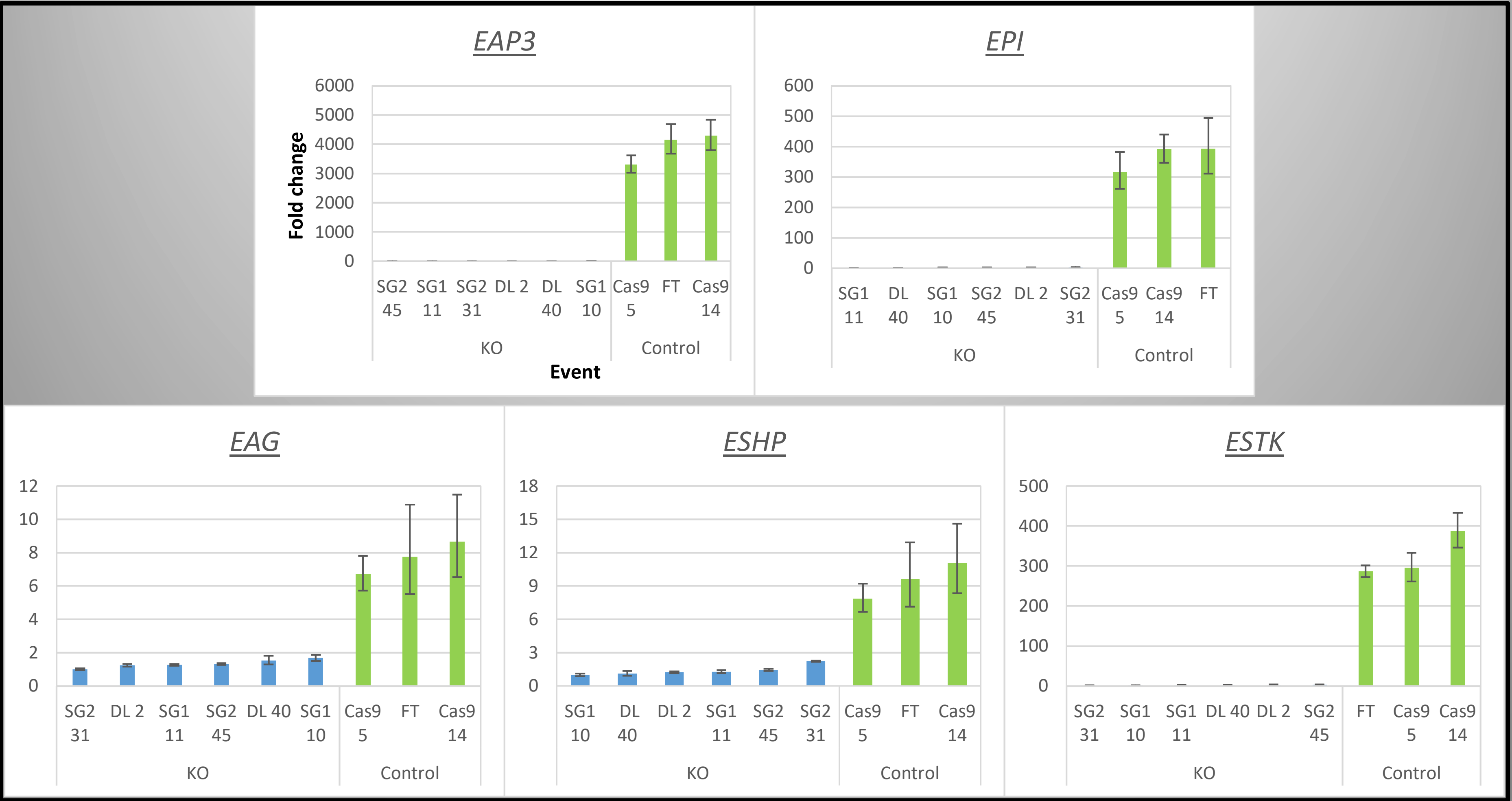
Distinct trajectories for wild type vs. CRISPR knock-out floral shoots



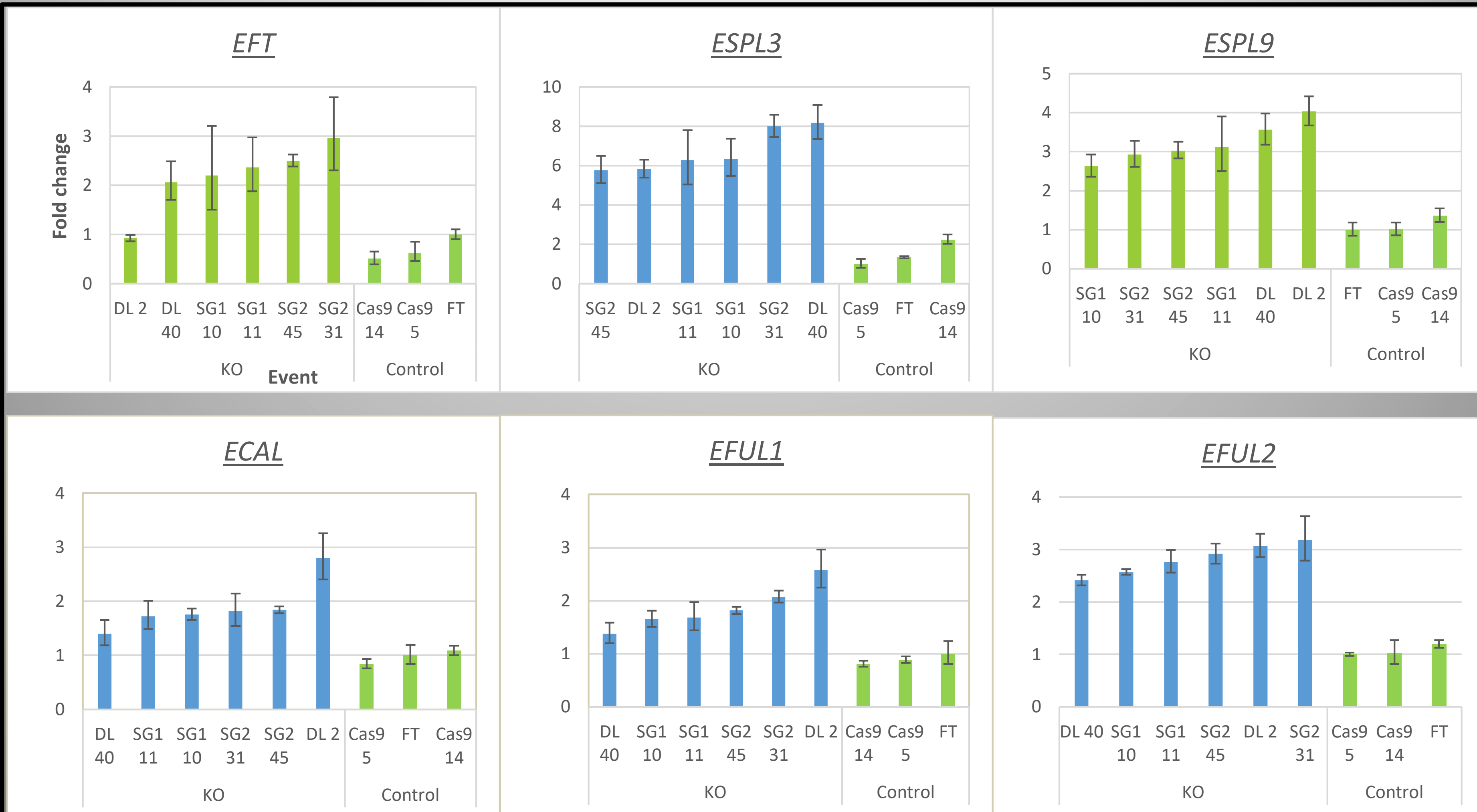
Knockout buds devoid of floral organs



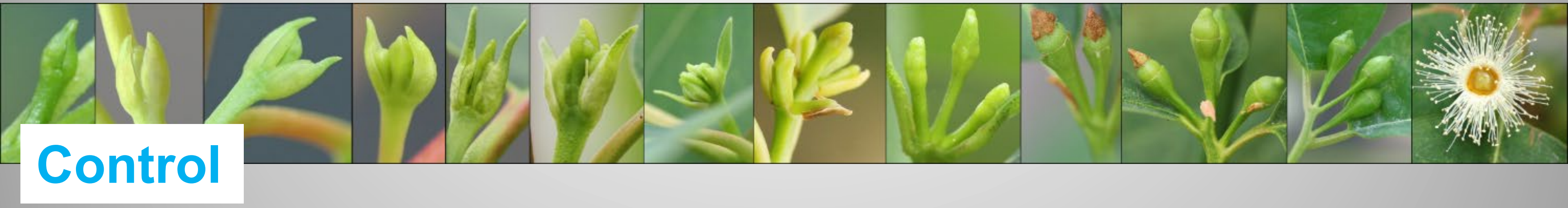
Knock-outs nearly devoid of floral meristem gene expression



But knock-outs with enhanced floral induction gene expression

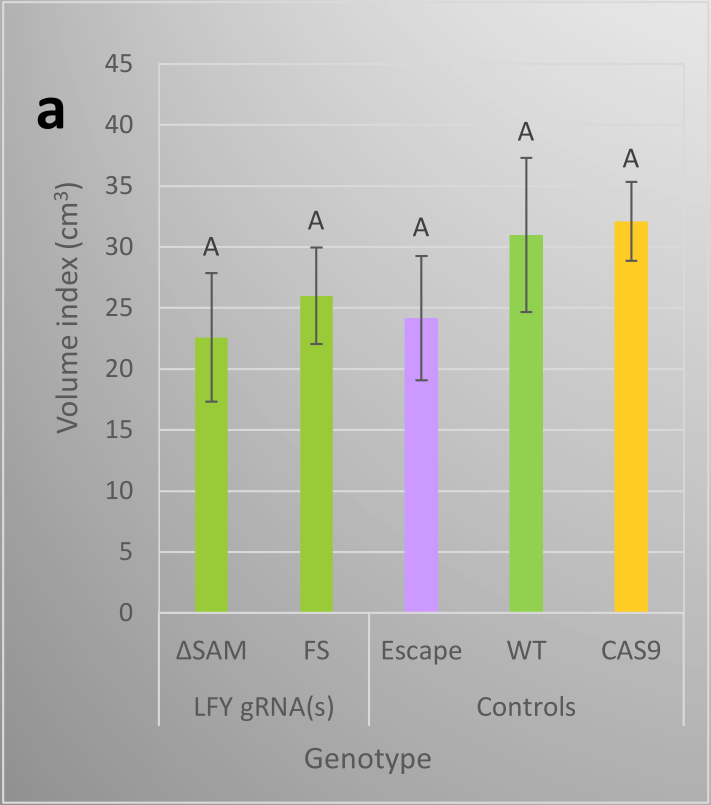


Summary view of floral shoot development in knockouts vs. wild-type



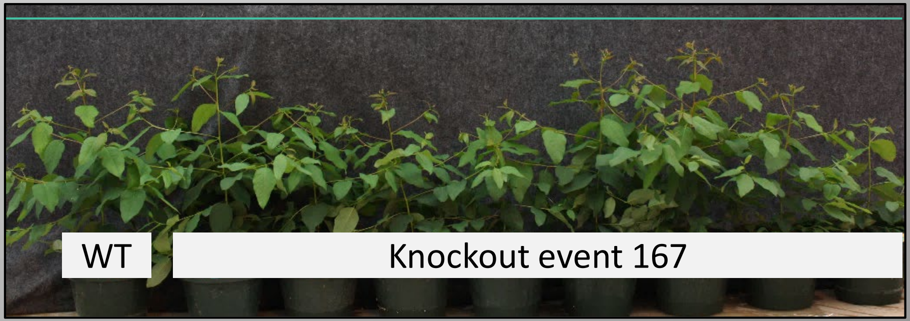
Vegetative growth and morphology in greenhouse unaffected by knock-out

a



b

c



Summary – *LFY* CRISPR in Eucalyptus

- Nearly 100% biallelic knockout rate
- Flower buds devoid of reproductive structures
- Partially indeterminate inflorescences
- No detectable vegetative effects
- Work underway to develop CRISPR excision and other methods for “clean” knock-outs

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