Transgenic trees

Remarkable progress, extraordinary constraints

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Clarifying terms

- Transgenic, genetically modified, genetically engineered
  - GMO, GEO, GE, GM
- Can modify gene structure or expression that are already present in breeders' gene pools
  - Cisgenics, intragenics, edited genes
- Can insert genes not within breeders' gene pools
- GE / GM used interchangably today to mean direct, asexual, heritable modification of DNA
  - A method not a product

Goals for today

- Value of GMO methods for trees and other woody perennials
  - Horticulture and forestry
- Overview of advanced GMO varieties in production or developmental pipelines
- Regulatory problems needing high level policy solutions

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Constraints to breeding with trees are great – GMO methods offer very significant additional tools

Constraints include

- Long breeding cycle
- Difficulty to inbreed / introgress new genes from hybrids
- Hard to identify dominant, major genes
- Common use of asexually propagated varieties of high value

GE of diverse value for trees

All demonstrated in the field

- Improved fruit quality/durability
- Resistance to insects and diseases
- Tolerance to salinity, cold, drought, and high temperature stresses
- Phytoremediation of environmental toxins
- Modified properties to improve processing of wood for biofuels, paper, or solid wood
- Tolerance to herbicides to reduce the environmental impacts, improve efficiency, or reduce costs of weed control treatments

GE of diverse value for trees

All demonstrated in the field

- Accelerated flowering for faster breeding and research
- Fertility control for reduced spread and improved growth rate
- Improved growth and yield
- Synthesis of new, renewable bioproducts such as plastics, enzymes, and fragrances

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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

HoneySweet plum with GE resistance to plum pox virus

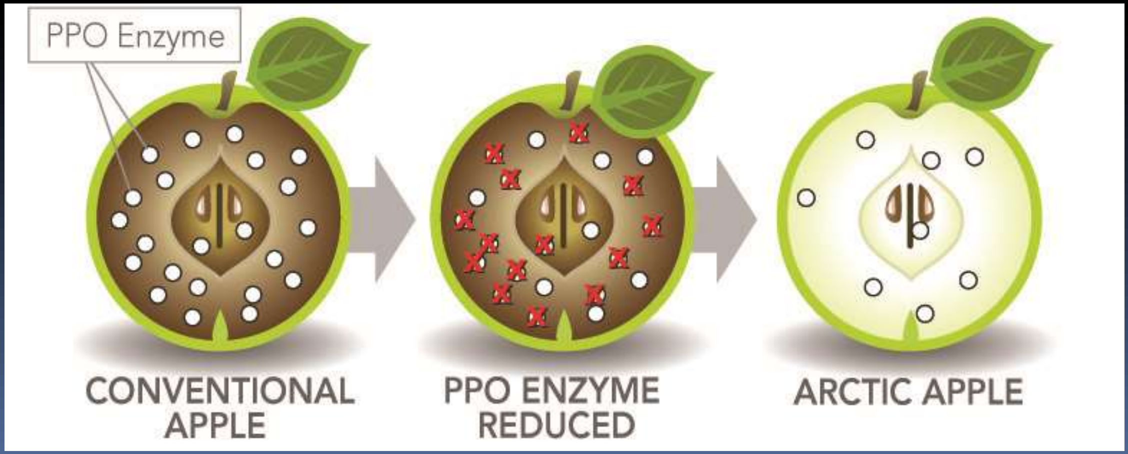
Ralph Scorza USDA-ARS

GE

Non-browning "Arctic Apple"

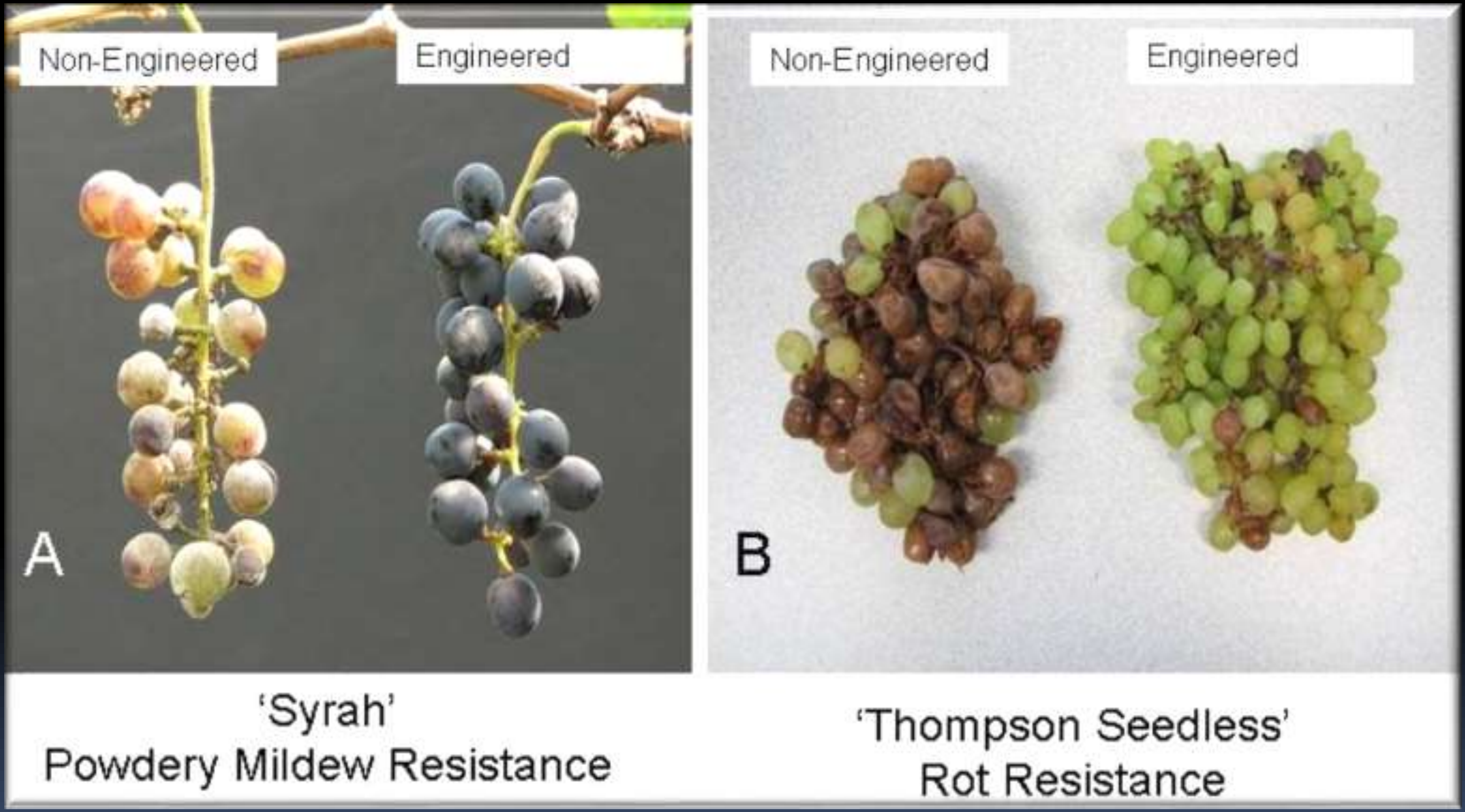
Suppression of native polyphenol oxidase gene expression

Courtesy of Jennifer Armen, Okanagan Specialty Fruits, Canada



Native grape genes used to produce fruit rot resistance

Grape **VvAlb gene** | **Grape VvTL-1 gene**



http://mrec.ifas.ufl.edu/grapes/genetics

Courtesy of Denis Gray, UF/IFAS Mid-Florida Research & Education Center

Native grape genes imparts black rot resistance in field trials

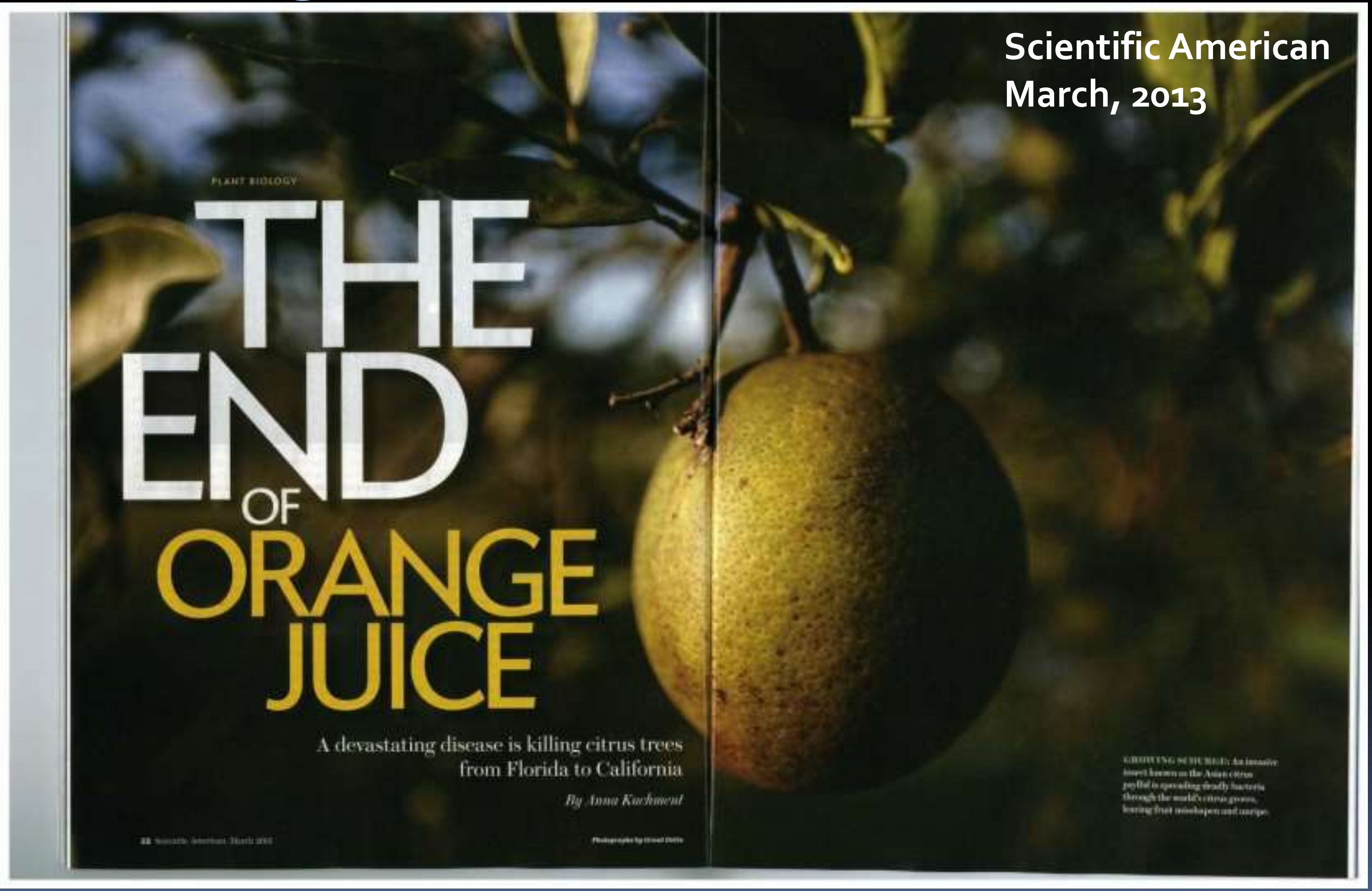
‘Thompson Seedless’ Control

‘Thompson Seedless’ containing VvTL-1

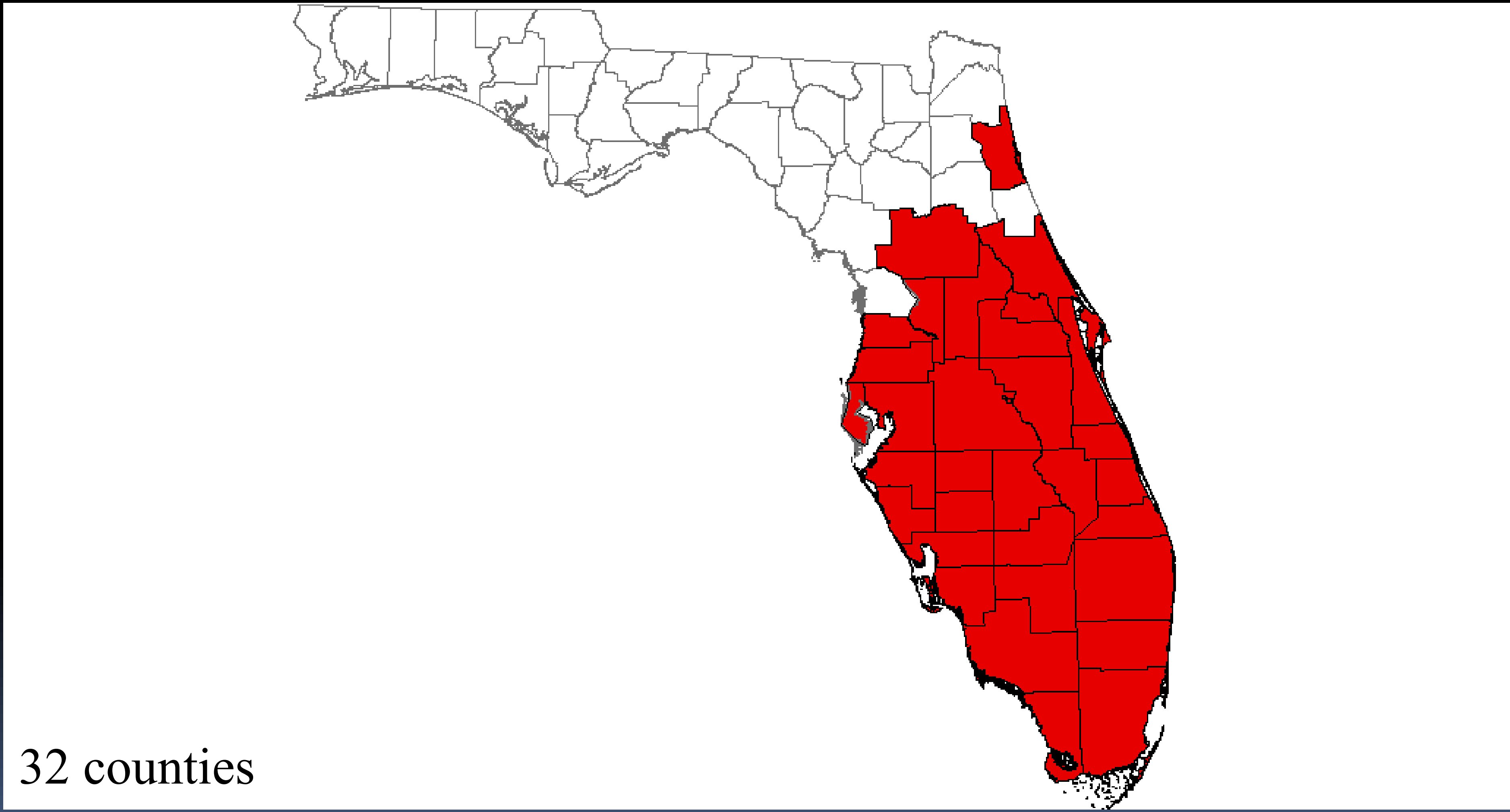
Courtesy of Denis Gray, UF/IFAS Mid-Florida Research & Education Center

http://mrec.ifas.ufl.edu/grapes/genetics

GMO-based resistance transgenes promising in citrus



Rapid spread throughout Florida and of great concern in other citrus growing areas



Courtesy of Eric Mirkov, Texas A & M

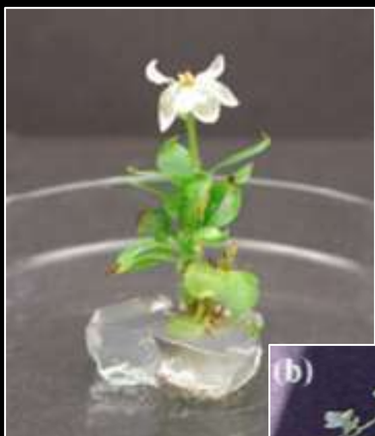
Defensin-like proteins from spinach promising

Insertion of a transgene that elevates natural systemic acquired resistance also promising

Courtesy of Manjul Dutt and Jude Grosser, Citrus Research and Education Center, Florida, USA

Overexpression of endogenous flowering genes induce early flowering in several tree species

Apple



Orange

Plum

Poplar

Rapid flowering of plum in the field to speed virus resistance breeding

Courtesy of Ralph Scorza, USDA ARS

Early flowering effective in eucalypts too….

And flowering accelerated in poplar by suppression of native genes

the plant journal

SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2010) doi: 10.1111/j.1365-313X.2010.04185.x

Populus CEN/TFL1 regulates first onset of flowering, axillary meristem identity and dormancy release in *Populus*

Rozi Mohamed[1,†,‡], Chieh-Ting Wang[2,†,§], Cathleen Ma[1], Olga Shevchenko[1], Sarah J. Dye[1], Joshua R. Puzey[2
Elizabeth Etherington[1], Xiaoyan Sheng[2], Richard Meilan[3], Steven H. Strauss[1] and Amy M. Brunner[2,*]

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[3]Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907-2061, USA

Insect resistant poplars commercially approved in China - Bt *cry1*

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

Currently creating new hybrids by crossing *Bt* poplar with other poplars

Courtesy of Menghu-Zhu Lu, Chinese Academy of Forestry

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

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.

Growth benefits despite low insect pressure during large field trial of resistant genotypes

Lignin-modified trees – much improved ethanol or pulp yields

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… …restières, 45075 Orléans, France… …Pierre Bourgin, Unité Mixte de Reche… …ry and Microbiology, Ghent Universi… …t Lansing, MI 48824; and [h]Departme… …at Lakes Bioenergy Research Cente…

Contributed by…

Lignin is one… enzymatic pr… *tremula* x *Pop*… tase (CCR), th… specific branc… field trials in… ture. Wood… of the red xy… regulation. S… conditions (n… simultaneous… that wood f… 161% increas… rial from the… trees, includi… yielded ~20%… down-regula… that *CCR* do… improve bion… 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… first step of the… hydroxycinnamoyl-… aldehydes (mainly… egulation of *CCR*… -13). *CCR*–down-… ange to wine-red… patches along the… l with a reduction… vels of ferulic acid

…ng the conversion…), we have evalu… lown-regulated in… trials were estab… ocess of obtaining… essential step in… in the laboratory… n because greenhouse-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

Significance

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

PLANT BIOLOGY

Freeze-tolerant *Eucalyptus*

Proposed for commercial deregulation in USA

Results from first winter in South Carolina

Control

Lead Line

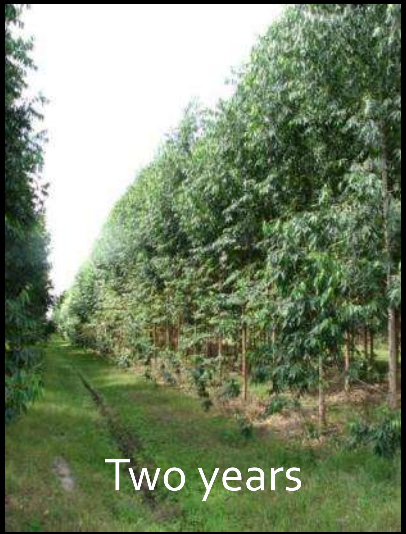
Results from second winter in Alabama

Lead Lines + Control

Field results indicate freezing tolerance to ~16°F (- 8° to - 9°C)

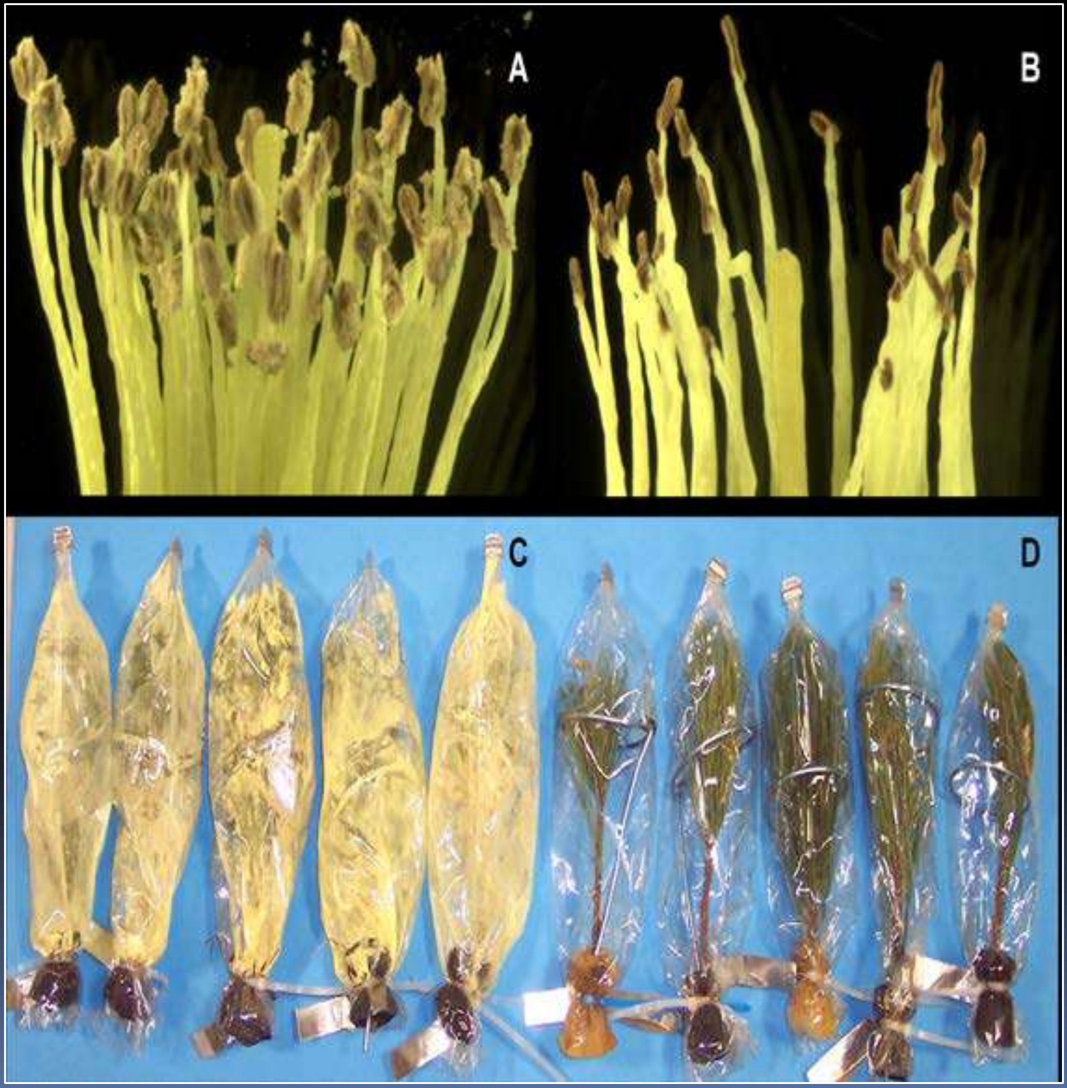
Provided by Arborgen

Many eucalypt field trials underway



Courtesy of Les Pearson, Arborgen

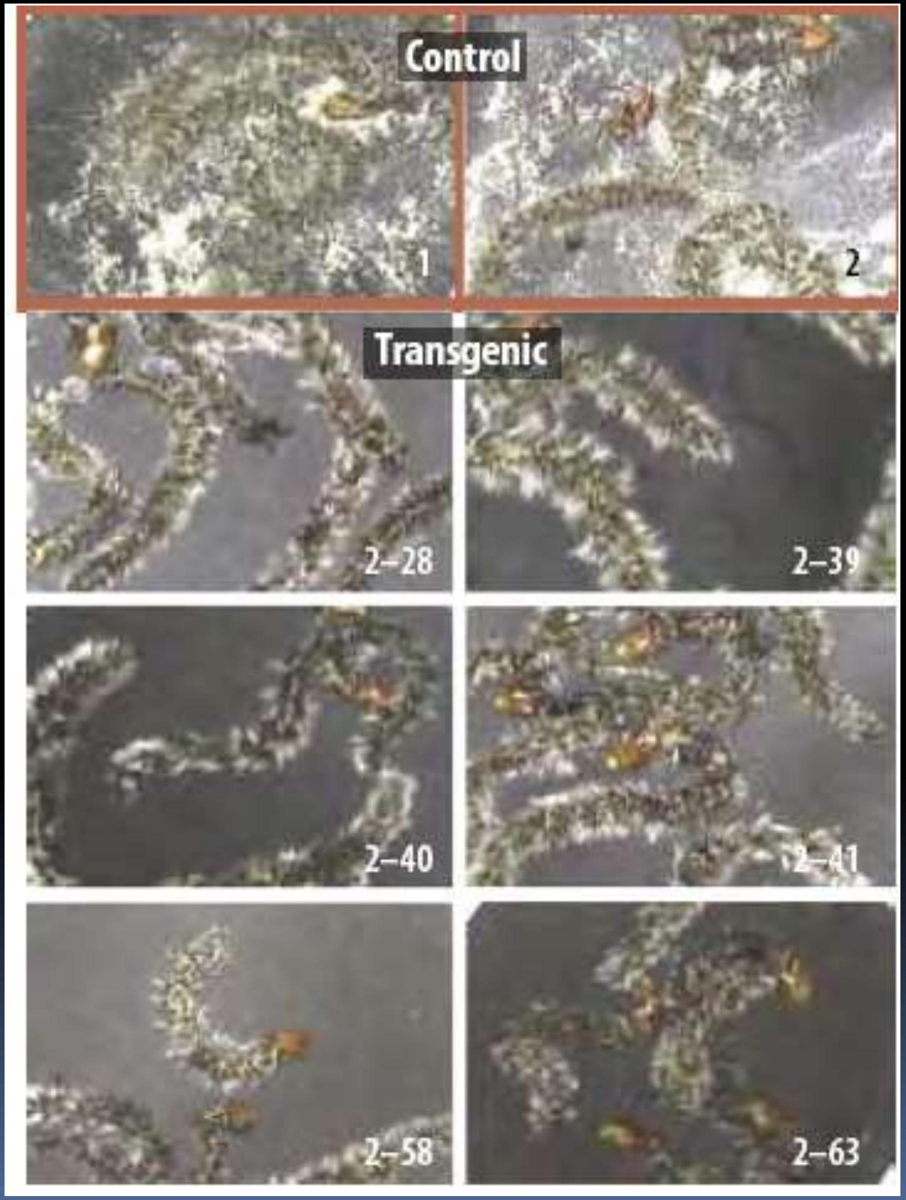
Field grown male-sterile eucalypts and pine - Arborgen



Complete and stable male-sterility over several years in the field

Similar results for poplar

Brunner et al. 2007, Elorriaga et al. 2014
Tree Genetics and Genomes



Complete sterility - Undeveloped catkins by suppression of native *LEAFY* gene in poplar (RNAi)

Klocko et al. 2014, American Soc. For Plant Biology, Portland, Oregon

Sterility a valuable tool for battling invasive, exotic forest trees: “Wilding” in New Zealand

GE appears to be a useful too 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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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

Goals for today

- Value of GMO methods for trees and other woody perennials
  - Horticulture and forestry
- Overview of advanced GMO varieties in production or developmental pipelines
- Regulatory problems needing high level policy solutions

There is hardly a trickle of GMO tree products compared to its scientific potential – why?

Social / market and regulatory barriers are great

Genetically modified arboriculture

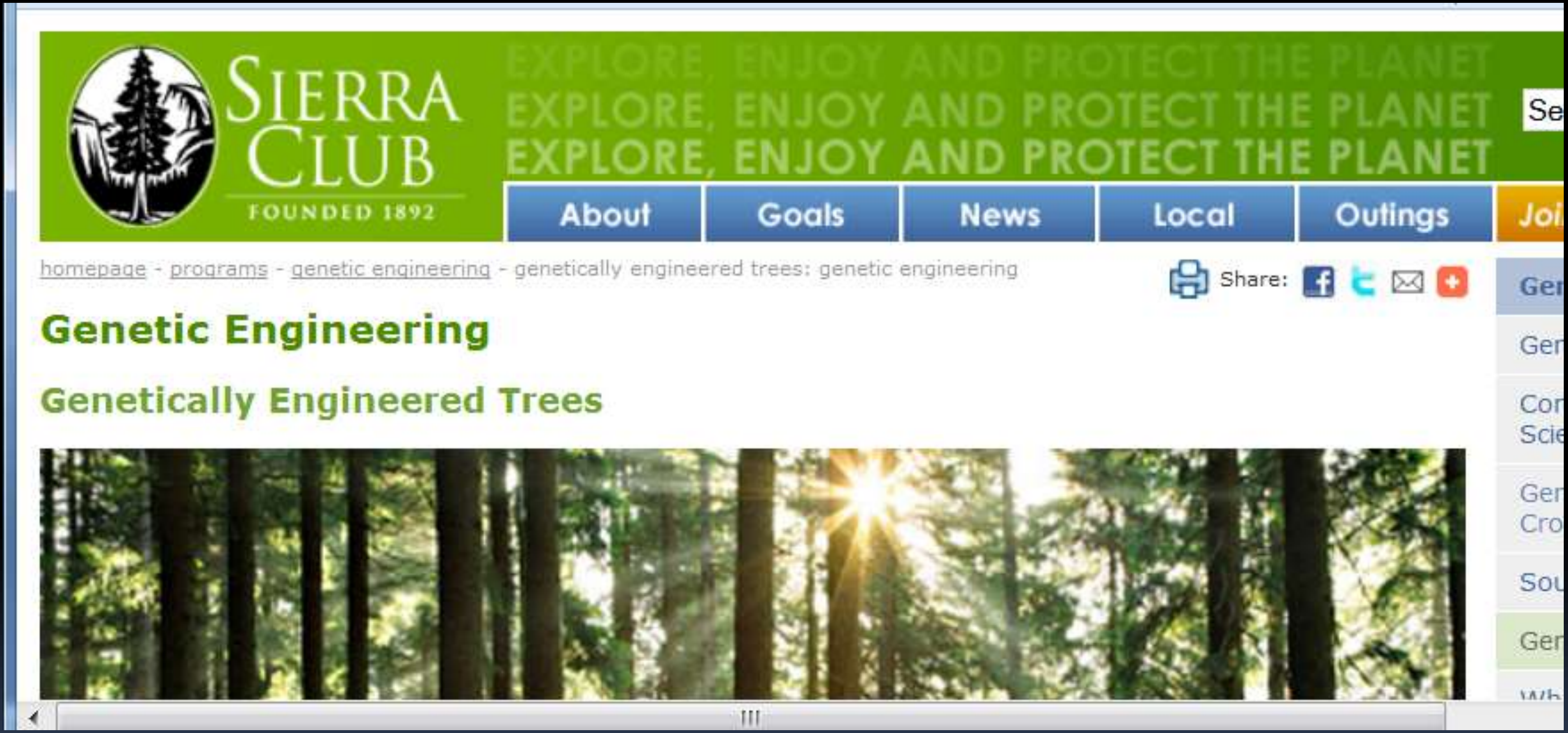
Down in the forest, something stirs

The Economist, 2005

Transgenic trees are controversial

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 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…”

Regulatory problems with GMO trees also severe

Event-specific decisions and costs

- Slowness/difficulty of introgression
- Need diverse genotypes transformed (varieties)
- Much smaller economic benefits to pay back regulatory costs from single events

Regulatory problems with GMO trees also severe

Presumption of harm from GE method during research and breeding

- All gene flow must be prevented during research
  - Some movement will occur due to incomplete domestication, wild and feral relatives, wide pollen and often seed movement
- Impedes or prevents stress resistance and other complex trait development
  - Require extensive field trials to test many concepts and insertion events
- No longer makes sense in era of precision breeding, cisgenics, intragenics

A serious regulatory problem under USA system

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

An international regulatory issue given Cartagena Protocol and trade

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.

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 ... the CBD

Millions of dollars of regulatory costs to use a gene we eat daily in spinach

The New York Times

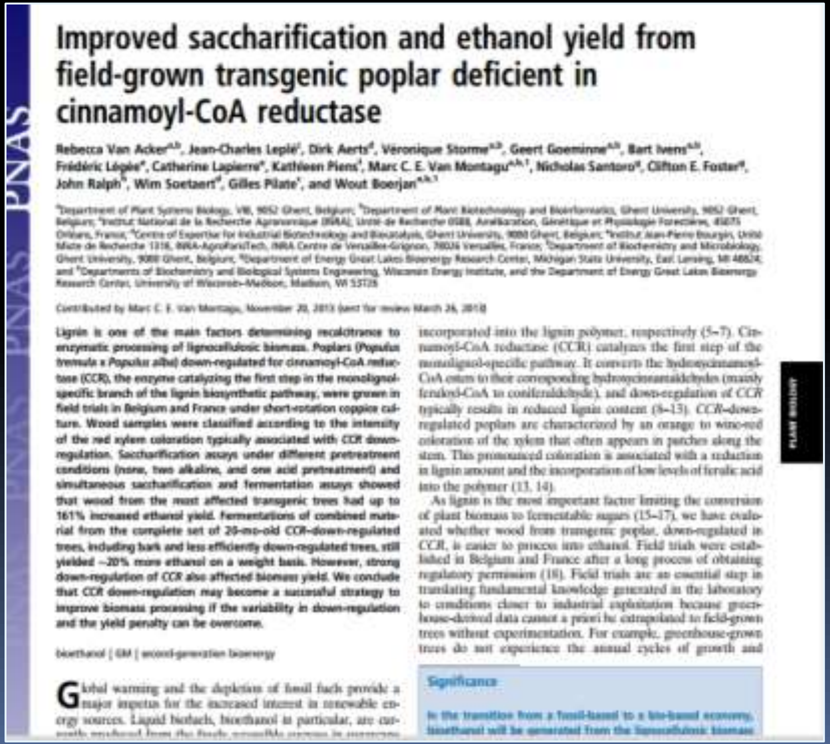
July 27, 2013

A Race to Save the Orange by Altering Its DNA

Lignin-modified trees

Concept proven, but much refinement needed

Type of gene, promoters, extent of modification, environment, stand age, genotype modified



PNAS

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

Rebecca Van Acker, Jean-Charles Leplé, Dirk Aerts, Véronique Storme, Geert Goeminne, Bart Ivens, Frédéric Légée, Catherine Lapierre, Kathleen Piens, Marc C. E. Van Montagu, Nicholas Santoro, Clifton E. Foster, John Ralph, Wim Soetaert, Gilles Pilate, and Wout Boerjan

Lignin is one of the main factors determining recalcitrance to enzymatic processing of lignocellulosic biomass. Poplars (*Populus tremula x Populus alba*) down-regulated for cinnamoyl-CoA reductase (CCR), the enzyme catalyzing the first step in the monolignol-specific branch of the lignin biosynthetic pathway, were grown in field trials in Belgium and France under short-rotation coppice culture. Wood samples were classified according to the intensity of the red xylem coloration typically associated with CCR down-regulation. Saccharification assays under different pretreatment conditions (none, two alkaline, and one acid pretreatment) and simultaneous saccharification and fermentation assays showed that wood from the most affected transgenic trees had up to 161% increased ethanol yield. Fermentations of combined material from the complete set of 20-mo-old CCR-down-regulated trees, including bark and less efficiently down-regulated trees, still yielded ~20% more ethanol on a weight basis. However, strong down-regulation of CCR also affected biomass yield. We conclude that CCR down-regulation may become a successful strategy to improve biomass processing if the variability in down-regulation 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 cur-

incorporated into the lignin polymer, respectively (5–7). Cinnamoyl-CoA reductase (CCR) catalyzes the first step of the monolignol-specific pathway. It converts the hydroxycinnamoyl-CoA esters to their corresponding hydroxycinnamaldehydes (mainly feruloyl-CoA to coniferaldehyde), and down-regulation of *CCR* typically results in reduced lignin content (8–13). *CCR*-down-regulated poplars are characterized by an orange to wine-red coloration of the xylem that often appears in patches along the stem. This pronounced coloration is associated with a reduction in lignin amount and the incorporation of low levels of ferulic acid into the polymer (13, 14).

As lignin is the most important factor limiting the conversion of plant biomass to fermentable sugars (15–17), we have evaluated whether wood from transgenic poplar, down-regulated in *CCR*, is easier to process into ethanol. Field trials were established in Belgium and France after a long process of obtaining regulatory permission (18). Field trials are an essential step in translating fundamental knowledge generated in the laboratory to conditions closer to industrial exploitation because greenhouse-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

Significance

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

Cold tolerant *Eucalyptus*

Concept proven, much refinement needed

Type of gene, promoters, extent of modification, environment, stand age, genotype modified

Provided by Arborgen

Pest epidemics increasing with travel and climate change

Need rapid use of all available tools, including GE – regulations make impossible

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

Gene targeting, cisgenics, intragenics coming along fast in genomic age

= increased precision, safer than breeding!


NEWS & VIEWS

PLANT BIOTECHNOLOGY

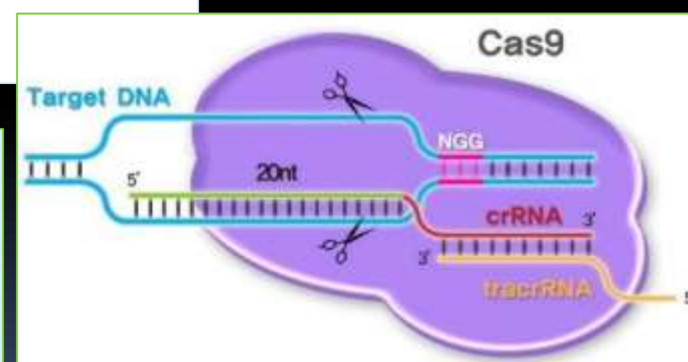
Zinc fingers on target

NATURE|Vol 459|21 May 2009

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

In summary

- Many examples show great progress on a wide variety of fronts
  - Despite very large social barriers and disinvestment over the last decade plus
- Extraordinary regulatory barriers based on the process rather than the product
  - Makes implementation of GE tools on a scale and speed relevant to need and benefit unworkable
- Need for fundamental regulatory change
  - **A start**: Adventitious presence allowances during research and development for cisgenes, intragenes, and edited genes with known, safe marker systems