Genetic engineering of coexistence: Containment technology to enable transgenic innovations in trees

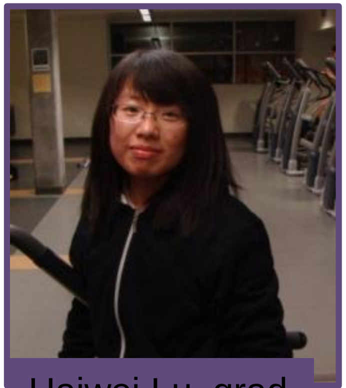
Steve Strauss, Professor
College of Forestry, Oregon State University
Steve.Strauss@OregonState.Edu

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

Cathleen Ma, transformation & greenhouse experiments

Kori Ault, program & field manager

Michael Dow, former post-doc, constructs



Haiwei Lu, grad student, ZFNs

Sarah Robertson, heat induction and flowering

Chad Washington, undergrad, field trials

USDA

NSF

The National
Science Foundation

United States
Department of
Agriculture

National Institute
of Food and
Agriculture

TBGRC

Sappi, Arborgen, Futuragene
Swetree, U. Pretoria

Advanced **Hardwood Biofuels** Northwest

Roadmap

- Why genetics and biotech matters for trees
- Why gene flow is an immense problem needing technical as well as policy solutions
- Evolving technology options
- Obstacles and progress in making them work for trees

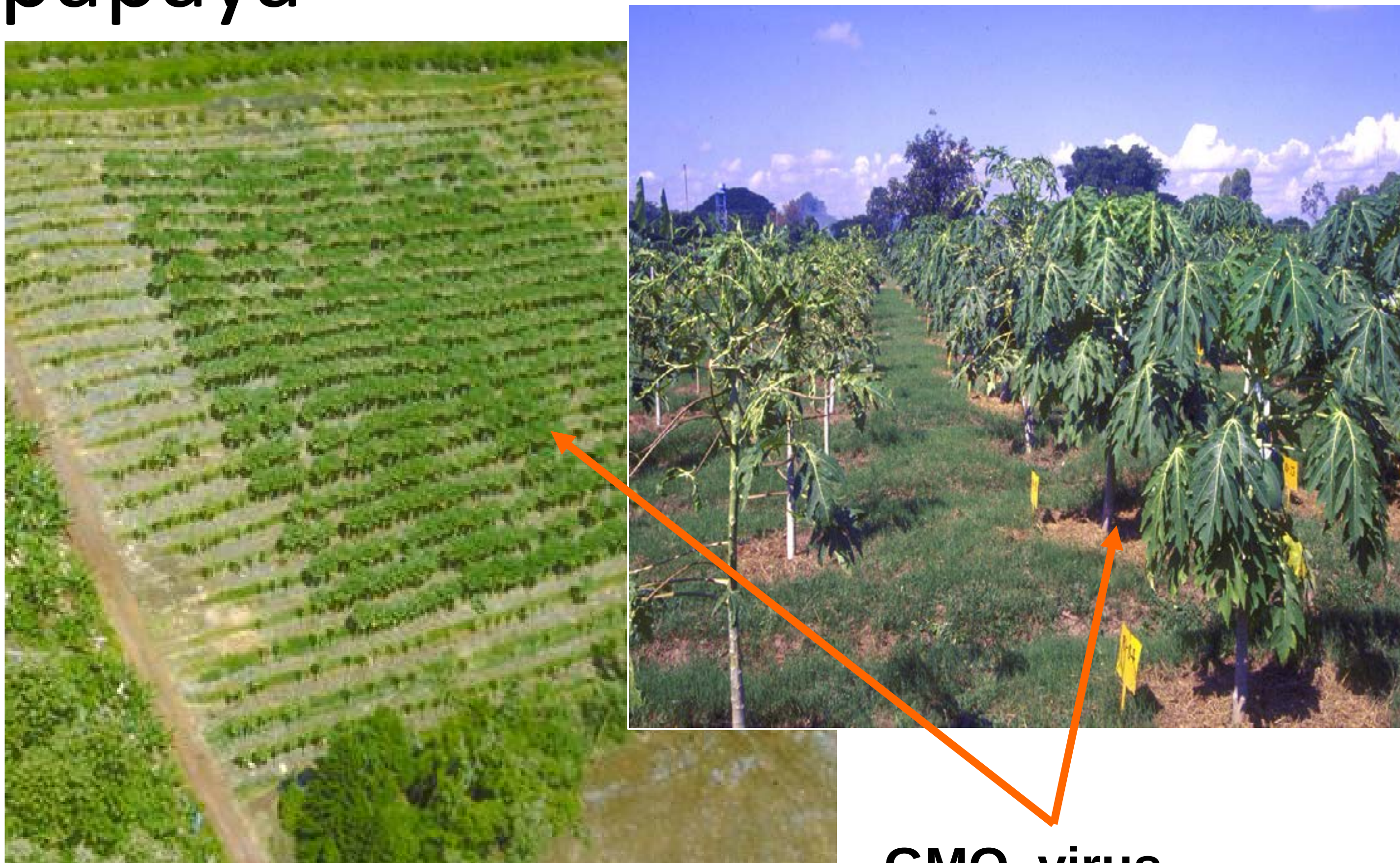
One generation of breeding Monterey pine (*Pinus radiata*) in New Zealand made striking changes in growth & form - enabling an industry

Hybridization and cloning enabled the poplar industry in the northwest USA

Exotics, hybridization, and cloning created the extraordinary eucalypt industry in Brazil

GMOs are doing it too: 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

Defensin-like proteins from spinach a promising solution to citrus greening

Courtesy of Eric Mirkov, Texas A & M

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 elm

American Chestnut most advanced case

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SCIENTIFIC AMERICAN
The New Century of the Brain
See Inside

The American Chestnut's Genetic Rebirth

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

By William Powell

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—

More In This Article

A New Generation of American Chestnut Trees May Redefine America's Forests

March 2014 issue
Scientific American

Courtesy of Bill Powell, SUNY Syracuse, USA

Modification of intact varieties is powerful for trees

Non-browning "Arctic Apple" -- Suppression of native polyphenol oxidase gene expression

Courtesy of Jennifer Armen, Okanagan Specialty Fruits, Canada

Insect resistant poplars commercially approved in China ~10 years ago - Bt *cry1*

- Trait stable
- Helps to protect non-Bt trees
- Reduced insecticide use
- Improved growth rate

Growth rate benefits substantial for Bt-poplars (cry3a) – >>10-20%

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

Abstract: The stability and value of transgenic pest resistance for promoting tree growth are poorly understood. These data are essential for determining if such trees could be beneficial to commercial growers in the face of substantial regulatory and marketing costs. We investigated growth and insect resistance in hybrid poplar expressing the *cry3Aa* transgene in two field trials. An initial screening of 502 trees comprising 51 transgenic gene insertion events in four clonal backgrounds (*Populus trichocarpa* × *Populus deltoides*, clones 24-305, 50-197, and 198-434; and *P. deltoides* × *Populus nigra*, clone OP-367) resulted in transgenic trees with greatly reduced insect damage. A large-scale study of 402 trees from nine insertion events in clone OP-367, conducted over two growing seasons, demonstrated reduced tree damage and significantly increased volume growth (mean 14%). Quantification of Cry3Aa protein indicated high levels of expression, which continued after 14 years of annual or biannual coppice in a clone bank. With integrated management, the *cry3Aa* gene appears to be a highly effective tool for protecting against leaf beetle damage and improving yields from poplar plantations.

Résumé : La stabilité et la valeur de la résistance transgénique aux ravageurs pour favoriser la croissance des arbres ne sont pas bien connues. Ces données sont essentielles si l'on veut déterminer dans quelle mesure de tels arbres transgéniques pourraient être profitables pour des producteurs commerciaux considérant les coûts substantiels reliés à la réglementation et la mise en marché de tels arbres. Les auteurs ont étudié la croissance et la résistance aux insectes de peupliers hybrides exprimant le

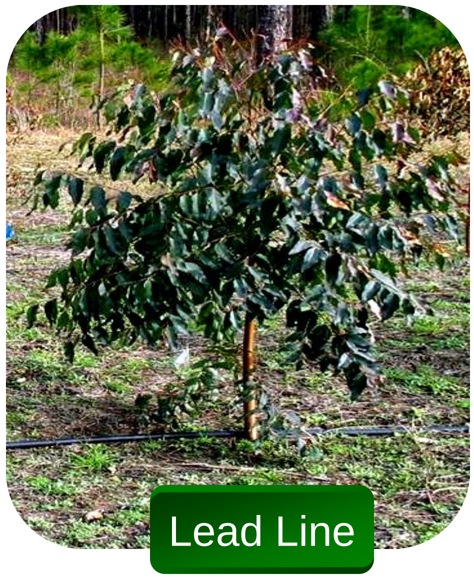
Can. J. For. Res. 44: 28–35 (2014) dx.doi.org/10.1139/cjfr-2013-0270

Published at www.nrcresearchpress.com/cjfr on 28 October 2013.

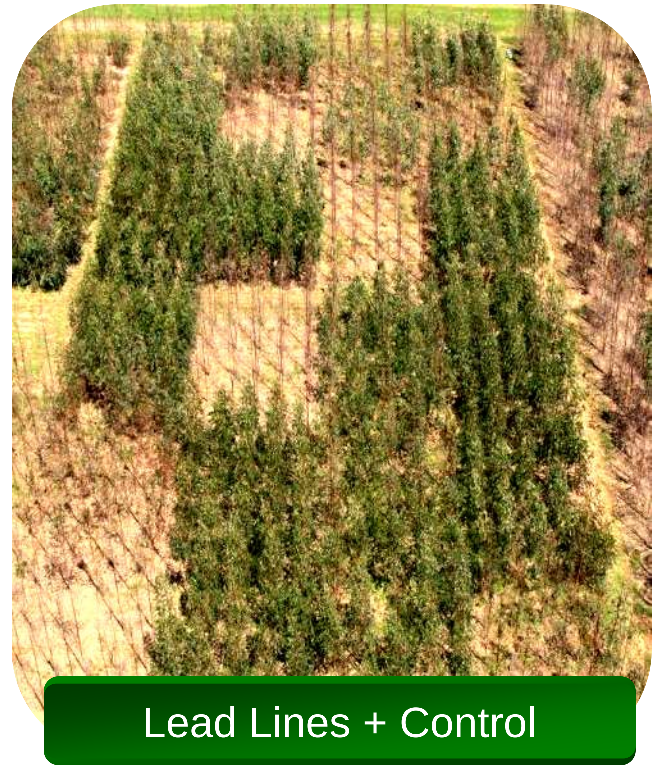
Freeze-tolerant *Eucalyptus*

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

Lignin-modified trees – improved ethanol or pulp yields

PNAS PNAS PNAS PNAS PNAS

Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase

Rebecca Van Acker[a,b], Jean-Charles Leplé[c], Dirk Aerts[d], Véronique Storme[a,b], Geert Goeminne[a,b], Bart Ivens[a,b], Frédéric Légée[e], Catherine Lapierre[e], Kathleen Piens[f], Marc C. E. Van Montagu[a,b,1], Nicholas Santoro[g], Clifton E. Foster[g], John Ralph[h], Wim Soetaert[d], Gilles Pilate[c], and Wout Boerjan[a,b,1]

[a]Department o … versity, 9052 Ghent,
Belgium; [c]Instit … orestières, 45075
Orléans, France … Pierre Bourgin, Unité
Mixte de Reche … ry and Microbiology,
Ghent Universi … st Lansing, MI 48824;
and [h]Departme … eat Lakes Bioenergy
Research Cente

Contributed by

Lignin is one
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ture. Wood
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that *CCR* do
improve bio
and the yield penalty can be overcome.

bioethanol | GM | second-generation bioenergy

Global warming and the depletion of fossil fuels provide a major impetus for the increased interest in renewable energy sources. Liquid biofuels, bioethanol in particular, are currently produced from the freely accessible sucrose in sugarcane

tively (5–7). Cin-
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hydroxycinnamoyl-
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egulation of *CCR*
-13). *CCR*–down-
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ng the conversion
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ocess of obtaining
essential step in
in the laboratory
because green-
house-derived data cannot a priori be extrapolated to field-grown trees without experimentation. For example, greenhouse-grown trees do not experience the annual cycles of growth and

PLANT BIOLOGY

Significance

In the transition from a fossil-based to a bio-based economy, bioethanol will be generated from the lignocellulosic biomass

Chemical feedstocks and biofuel sources?

The Seattle Times
Winner of Nine Pulitzer Prizes

Local News

Originally published Sunday, February 9, 2014 at 9:10 PM

Rose scent in poplar trees? WSU turns to genetic engineering

A WSU team aims to turn poplars and other fast-growing trees into living factories that churn out valuable chemicals.

By Sandi Doughton

Seattle Times science reporter

Sniff the air around Norman Lewis' experimental poplars, and you won't pick up the scent of roses.

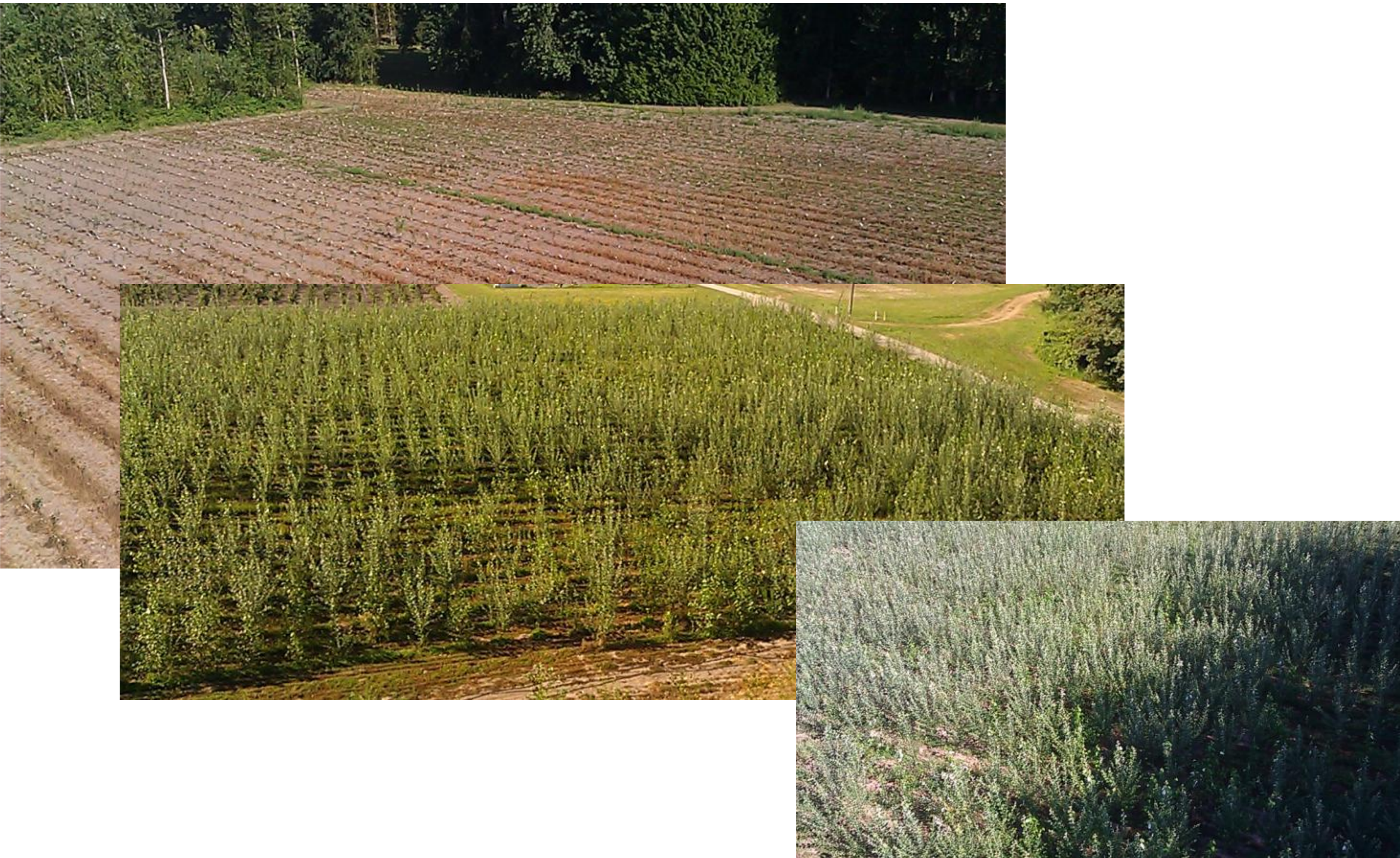
But inside the saplings' leaves and stems, cells are hard at work producing the chemical called 2-phenylethanol— which by any other name would smell as sweet.

Sweeter still is the fact that perfume and cosmetics companies will pay as much as $30 an ounce for the compound that gives roses their characteristic aroma. Because what Lewis and his colleagues at Washington State University are really chasing is the smell of money.

Production of 2-phenylethanol

Lignin reduction

Large scale field trials of a variety of genes and insertions underway



Conventional genetics has made a huge difference in forestry and agricultural production from trees – there is every indication that GMOs could have as large or a larger impact on production and sustainability if allowed to by society

Roadmap

- Why genetics and biotech matters for trees
- **Why gene flow is an immense problem needing technical as well as policy solutions**
- Evolving technology options
- Obstacles and progress in making them work for trees

Gene flow is ubiquitous in agriculture – with or without GMOs – pollen, seed, and vegetative

Slides courtesy of Wayne Parrott, Univ. Georgia

Gene flow greater for many trees

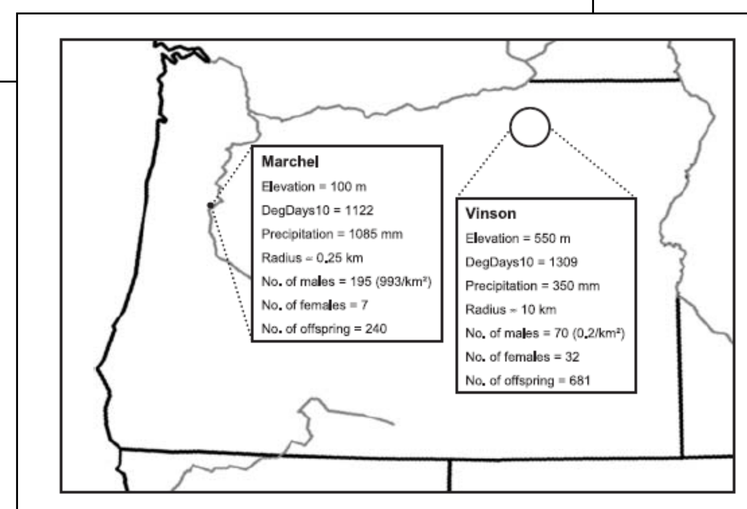
Molecular Ecology (2009) **18**, 357–373

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*



In poplar, ~50% of pollen comes from >1 km to >10 km

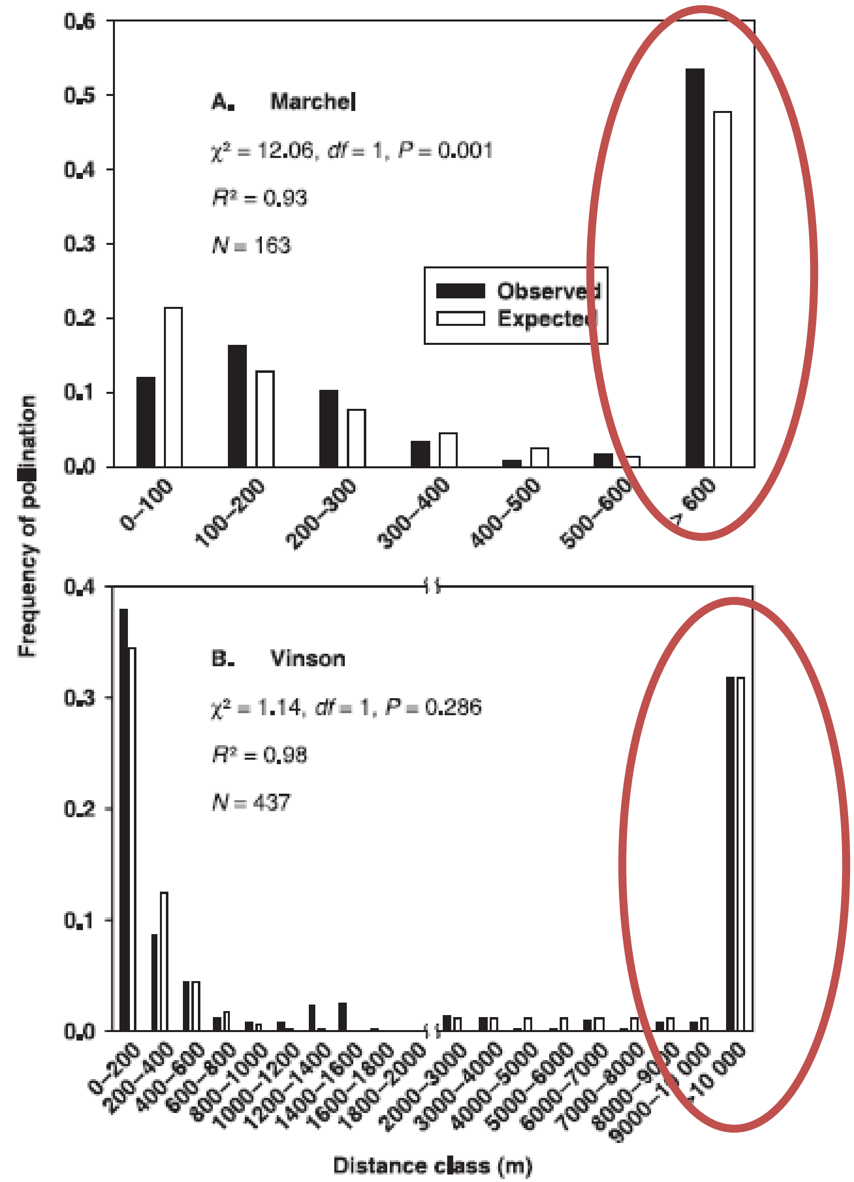


Fig. 5 Observed vs. expected pollination frequencies based on predictions from a mixed probability density function, whose parameters were estimated based on paternity analysis data.

Cottonwood seeds can fly and float far

Big wind and big storms can move seed far

Tree gene flow extensive

- Distance – large, often wind pollinated
- Less domesticated than many crops – establishment in wild possible
- Entry into wild lands
  - Often keystone species – ecologically dominant so with potential effects on many other organisms
- Regulatory approval challenging
  - Difficult to estimate effects, fitness during contained field studies (if possible to do at all)

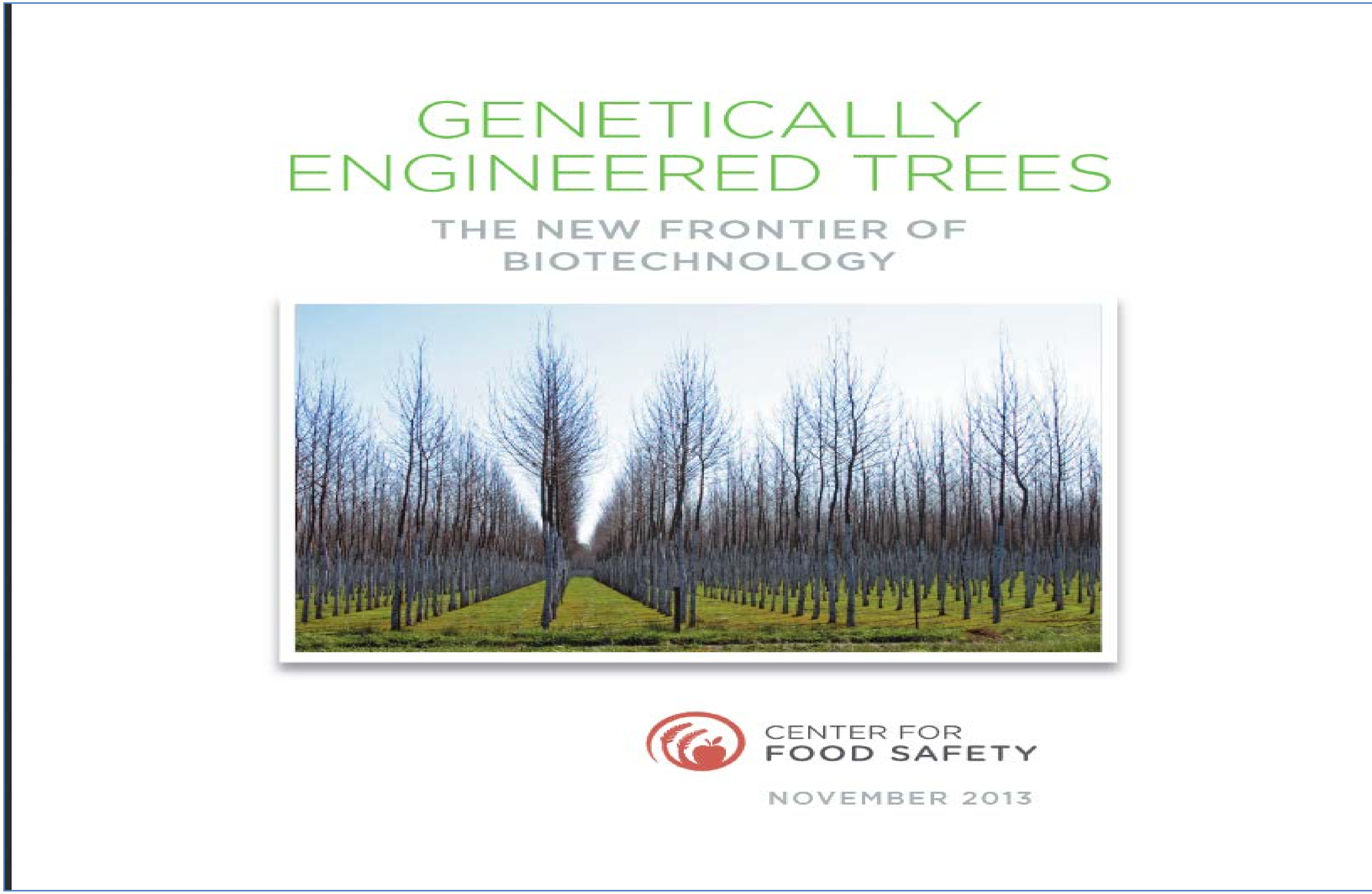
Coexistence is especially hard when ideologies conflict

Forest trees with their own anti-GMO activism

Genetically modified arboriculture

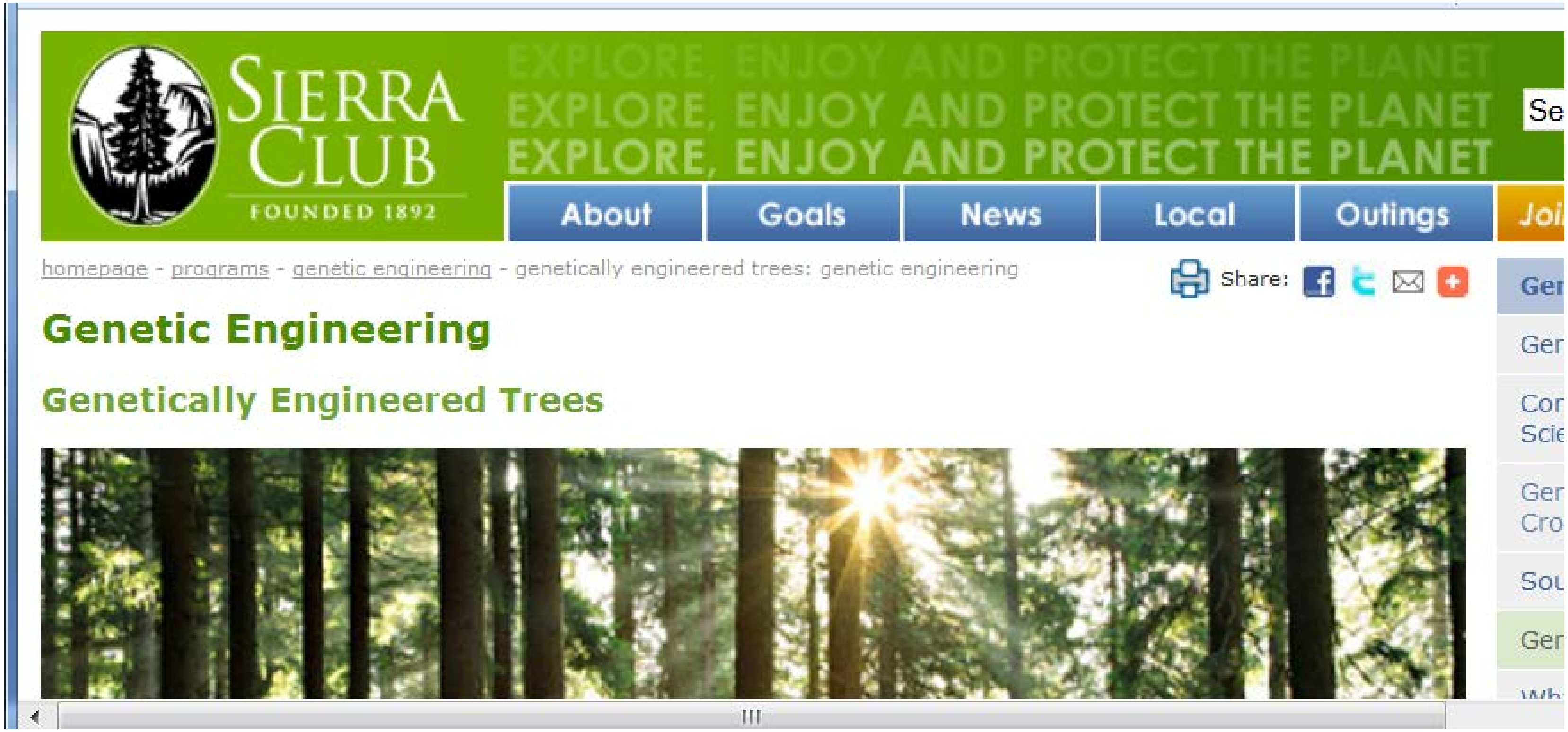
Down in the forest, something stirs

The Economist, 2005



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 creates a gene flow and research conundrum

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…”

No research exemptions

International treaties push for stringent regulations, focus on gene flow

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.

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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 ... mandate in the CBD

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NO GE Trees!

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Nearly 150 organizations around the world responded to the social and ecological threats of GE trees by demanding a global ban on the release of GE trees into the environment. These organizations, gathered in only 1 week's time and only from countries where research on the genetic modification of trees is being carried out (or has in recent years), are listed below, and an excerpt of the statement is found on the following page. The language being considered by SBSTTA at this point regarding GE trees is a big step backward from the decision on GE trees at COP-8. The decision to apply the precautionary approach to GE trees must be strengthened into a moratorium, not watered down. Delegates wishing to learn more about the impacts of GE trees are invited to attend a side event on the issue today at lunch in the Green Room.

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5. Agenda 21 Anil&Azul - Rio de Janeiro, Brazil
6. Agenda Regional de La Araucanía, Chile
7. Agrupación ambientalista Koyam Newen, Chile
8. Agrupación de jóvenes profesionales mapuche Konapewman, Chile
9. Alianza por una Mejor Calidad de Vida (RAP-Chile), Chile
10. Amigos de la Tierra España – Friends of the Earth Spain, Spain
11. AOPA - Associação para o Desenvolvimento da Agroecologia, Brazil
12. Argonautas Ambientalistas da Amazônia, Brazil
13. AS-PTA Assessoria e Serviços a Projetos em Agricultura Alternativa, Brazil
14. Associação de Programas em Tecnologias Alternativas-APTA, Brazil

24. Carbon Trade Watch, International
25. CAxTIERRA (Comisión de Apoyo X Tierra), Uruguay
26. Centro de Agricultura Alternativa do Norte de Minas - CAA NM, Brazil
27. Centro de Defesa dos Direitos Humanos – CDDH, Brazil
28. Centro de Estudos Ambientais (CEA), Brazil
29. CENTRO ECOLOGICO BORDE RIO, Chile
30. Centro Federal de Educação Tecnológica de Rio Pomba (CEFET-Rio Pomba), Brazil
31. CLOC (Coordinadoria LatinoAmericana de las Organizaciones del Campo), Republica Dominicana

32. Coalition for Safe Food, Powell River, British Columbia, Canada
33. COATI - Centro de Orientação Ambiental Terra Integrada - Jundiaí, Brazil
34. CODEFF / Amigos de la Tierra, Chile
35. Comissão Pastoral da Terra - Diocese Itabuna/Bahia, Brazil
36. Coorporación Unión Araucana "XAPELEAI TAIÑ KIMVN", Padre Las Casas, Chile
37. Crescente Fértil, Brazil
38. Cumberland Countians for Peace & Justice, USA
39. Development Fund, Norway
40. Dogwood alliance, USA
41. Down to Earth - the International Campaign for Ecological Justice in IndonesiaUnited Kingdom
42. Ecodevelop - Publikation und Dienstleistung für ökosoziale Entwicklung, Germany
43. Ecologistas en Acción, Madrid, Spain
44. ESPLAR - CENTRO DE PESQUISA E ASSESSORIA, Brazil
45. ETC Group, Canada
46. Fair-Fish, Switzerland
47. Federação de Órgãos Para

57. Forum Ökologie & Papier, Germany
58. Friends of the Earth (England, Wales and Northern Ireland), United Kingdom
59. Friends of the Earth Australia
60. Friends of the Earth Europe
61. Fundação Vitória Amazônica, Brazil
62. Fundacion Sociedades Sustentables de Chile, Chile
63. Gaia Foundation, International
64. GE Free New Zealand, Aotearoa/New Zealand
65. GEEMA - Grupo de Estudos em Educação e Meio Ambiente, Rio de Janeiro, Brazil
66. GENANET - focal point gender, environment, sustainabilty, Germany
67. Gene ethical Network, Germany
68. Gesellschaft für Ökolgische Forschung, Munich, Germany
69. Global Forest Coalition, International
70. Global Justice Ecology Project, International
71. GM Freeze, United Kingdom
72. GM-Free Dorset Campaign, United Kingdom
73. Green Press Initiative, USA
74. Greenpeace, International
75. Grupo Ambientalista da Bahia - Gambá, Brazil
76. Grupo Mamangava, Brazil
77. GT Ambiente / AGB-Rio e AGB-Niteroi, Brazil
78. IDESA (Instituto de Desenvolvimento Social e Ambiental), Brazil
79. Indiana Forest Alliance, USA
80. Indigenous Environmental Network (IEN), USA/Canada
81. Institute for Responsible Technology, USA
82. Institute for Social Ecology, USA
83. Instituto Ambiental Viramundo - Ceará, Brazil
84. Instituto para o Desenvolvimento Ambiental - IDA, Brazil
85. International Tribal Association, USA
86. Kentucky Heartwood, USA
87. Latin American Network Against Monoculture Tree Plantations, International
88. Les Amis de la Terre (Friends of the Earth France), France

96. Network for Environmental & Economic Responsibility, United Church of Christ, USA
97. Ngualien Pelu Mapu / protectores de la tierra, Chile
98. Northern Heritage Association, Finland
99. Northwest Resistance Against Genetic Engineering, USA
100. Northwoods Wilderness Recovery, USA
101. OroVerde - Tropical Forest Foundation, Germany
102. Pacific Indigenous Peoples Environment Coalition (PIPEC), Aotearoa/New Zealand
103. Plataforma Transgenicos Fora (Portuguese GM-Free Coalition), Portugal
104. Prairie Red Fife Organic Growers Cooperative Ltd., Canada
105. Prodema - UFC, Brazil
106. RAE - Rede de Educação Ambiental Escolar, Rio de Janeiro, Brazil
107. Rainforest Relief, USA
108. Red por una América Latina Libre de Transgénicos, Ecuador
109. Rede Ambiental do PiauÃ - REAPI, Brazil
110. Rede de Educadores Ambientais da Baixada de Jacarepaguá, Rio de Janeiro, Brazil
111. Rede de Integração Verde, Brazil
112. Rettet den Regenwald, Germany
113. Rising Tide North America, USA
114. Robin Wood, Germany
115. Safe Alternatives for our Forest Environment (SAFE)USA
116. Sierra Club, USA
117. Sindicato dos Trabalhadores de Rio Pardo de Minas - MG, Brazil
118. Sociedade Angrense de Proteção Ecológica, Brazil
119. Society for a Genetically Engineered British Columbia, Canada
120. Soil Association, USA
121. Stop GE Trees Campaign, International
122. Terra de Directos, Brazil
123. UITA - Unión Internacional de Trabajadores de la Alimentación y la Agricultura, International

134. World Rainforest Movement, International
135. Worldforests, Scotland
136. Worldview, USA
137. Xarxa de l'Observatori del Deute en la Globalització, Cataluña, Estado español

GE Tree Statement

Below is a brief description of the statement and letter signed by 137 groups.

Statement signatories begin by stating that their *"concern is based on the fact that the genetic manipulation being undertaken is aimed at consolidating and further expanding a model of monoculture tree plantations that has already proven to result in serious social and environmental impacts in many of our countries."*

The statement then provides a number of examples on how current research would impact on the environment, given that trees are being genetically manipulated for.

The signatories remind country delegates that "the last Conference of the Parties to the Convention on Biological Diversity (COP-8) adopted decision VIII/19", which *"recommends Parties to take a precautionary approach when addressing the issue of genetically modified trees"* and urge them *"to definitely ban GE trees - including fields trials – because of the serious risks they pose on the Planet's biological diversity."*

Full letter and signatories available at:
http://www.wrm.org.uy/actors/BDC/SBSTT

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13. AS-PTA Assessoria e Serviços a Projetos em Agricultura Alternativa, Brazil
14. Associação de Programas em Tecnologias Alternativas-APTA, Brazil

24. Carbon Trade Watch, International
25. CAxTIERRA (Comisión de Apoyo X Tierra), Uruguay
26. Centro de Agricultura Alternativa do Norte de Minas - CAA NM, Brazil

...Organizaciones del Campo), Republica Dominicana

32. Coalition for Safe Food, Powell River, British Columbia, Canada
33. COATI - Centro de Orientação Ambiental Terra Integrada - Jundiaí, Brazil

... Down to Earth - the International Campaign for Ecological Justice in IndonesiaUnited Kingdom
42. Ecodevelop - Publikation und Dienstleistung für ökosoziale Entwicklung, Germany
43. Ecologistas en Acción, Madrid, Spain
44. ESPLAR - CENTRO DE PESQUISA E ASSESSORIA, Brazil
45. ETC Group, Canada
46. Fair-Fish, Switzerland
47. Federação de Órgãos Para

57. Forum Ökologie & Papier, Germany
58. Friends of the Earth (England, Wales and Northern Ireland), United Kingdom
59. Friends of the Earth Australia
60. Friends of the Earth Europe
61. Fundação Vitória Amazônica, Brazil
62. Fundacion Sociedades Sustentables de Chile, Chile
63. Gaia Foundation, International
64. GE Free New Zealand, Aotearoa/New Zealand
65. GEEMA - Grupo de Estudos em Educação e Meio Ambiente, Rio de Janeiro, Brazil
66. GENANET - focal point gender, environment, sustainability, Germany
67. Gene ethical Network, Germany
68. Gesellschaft für Ökologische Forschung, Munich, Germany
69. Global Forest Coalition, International
70. Global Justice Ecology Project, International
71. GM Freeze, United Kingdom
72. GM-Free Dorset Campaign, United Kingdom
73. Green Press Initiative, USA
74. Greenpeace, International
75. Grupo Ambientalista da Bahia -

83. Instituto Ambiental Viramundo - Ceará, Brazil
84. Instituto para o Desenvolvimento Ambiental - IDA, Brazil
85. International Tribal Association, USA
86. Kentucky Heartwood, USA
87. Latin American Network Against Monoculture Tree Plantations, International
88. Les Amis de la Terre (Friends of the Earth France), France

96. Network for Environmental & Economic Responsibility, United Church of Christ, USA
97. Ngualien Pelu Mapu / protectores de la tierra, Chile
98. Northern Heritage Association, Finland
99. Northwest Resistance Against Genetic Engineering, USA
100. Northwoods Wilderness Recovery, USA
101. OroVerde - Tropical Forest Foundation, Germany
102. Pacific Indigenous Peoples Environment Coalition (PIPEC), Aotearoa/New Zealand
103. Plataforma Transgenicos Fora (Portuguese GM-Free Coalition), Portugal
104. Prairie Red Fife Organic Growers Cooperative Ltd., Canada
105. Prodema - UFC, Brazil
106. RAE - Rede de Educação Ambiental Escolar, Rio de Janeiro, Brazil
107. Rainforest Relief, USA
108. Red por una América Latina Libre de Transgénicos, Ecuador
109. Rede Ambiental do PiauÃ - REAPI, Brazil

118. Sociedade Angrense de Proteção Ecológica, Brazil
119. Society for a Genetically Engineered British Columbia, Canada
120. Soil Association, USA
121. Stop GE Trees Campaign, International
122. Terra de Directos, Brazil
123. UITA - Unión Internacional de Trabajadores de la Alimentación y la Agricultura, International

134. World Rainforest Movement, International
135. Worldforests, Scotland
136. Worldview, USA
137. Xarxa de l'Observatori del Deute en la Globalització, Cataluña, Estado español

GE Tree Statement

Below is a brief description of the statement and letter signed by 137 groups.

Statement signatories begin by stating that their *"concern is based on the fact that the genetic manipulation being undertaken is aimed at consolidating and further expanding a model of monoculture tree plantations that has already proven to result in serious social and environmental impacts in many of our countries."*

The statement then provides a number of examples on how ... would impact on ..., given that trees ...tically manipulated

...s remind country ...he last Conference ...the Convention on ...versity (COP-8) ...n VIII/19", which *"recommends Parties to take a precautionary approach when addressing the issue of genetically modified trees"* and urge them *"to definitely ban GE trees - including fields trials – because of the serious risks they pose on the Planet's biological diversity."*

Full letter and signatories available at: http://www.wrm.org.uy/actors/BDC/SBSTT...

…global ban on the release of GE trees into the environment…" = NO FIELD RESEARCH

NO GE Trees!

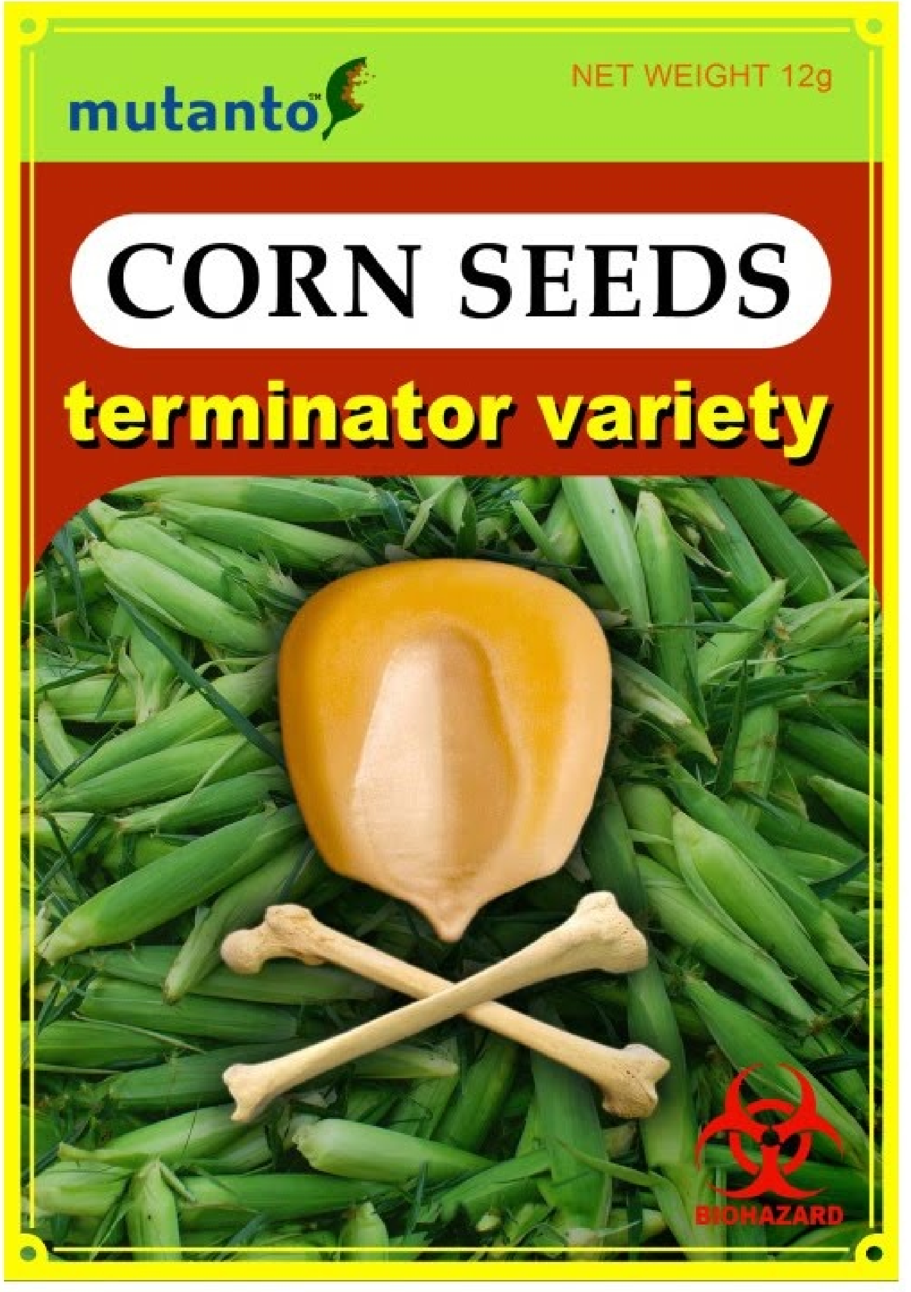
NO Case by Case!

Nearly 150 organizations around the world responded to the social and ecological threats of GE trees by demanding a global ban on the release of GE trees into the environment. These organizations, gathered in only 1 week's time and only from countries where research on the genetic modification of trees is being carried out (or has in recent years), are listed below, and an excerpt of the statement is found on the following page. The language being considered by SBSTTA at this point regarding GE trees is a big step backward from the decision on GE trees at COP-8. The decision to apply the precautionary approach to GE trees must be strengthened into a moratorium, not watered down. Delegates wishing to learn more about the impacts of GE trees are invited to attend a side event on the issue today at lunch in the Green Room.

Unpopularity of gene flow restriction technologies

“The Destruction of Our Food - GMO and Terminator Seeds….

“Ever since I found out about terminator seeds, I have understood how famine could take over the planet as predicted in the Bible.”



‘TERMINATOR’

Regulatory confusion, obstacles at national and international levels

The Phantom Forest: Research on Gene-Altered Trees Leaps Ahead, into a Regulatory Limbo

STEVE NASH

At an industrial park in Walnut Creek, California, technicians and robots are sorting through the 550 million base pairs of genetic code in poplar DNA to sequence a tree genome for the first time. They are poised to unlock a fine, full toolbox for the work of genetic engineering in trees.

In Vermont, a group called Action for Social and Ecological Justice has just kicked off a national campaign to pressure companies to ban research on genetically engineered (GE) trees. The Sierra Club, the World Wildlife Fund, and the American Lands Alliance, among others, have called for a moratorium on commercialization of GE trees.

In Wa… key resp… logical s… response…

More than 200 notices of field trials have been filed with federal regulators for lab-engineered fruit, nut, and forest trees—also known as genetically modified, biotech, or transgenic trees. But aside from a virus-resistant, bushlike papaya tree grown in Hawaii, no one has yet sought regulatory approval for commercial use of a gene-altered tree.

Westvaco Corporation, and two New Zealand firms. Arborgen estimates that, if tests go very well, the product could be ready for the market in a decade.

Cloned cathedrals

Tinkering with tree DNA presents some issues for research and for public policy, however. Casting an uncertain light over

462 BioScience • *May 2003 / Vol. 53 No. 5*

Roadmap

- Why genetics and biotech matters for trees
- Why gene flow is an immense problem needing technical as well as policy solutions
- **Evolving technology options**
- Obstacles and progress in making them work for trees

Many options for containment technologies – V-GURTs

Plant Biotechnology Journal

Plant Biotechnology Journal (2014), pp. 1–11

doi: 10.1111/pbi.12242

Review article

Genetic use restriction technologies: a review

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Department of Crop Systems, Forestry and Environmental Sciences, University of Basilicata, Potenza, Italy

Received 9 March 2014;
revised 16 July 2014;
accepted 17 July 2014.
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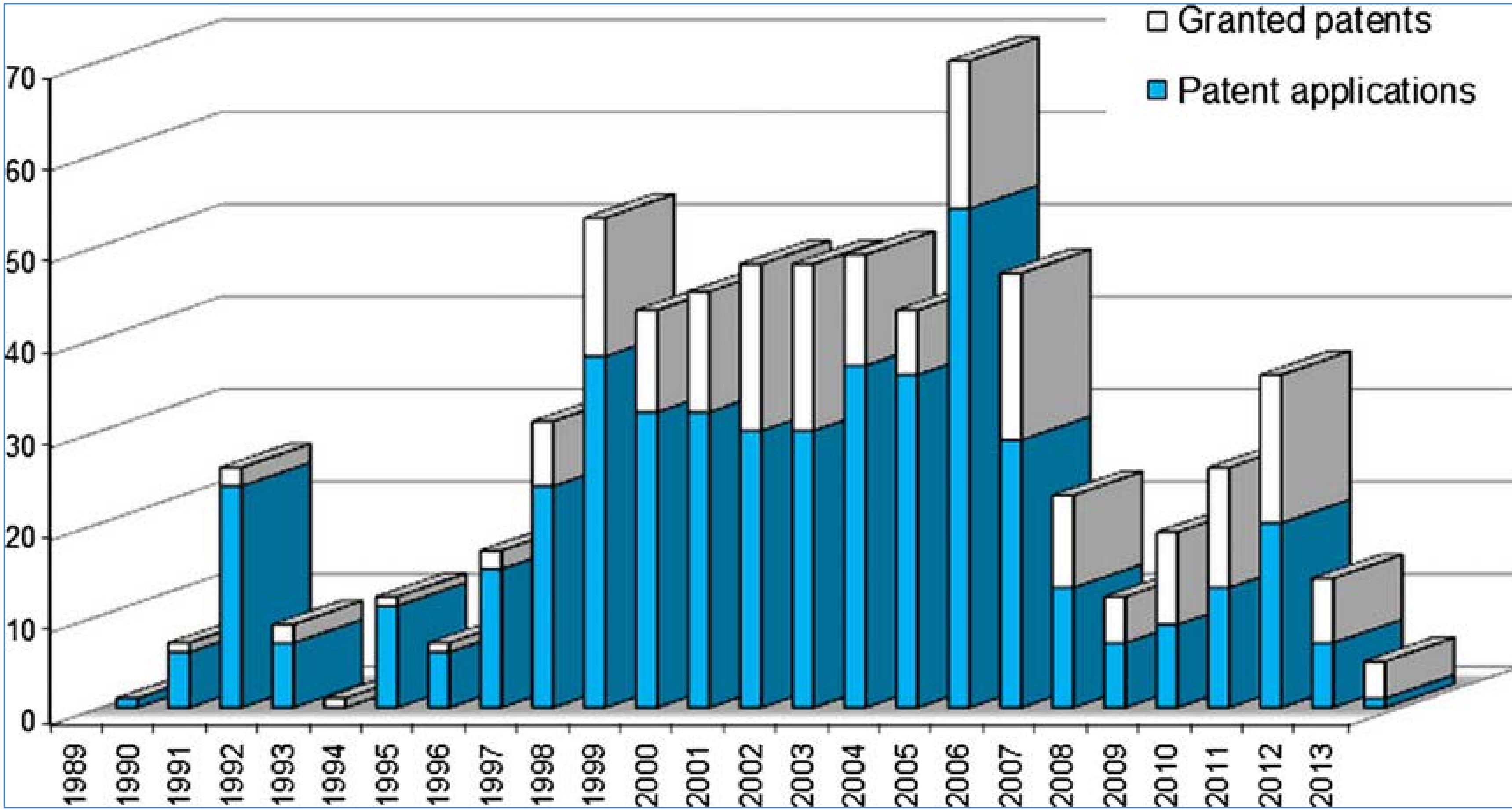
fax +39 0971205378;
email lombluca@yahoo.it)

Keywords: V-GURTs, T-GURTs, intellectual property, seed saving.

Summary

Genetic use restriction technologies (GURTs), developed to secure return on investments through protection of plant varieties, are among the most controversial and opposed genetic engineering biotechnologies as they are perceived as a tool to force farmers to depend on multinational corporations' seed monopolies. In this work, the currently proposed strategies are described and compared with some of the principal techniques implemented for preventing transgene flow and/or seed saving, with a simultaneous analysis of the future perspectives of GURTs taking into account potential benefits, possible impacts on farmers and local plant genetic resources (PGR), hypothetical negative environmental issues and ethical concerns related to intellectual property that have led to the ban of this technology.

Investment in GURTs have rapidly declined, little field research, no commercial use



Lombardo 2014 / Plant Biotechnology Journal

Focus on genetic containment via complete bisexual sterility – vegetative propagation, vegetative harvest – poplar, eucalypts, pine

Options for genetic containment via complete, constitutive, bisexual sterility

- Controlled cell/tissue ablation
  – Floral developmental promoter
- Floral gene malfunction
  – RNA suppression (RNAi)
  – Protein disruption (dominant negative)
  – Gene mutation (ZFN, TALEN, CRISPR)
- Floral transgene excision (recombinase)

Roadmap

- Why genetics and biotech matters for trees
- Why gene flow is an immense problem needing technical as well as policy solutions
- Evolving technology options
- **Obstacles and progress in making them work for trees**

Complexities of containment system development

- Delay to onset of flowering in research (trees)
- Isolation during research in field
  - Regulations
  - Legal/business risks of adventitious presence
- Stability and efficiency in field?
  - Does it work? All genotypes / environments? Consistent over years and parts of trees?
  - Pleiotropic effects on vegetative growth?
- Ecological impacts of pollen/seed/fruit removal or modification?
  - Pollinators, biodiversity, mitigation options
  - Public / market acceptance

How much sterility is sufficient?

Even modest infertility can have a big impact on spread

New Phytologist

Research

Gene flow and simulation of transgene dispersal from hybrid poplar plantations

Stephen P. DiFazio[1], Stefano Leonardi[2], Gancho T. Slavov[1,3,4], Steven L. Garman[5,6], W. Thomas Adams[6] and Steven H. Strauss[6]

[1]Department of Biology, West Virginia University, Morgantown, WV 26506-6057, USA; [2]Dipartimento di Scienze Ambientali, Università di Parma, 43100 Parma, Italy; [3]Department of Dendrology, University of Forestry, Sofia 1756, Bulgaria; [4]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth SY23 3EB, UK; [5]National Park Service, PO Box 848, Moab, UT 84532, USA; [6]Department of Forest Ecosystems and Society, Oregon State University, 3180 SW Jefferson Way, Corvallis, OR 97331, USA

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Received: *1 August 2011*
Accepted: *14 November 2011*

Summary

- Gene flow is a primary determinant of potential ecological impacts of transgenic trees. However, gene flow is a complex process that must be assessed in the context of realistic genetic, management, and environmental conditions.
- We measured gene flow from hybrid poplar plantations using morphological and genetic markers, and developed a spatially explicit landscape model to simulate pollination, dispersal, establishment, and mortality in the context of historical and projected disturbance and land-

Regulations for field trials assume containment, generally do not assess ecological risks or benefits of particular genes or traits

Can you adequately test containment technologies in the field?

Unexpected summer flowering of semi-dwarf transgenic poplar in field trial

The upright summer "catkins" and veg-catkin transition structures

This field trial had been appended to a larger APHIS permit that permitted flowering in this location and with this genotype (incompatible with wild relatives, female tree) -- but APHIS was unsure if this meant the appended trial also could flower legally

Being a good soldier, I faithfully and immediately reported this unexpected occurrence (as the permit requires)

Then discussed what to do about it with APHIS regulatory science contacts for several days

I wanted to leave the catkins for study, as they were interesting, risk seemed to be zero, and would be difficult to remove

I pointed out the layers of safety from the genes (dwarfism, fitness reduced) and biology (lack of pollen or receptive females in summer, no seed dormancy) to APHIS

The APHIS scientists agreed, but they felt, legally, they must report it to the **compliance branch** as a permit violation/release….

© AP Photo/Rogelio V. Solis

Thankfully a science colleague at APHIS alerted me that the report to Compliance had occurred prior to a visit and action

Rather than risk arrest, fines, and who knows what else by federal agents…..

Including what would be sure to be highly publicized as major disregard for the rules and the environment, and thus a call for much stricter regulations…

The same day, all students in our lab were dispatched to manually remove every "catkin"

And the same in spring and beyond….

Students removing catkins from transgenic trees

We documented for APHIS that “All removed flowers were collected and brought back to the lab, then autoclaved”

Thank goodness, the federal agents never came to arrest me over the grave “violation”

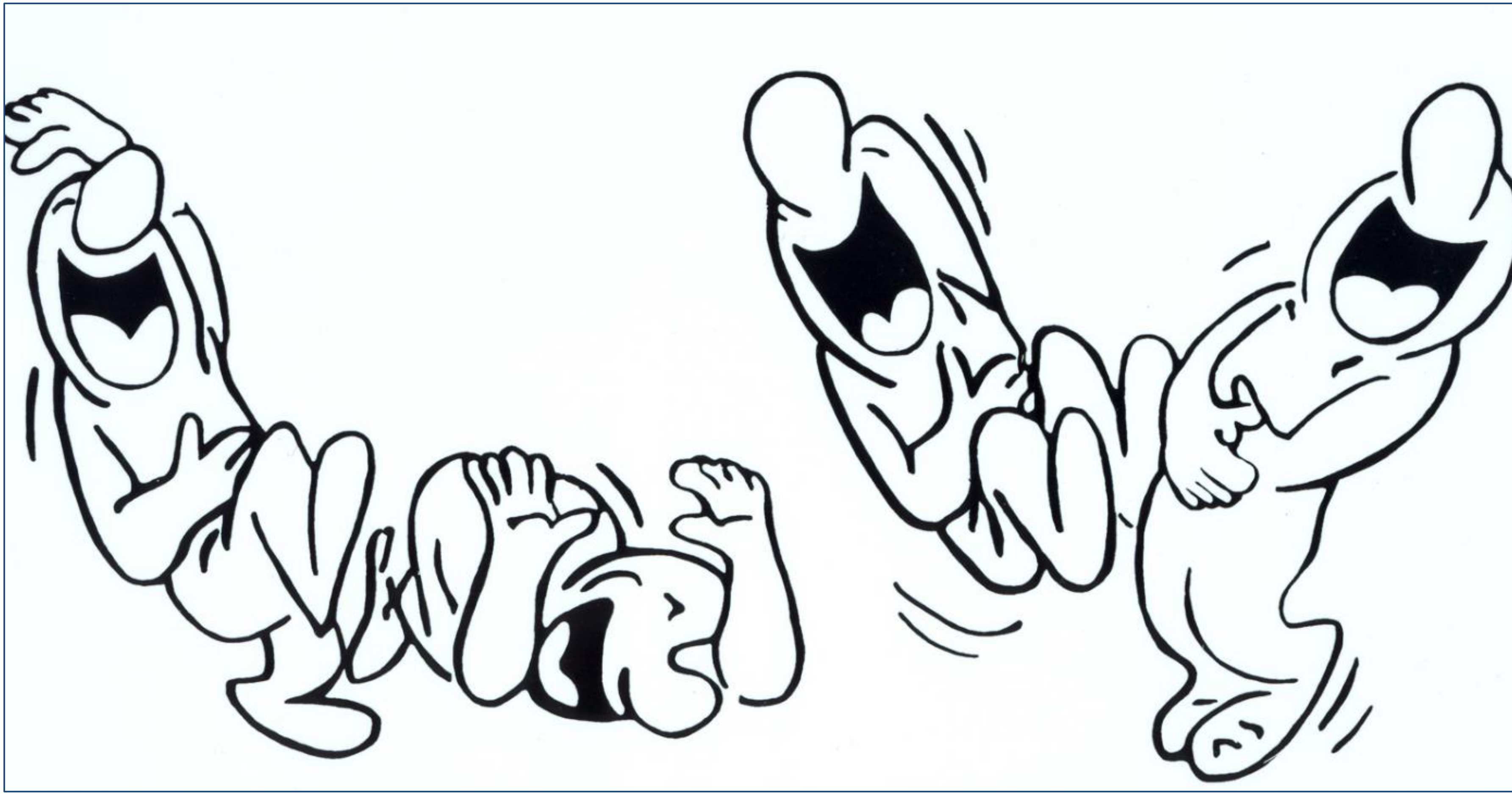
A powerful lesson about the letter of the law, and the reality that GE methods are considered evil and dangerous until proven otherwise, period

Biology, safety, and benefit are irrelevant

One answer is to deregulate it for science

Containment of every pollen grain and seed during field research would not be required

So I visited APHIS and suggested this given the increased safety of the trait and benefits of improved knowledge



They discussed how **each gene insertion event** needs a pile of data, and now certainly an EIS (environmental impact statement), to withstand lawsuits

And getting this data requires the years of research (that is what we are trying to find a way to obtain!)

Regulations a debilitating impediment to breeding progress with GMOs – as it requires field research with ~perfect containment

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

2010 / Vol. 60 No. 9 • BioScience 729

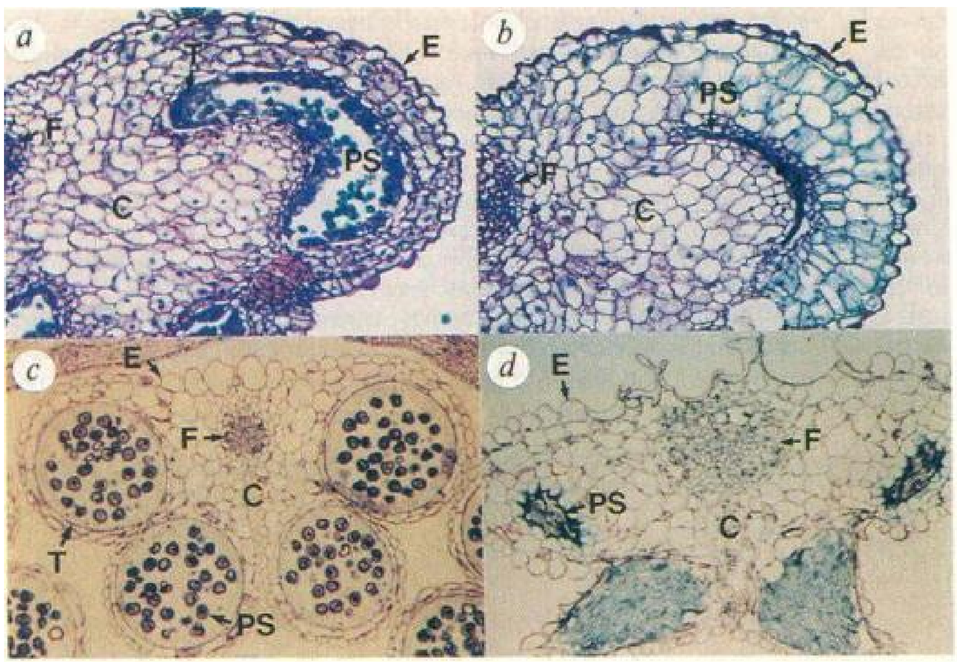
Progress

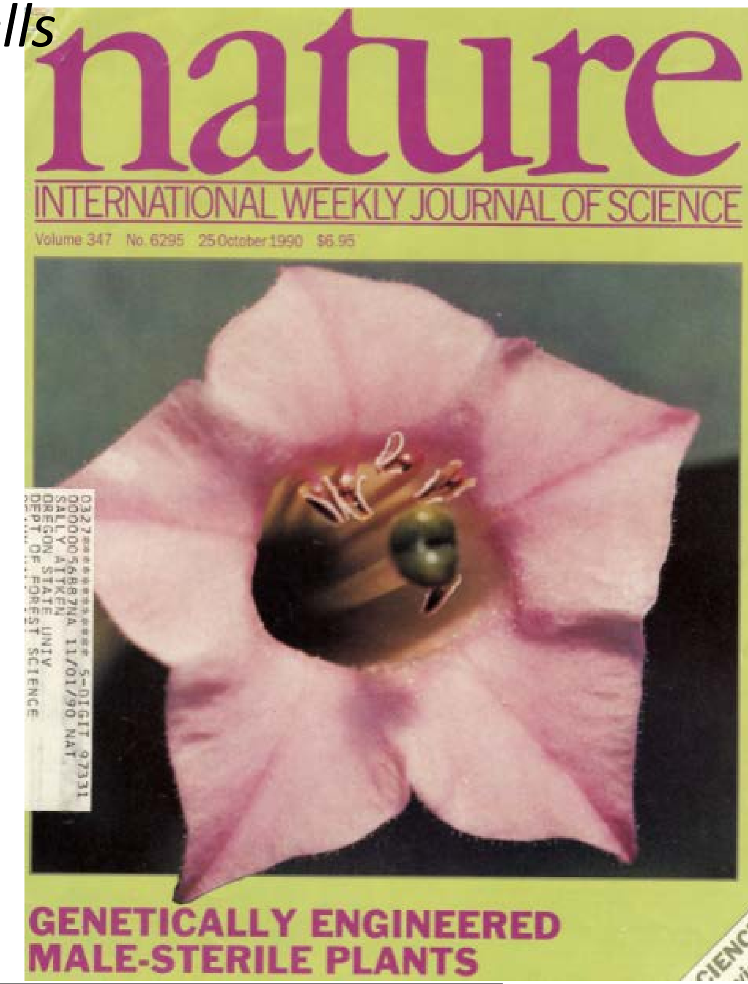
- Male-sterility
- Accelerated flower induction
- RNAi suppression
- Site-directed mutagenesis

First demonstration of transgenic male-sterility -- via "ablation" method

Cell-disrupting genes turned on in floral cells

Anther promoter::RNAse





Induction of male sterility in plants by a chimaeric ribonuclease gene

Celestina Mariani*, Marc De Beuckeleer*, Jessie Truettner†,

Jan Leemans* & Robert B. Goldberg†‡

A successful 10 year study: Male- sterility

Tree Genetics & Genomes (2014) 10:1583–1593
DOI 10.1007/s11295-014-0781-6

ORIGINAL PAPER

A tapetal ablation transgene induces stable male sterility and slows field growth in *Populus*

Estefania Elorriaga · Richard Meilan · Cathleen Ma · Jeffrey S. Skinner · Elizabeth Etherington · Amy Brunner · Steven H. Strauss

Received: 20 March 2014 /Revised: 18 July 2014 / Accepted: 18 July 2014 /Published online: 13 August 2014
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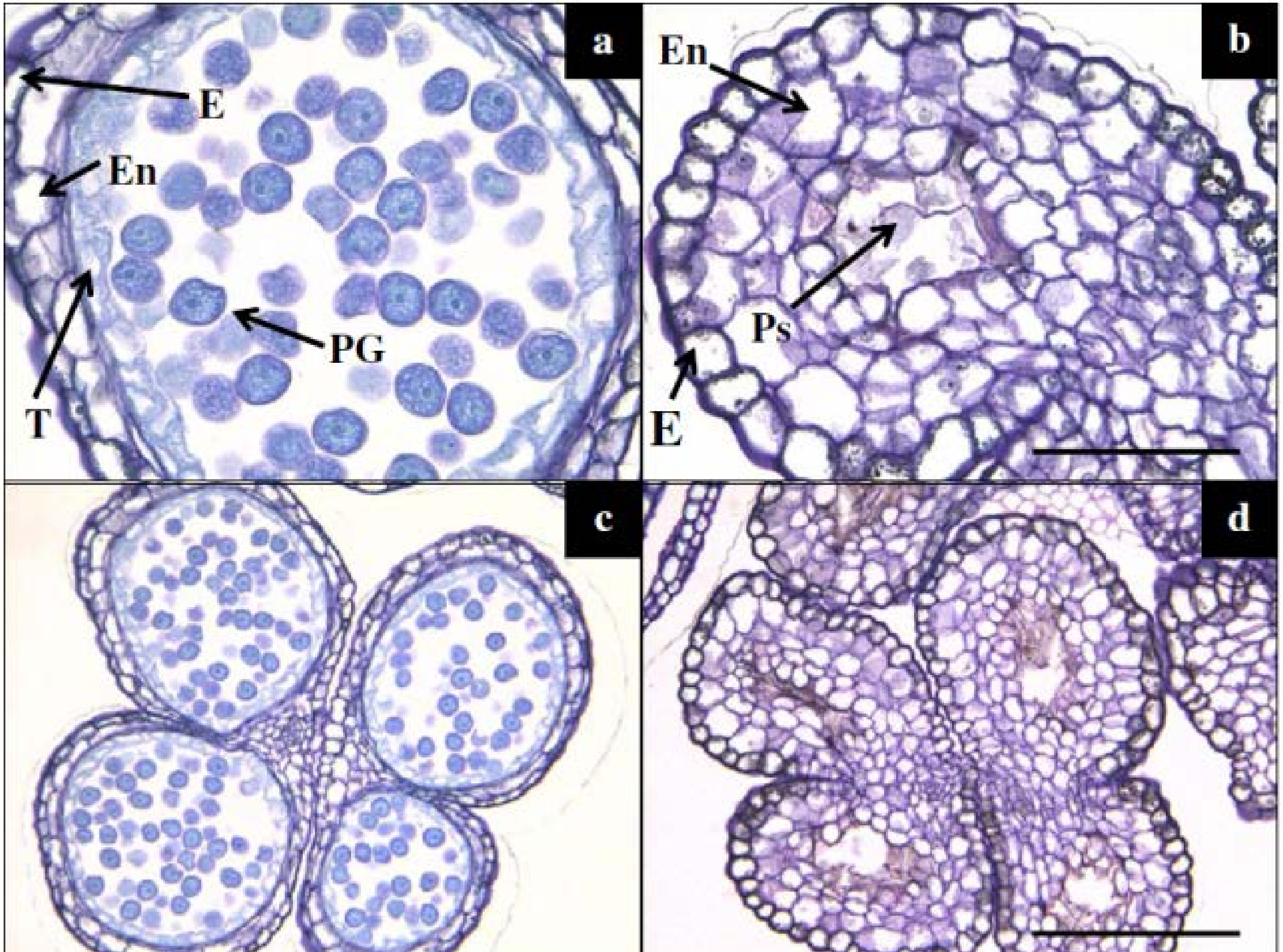
Abstract The field performance of genetic containment technologies–considered important for certain uses of transgenic trees in forestry–is poorly known. We tested the efficiency of a barnase gene driven by the *TA29* tapetum-dominant promoter for influencing growth rate and inducing male sterility in a field trial of transgenic hybrid poplar (*Populus tremula* × *Populus tremuloides*). When the growth of 18 transgenic event grew significantly more slowly than the control. In contrast, when we compared the growth of transgenic trees containing four kinds of *β-glucuronidase* (GUS) reporter gene constructs to non-transgenic trees—all of which had been produced using the same transformation method and poplar clone and grown at the same field site—there were no statistically significant differences in growth after three grow-

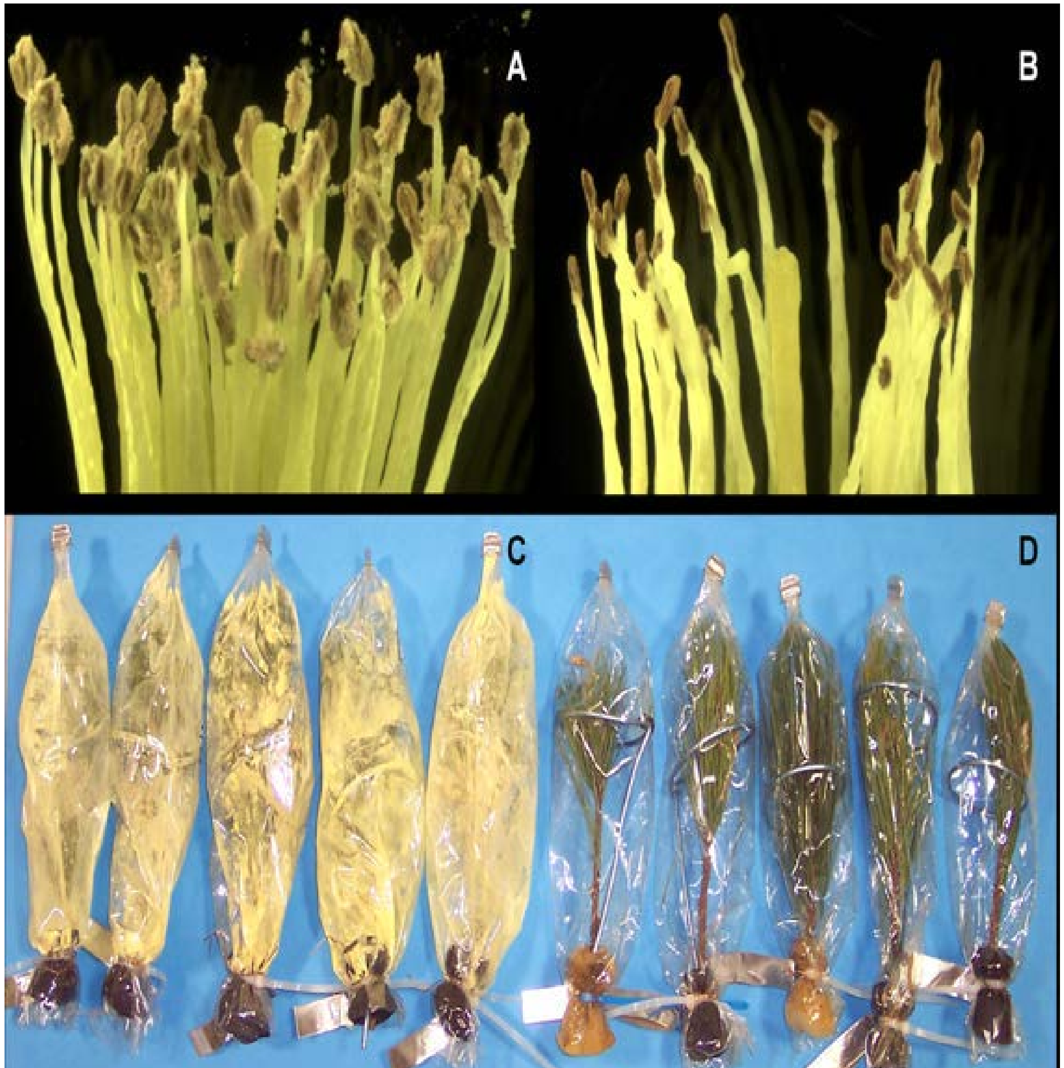
Harvesting the flowers high up in tree crowns in the wet, stormy Oregon spring is not so much fun

Pollen-less catkins in 8 yr-old male poplars in Oregon with same sterility gene

Tapetal collapse

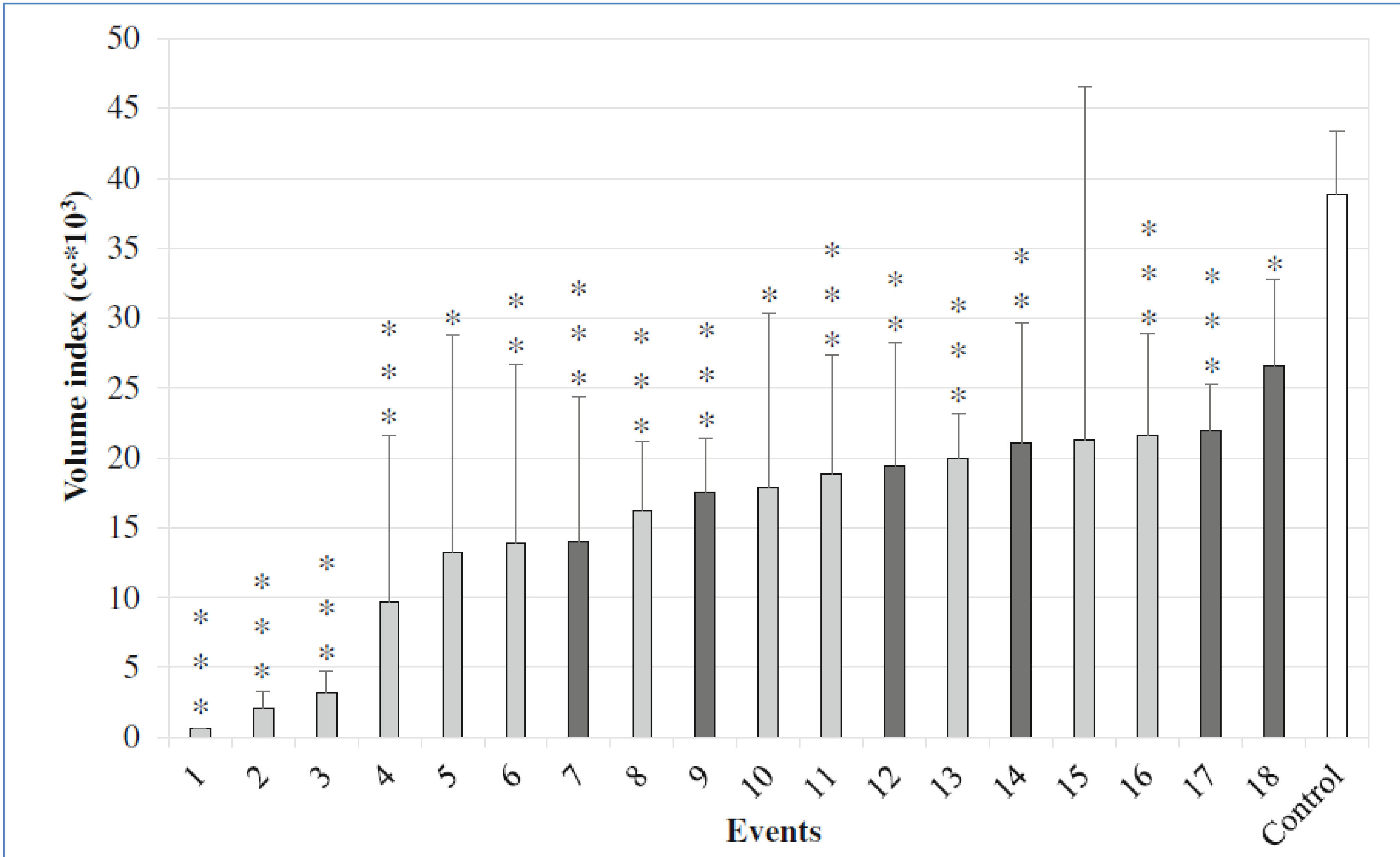


Male-sterility approach works well in eucalypts and pine as well - Arborgen



Anther-specific promoter driving expression of a strong RNAse prevents pollen maturation and release

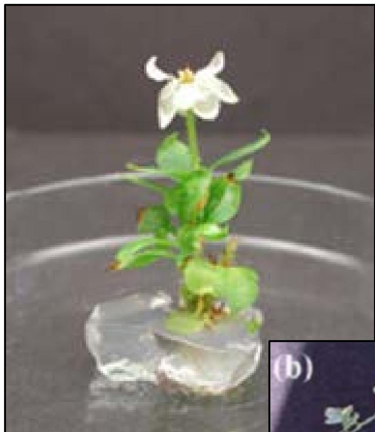
Pleiotropy: Deleterious effects of barnase on tree growth



An answer to speed research?

Overexpression of endogenous flowering genes induces early flowering in trees

Apple



Orange

Plum

Poplar

Flowering locus T (FT) to accelerate flowering in poplar – Heat induced by heat-shock promoter

LB	NOSp::NPTII::NOSt	GmHsp	FT sequence	35St	RB

Cotransformed *FT/RNAi* led to interesting, but very rare, floral alterations (a few among hundreds tested)

Dominant negative proteins highly effective

p*PTLF::PTLF-EAR/FT* led to the formation of “leafy” sterile shoots or flowers – but also disturbed vegetative growth due to *FT* overexpression

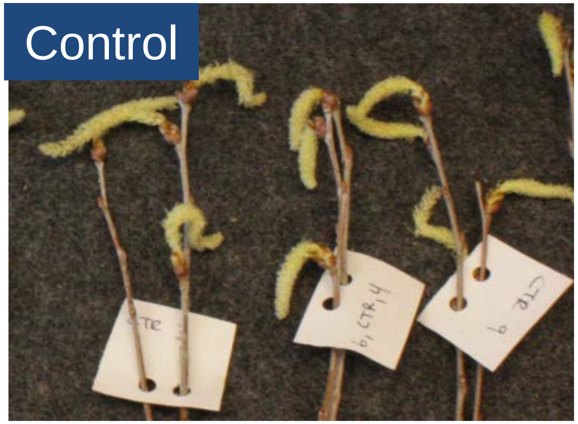
Gene suppression: Is it effective and stable in the field?

RNAi field trial of poplar in Oregon: 25 constructs, 3 genotypes, 4,000 trees, 9 acres

High rate of survival, excellent tree growth in most places on field site

Early flowering genotype: Floral buds visible during winter, enabling early assessment of flowering

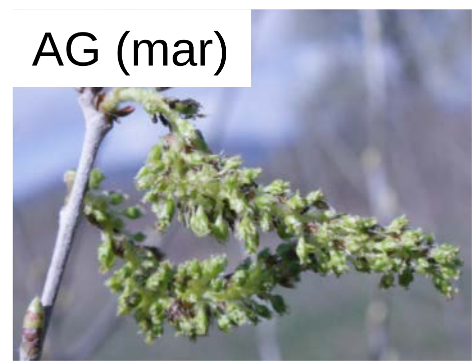
Flushing of dormant buds in lab uncovered modified catkin morphology



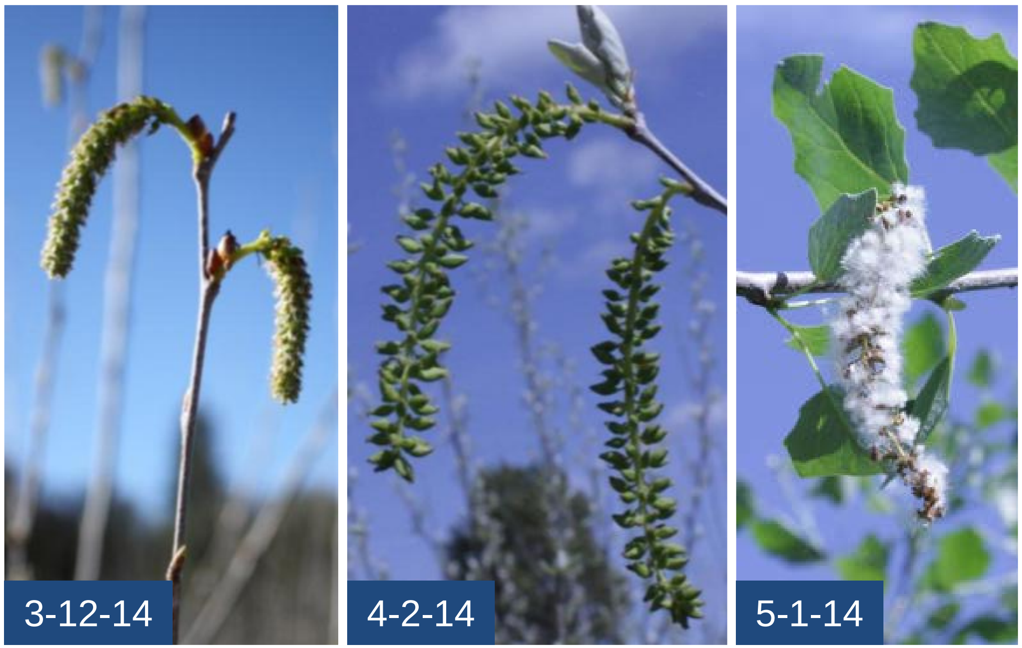
Events from four RNAi constructs targeting *LFY* and/or *AG* have modified floral phenotypes

Construct type	Gene(s) targeted	Floral phenotype
RNAi	LFY	Tiny, no stigma or ovules
RNAi	AG and LFY	Tiny, replicated, no ovules
RNAi	AG	Replicated, no ovules
RNAi	AG (mar)	Replicated, no ovules

Similar catkin phenotypes were also observed in the field



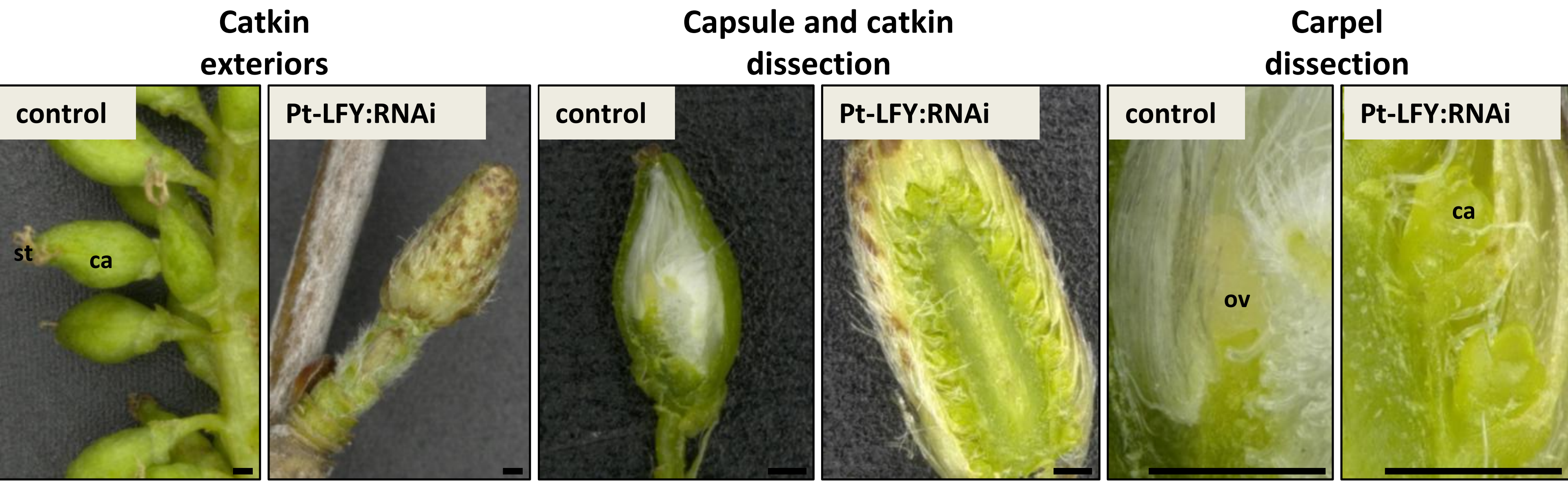
After maturation, RNAi:*LFY* catkins remained tiny and did not produce seeds or cotton



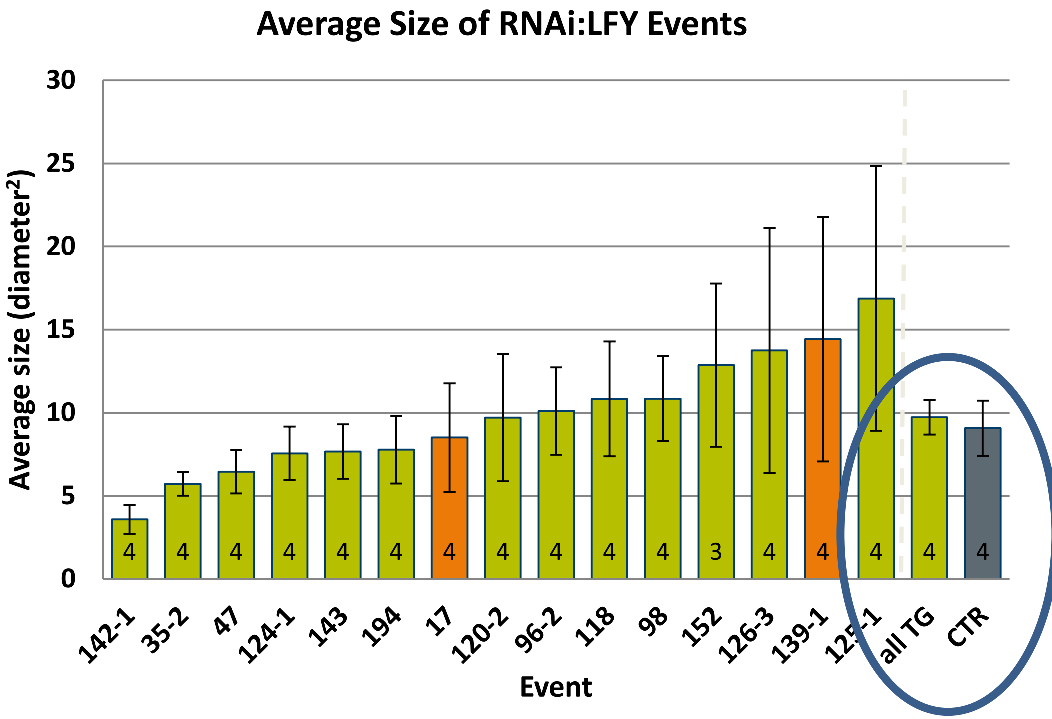
Control

RNAi-LFY

Tiny RNAi:*LFY* catkins lack stigmas, ovules, and cotton



Pleiotropy? RNAi:*LFY* trees had normal vegetative growth



Site directed mutagenesis, gene targeting, coming along fast


NEWS & VIEWS

NATURE|Vol 459|21 May 2009

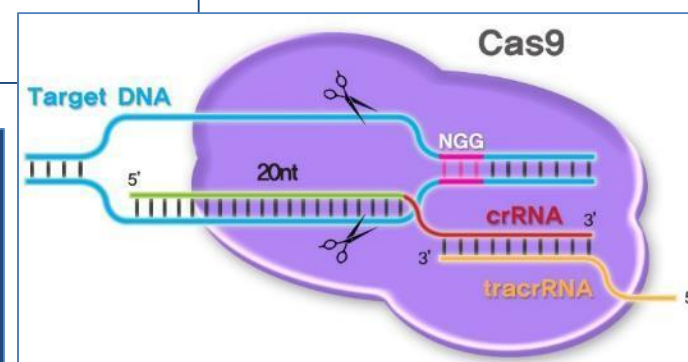
PLANT BIOTECHNOLOGY

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

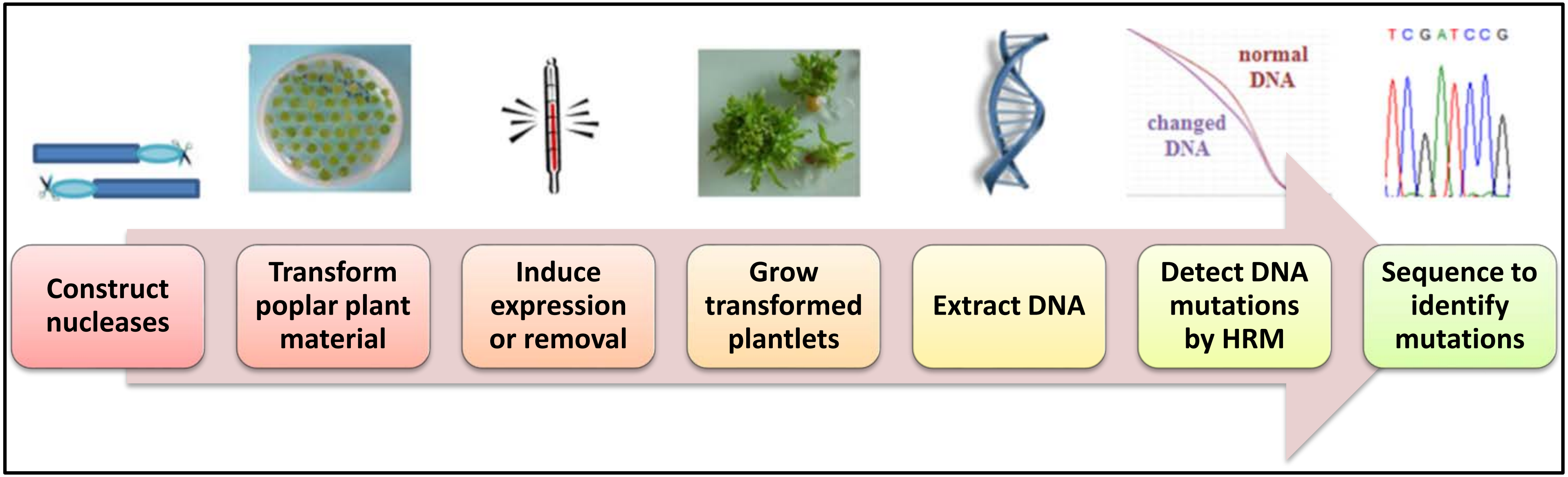


TALENs

Site directed mutagenesis might be an ideal method for containment

- Reported highly efficient – biallelic mutations common = complete loss of gene function
- Physical damage to floral gene/s should be far more reliable than modified/suppressed gene expression or protein function
- More predictable from new regenerant to flowering tree to speed breeding, avoid regulatory problems
- Inducible recombinases enable asexual removal from genome?

Experimental overview



Lessons from site-directed mutagenesis of poplar *AGAMOUS* and *LEAFY* genes

- Employed four heat-induced ZFNs
- ZFNs have deleterious effects on viability
  - ZFN transformation rates are low
  - Some constructs far more deleterious than others
- ZFNs had low rates of mutagenesis
  - < 0.5% per allele per explant (~260 stable transformants tested to date)
- New gene-targeting technologies better?
  - Lower cost, easier cloning
  - Studies of TALENs and CRISPRs underway

Summary

- GE a powerful technology for trees – no surprise
- Gene flow biology makes GE research and commercial use extremely difficult
- Ideology/activism against GE crops/trees and containment technology amplifies problems
- Transgene-induced flowering speeds research, but compromises phenotypes (RNAi, DNM protein)
- RNAi against *LFY* highly promising
- Site directed nucleases so far disappointing (ZFN), but technology rapidly improving
- Valuable new tools, but depth of ideological divide over GMOs requires social, policy change to matter