Gene editing in agriculture and forestry: Why the fuss?

Steve Strauss
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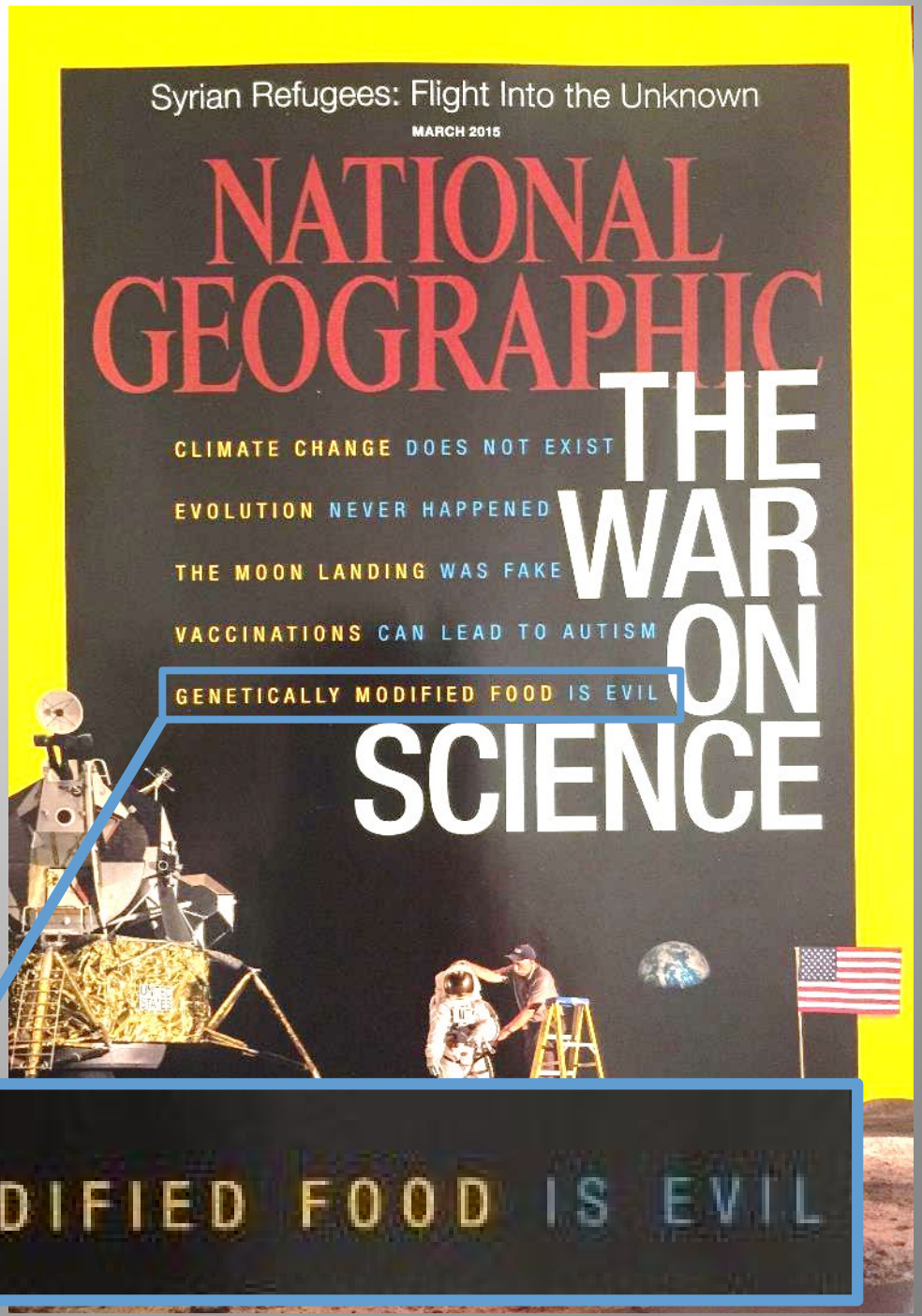
Agenda

- Setting the stage for “the fuss”
- The science
- Some recent research

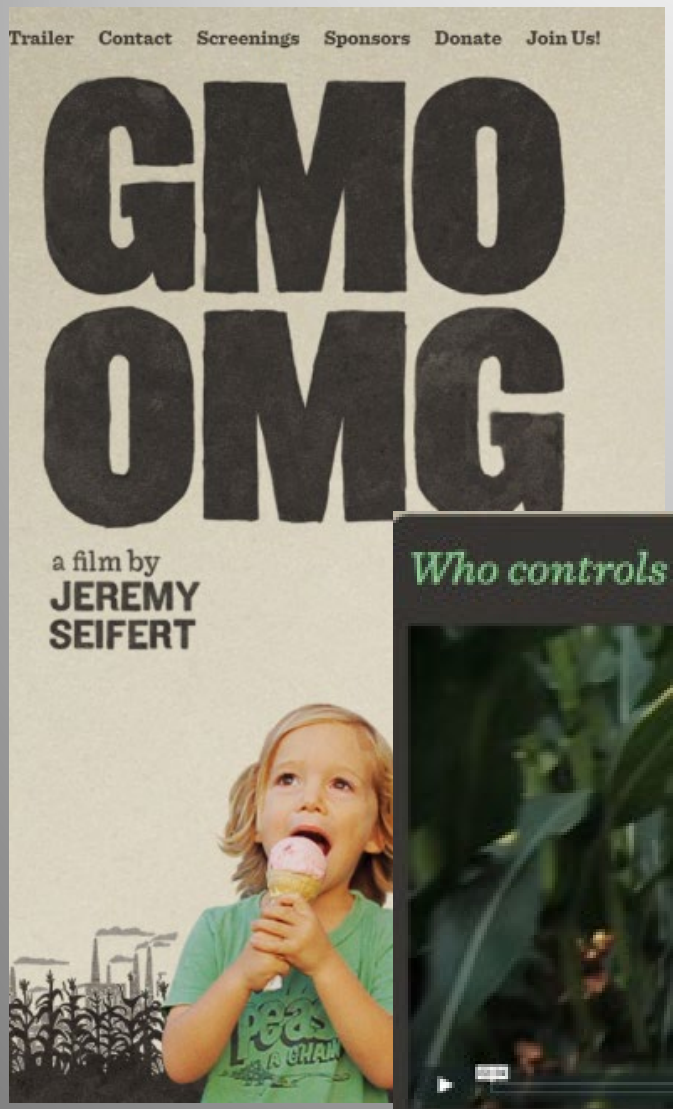
"GMO" has taken on a social stigma that has nothing to do with science – economics, environment, or food safety

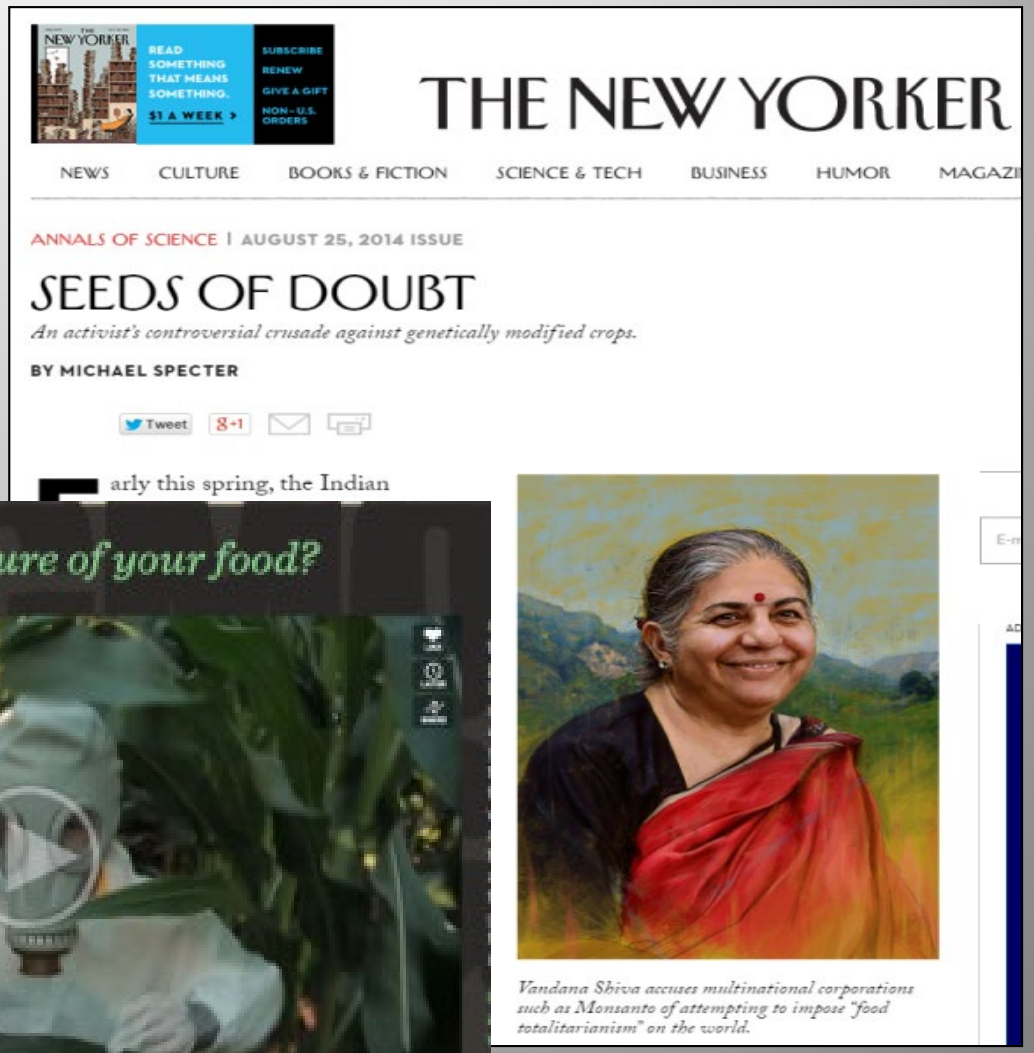
GMOs one of the "fake news - fake science" issues

It's hard to tell what science is saying amidst all the noise

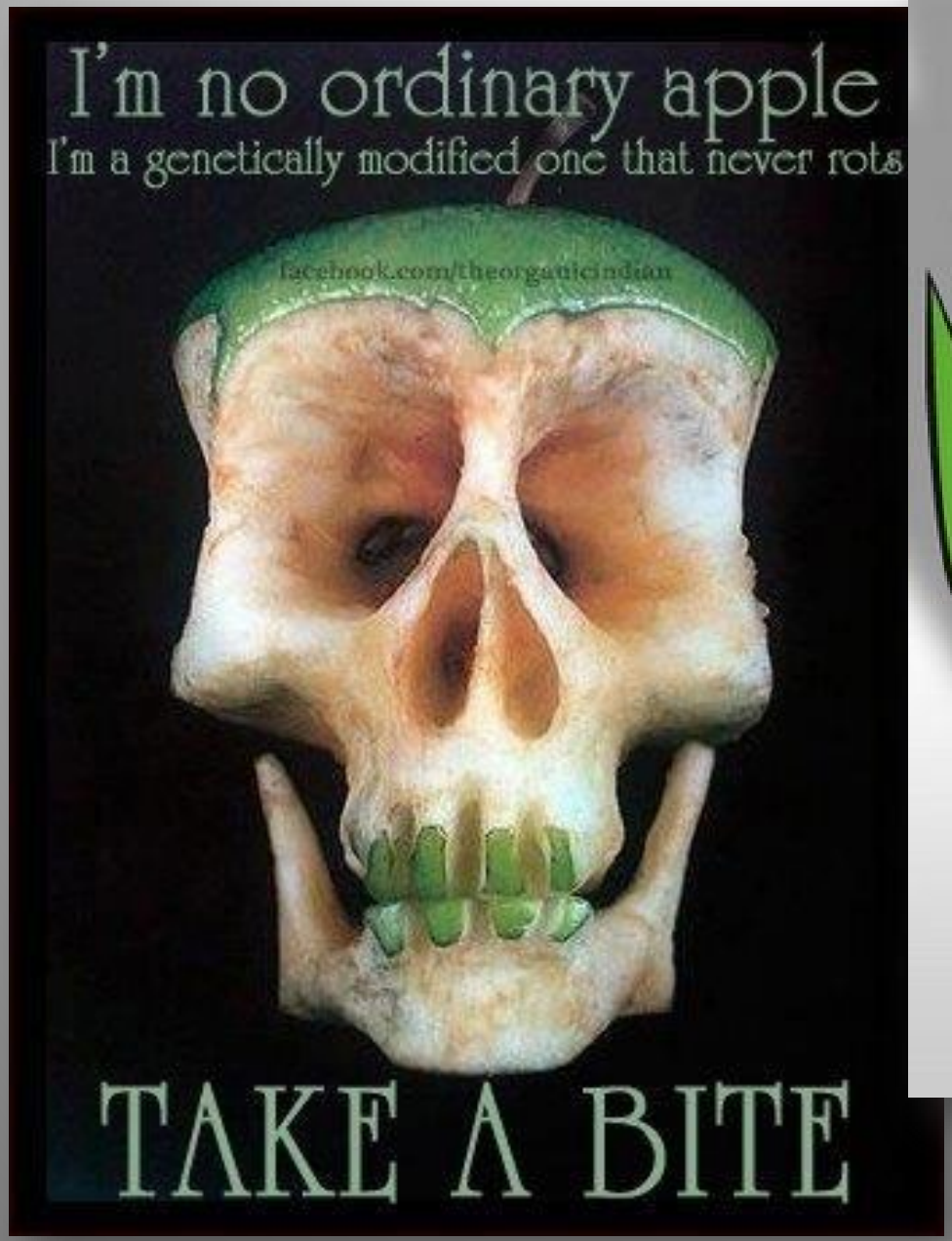


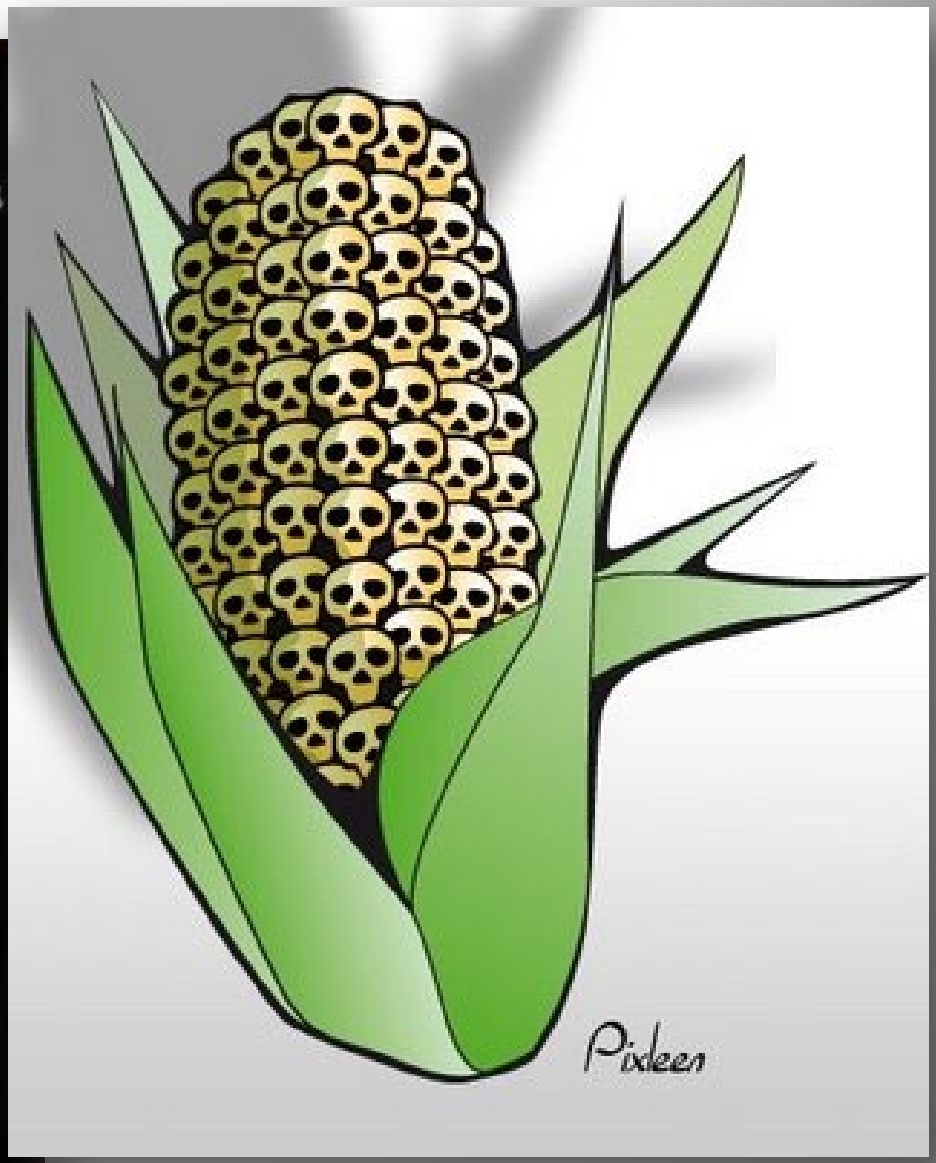
There are numerous myths that are rampant and recycled in media



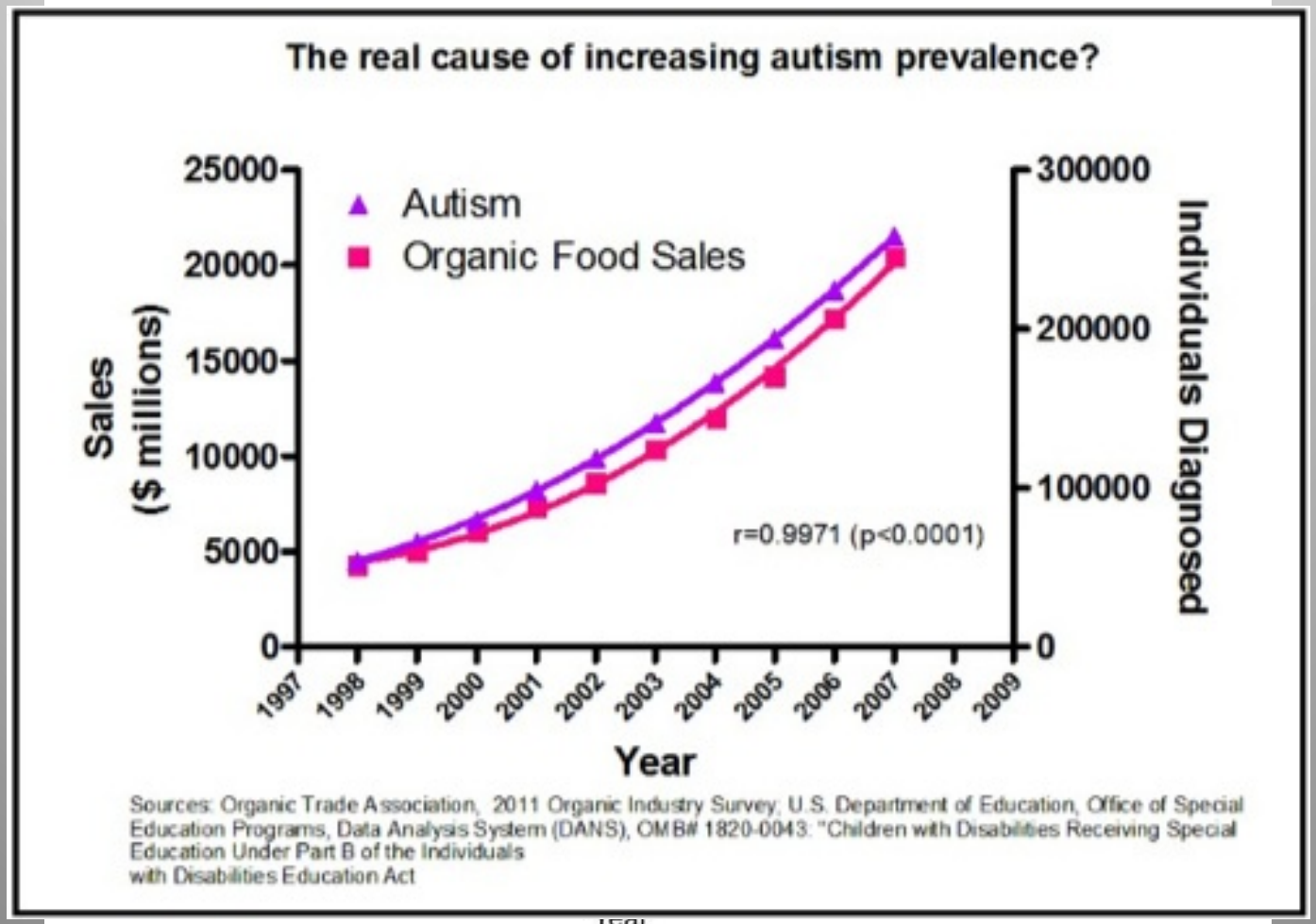


And many more…





Much pseudo-science: “Half of all children will be Autistic by 2025 due to Roundup warns MIT scientist”



Food Evolution movie debunks the “data” of the extreme anti-GMO left

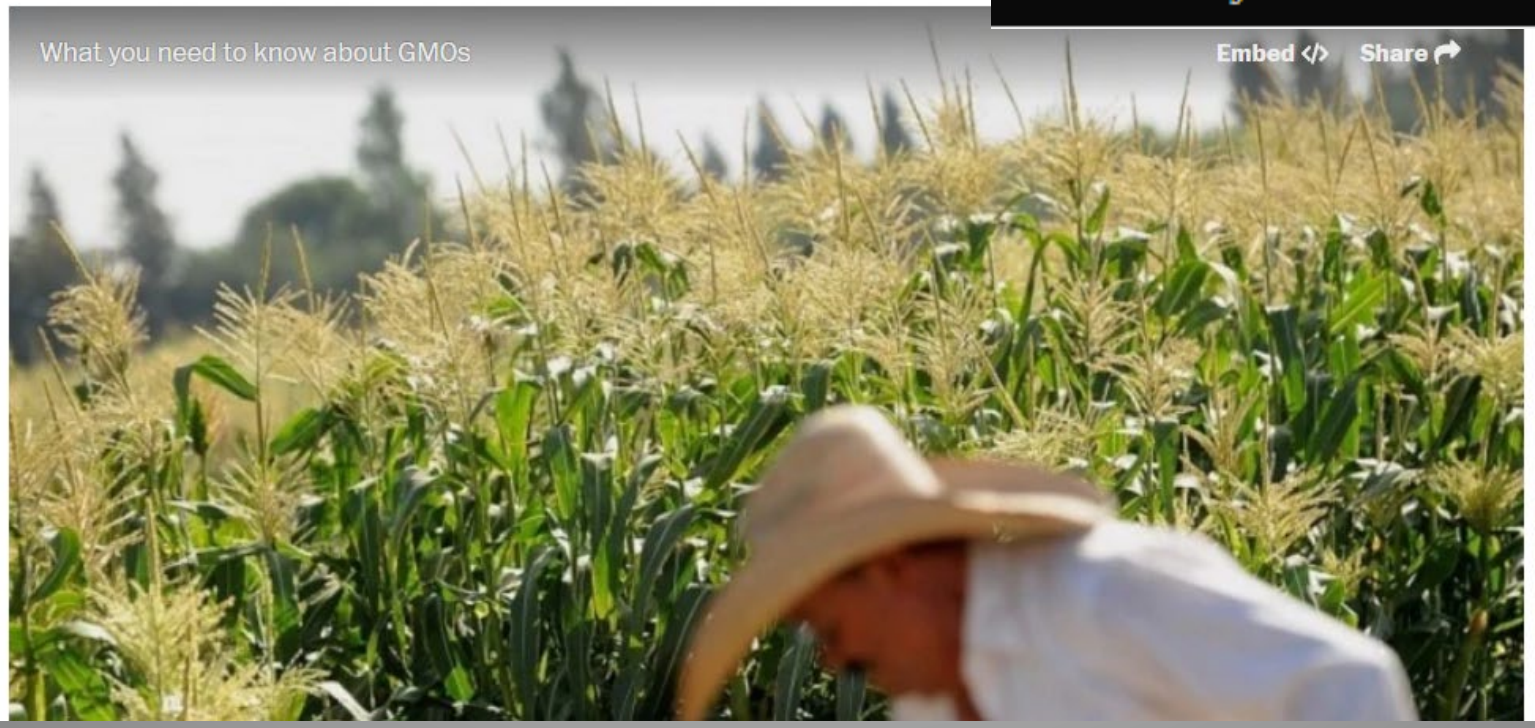
Some scientists try to change perception of GMOs

Speaking of Science

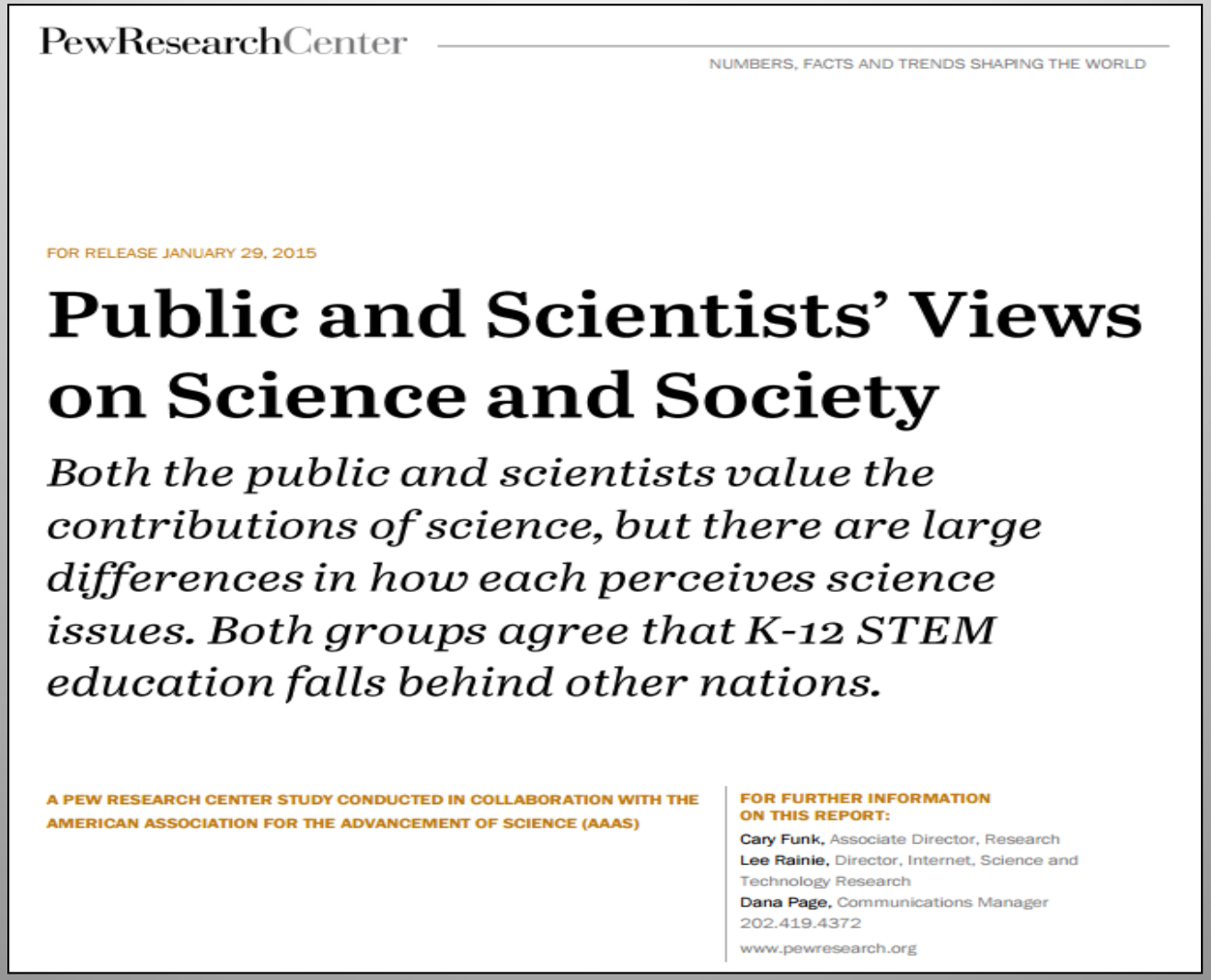
107 Nobel laureates sign letter blasting Greenpeace over GMOs

By Joel Achenbach June 30, 2016

The Washington Post
Democracy Dies in Darkness



Pew Survey on views of controversial science issues - 2015



PewResearchCenter

NUMBERS, FACTS AND TRENDS SHAPING THE WORLD

FOR RELEASE JANUARY 29, 2015

Public and Scientists' Views on Science and Society

Both the public and scientists value the contributions of science, but there are large differences in how each perceives science issues. Both groups agree that K-12 STEM education falls behind other nations.

A PEW RESEARCH CENTER STUDY CONDUCTED IN COLLABORATION WITH THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (AAAS)

FOR FURTHER INFORMATION ON THIS REPORT:
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202.419.4372
www.pewresearch.org

http://www.pewinternet.org/2015/01/29/public-and-scientists-views-on-science-and-society/

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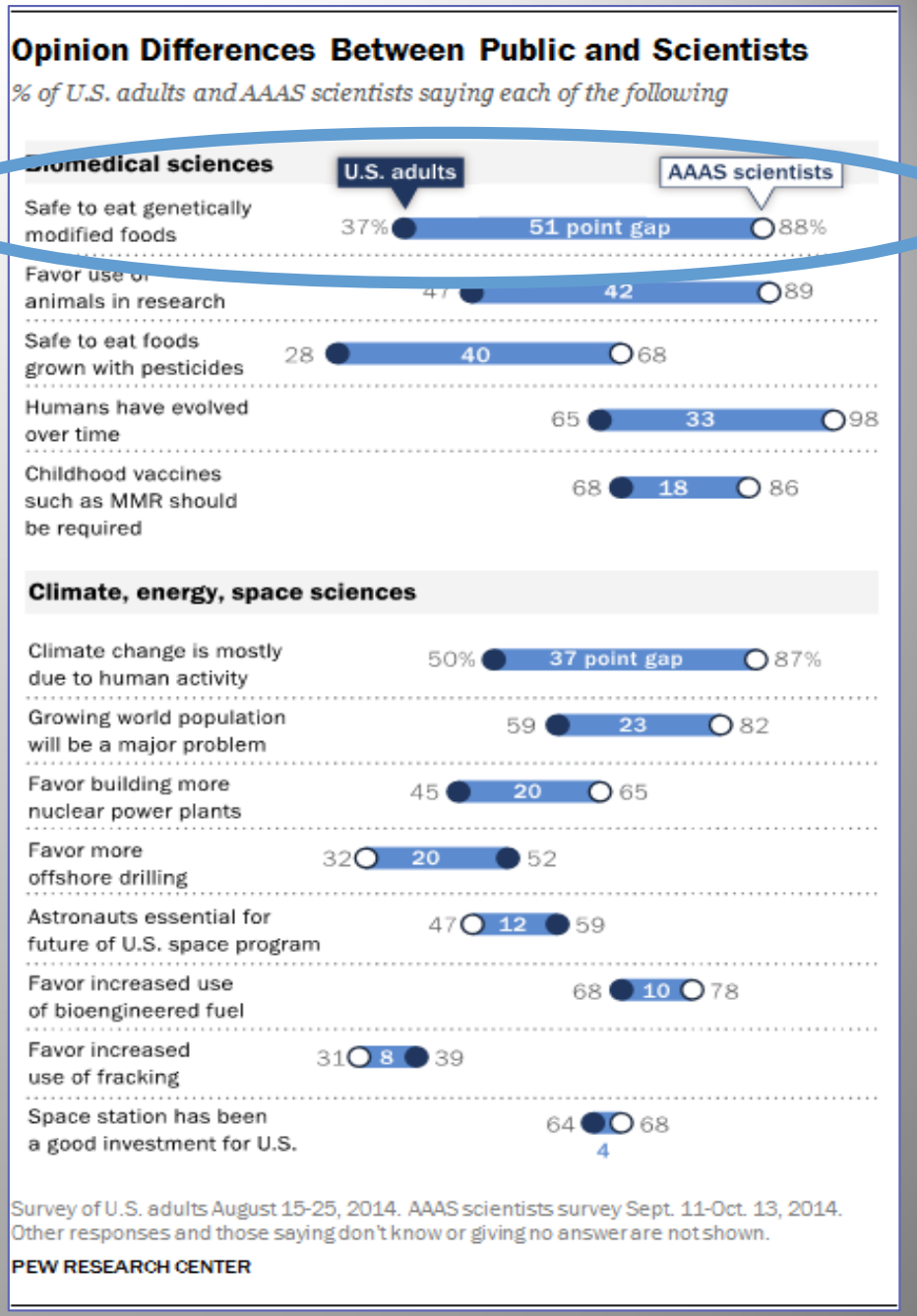
PUBLICATIONS | TOPICS | PRESENTATIONS | INTERACTIVES | KEY INDICATORS

JANUARY 28, 2015

PUBLIC AND SCIENTISTS' VIEWS ON SCIENCE AND SOCIETY

88% of AAAS scientists say genetically modified foods are safe to eat; only 37% of the public agrees

GMOs the largest scientist-public gap, 51%, of any issue surveyed



http://www.pewinternet.org/2015/01/29/public-and-scientists-views-on-science-and-society/

2018 - Unease with GMO safety growing?

FEATURED

Survey shows growing distrust of GMO safety

By MATEUSZ PERKOWSKI Capital Press Dec 1, 2018

A recent survey has detected a sharp uptick in the percentage of Americans who consider foods with genetically engineered ingredients to be worse for their health.

The 2018 survey by the Pew Research Center found that 49 percent of respondents viewed such foods as less healthful than those without genetically engineered ingredients, up from 39 percent just two years earlier.

GMO is a victim of social forces far beyond its scientific domain

- *"It is accurate to say that many of the real ethical issues [of GMOs in agriculture] have little to do with the use of transgenic technologies"* (Burkardt et al. 2005, Agricultural Ethics, CAST)

CAST

COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY

ISSUE PAPER

NUMBER 29 FEBRUARY 2005

AGRICULTURAL ETHICS

INTRODUCTION

It is widely known that agriculture has a long history. Starting approximately 12,000 years ago, the domestication of plants and animals began independently in several different places, including centers in West Asia, East Asia, Central America, and South America. Domestication also may have occurred in other locations, although convincing archeological evidence has not been found. In the

TASK FORCE MEMBERS: **Jeffrey Burkhardt, Chair**, Department of Food and Resource Economics, University of Florida, Gainesville; **Gary Comstock**, Department of Philosophy and Religion, North Carolina State University, Raleigh; **Peter G. Hartel**, Department of Crop and Soil Sciences, University of Georgia, Athens; **Paul B. Thompson**, Department of Philosophy, Michigan State University, East Lansing; REVIEWERS: **Maarten J. Chrispeels**, Center for Molecular Agriculture, University of California–San Diego; **Charles C. Muscoplat**, College of Agricultural, Food and Environmental Sciences, University of Minnesota, St. Paul; **Robert Streiffer**, Department

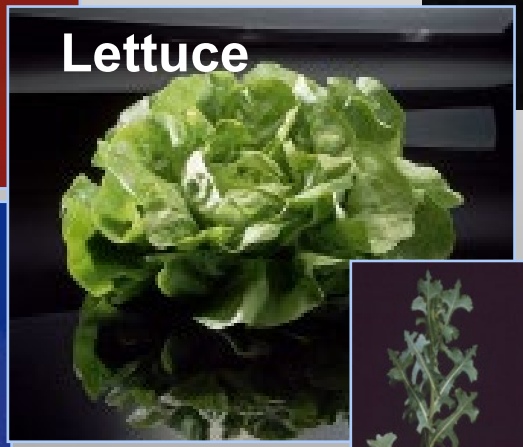
commented on the importance of agricultural knowledge in the quest for the "good life" by the individual and the polity. The fundamental value of agriculture was highlighted by Enlightenment thinkers from John Locke to Thomas Jefferson, who underscored the political, economic, and philosophical importance of "tillers of the soil" (Spiegel 1991). In the United States, problems faced by farmers became the focus of the nine-

As with any environmental technology in today's world, the science of benefits, tradeoffs, and harms is complex – No silver bullets

Agenda

- Setting the stage for “the fuss”
- The science
- Some recent research

These are highly genetically modified but not GMO



Many plant varieties derived from induced mutations – not GMO

Calrose 76 semi-dwarf rice

Over 3,000 crop varieties derived from mutagenesis have been commercialized

High oleic sunflower

Rio Red grapefruit

Domesticated animals are radically modified – not GMO





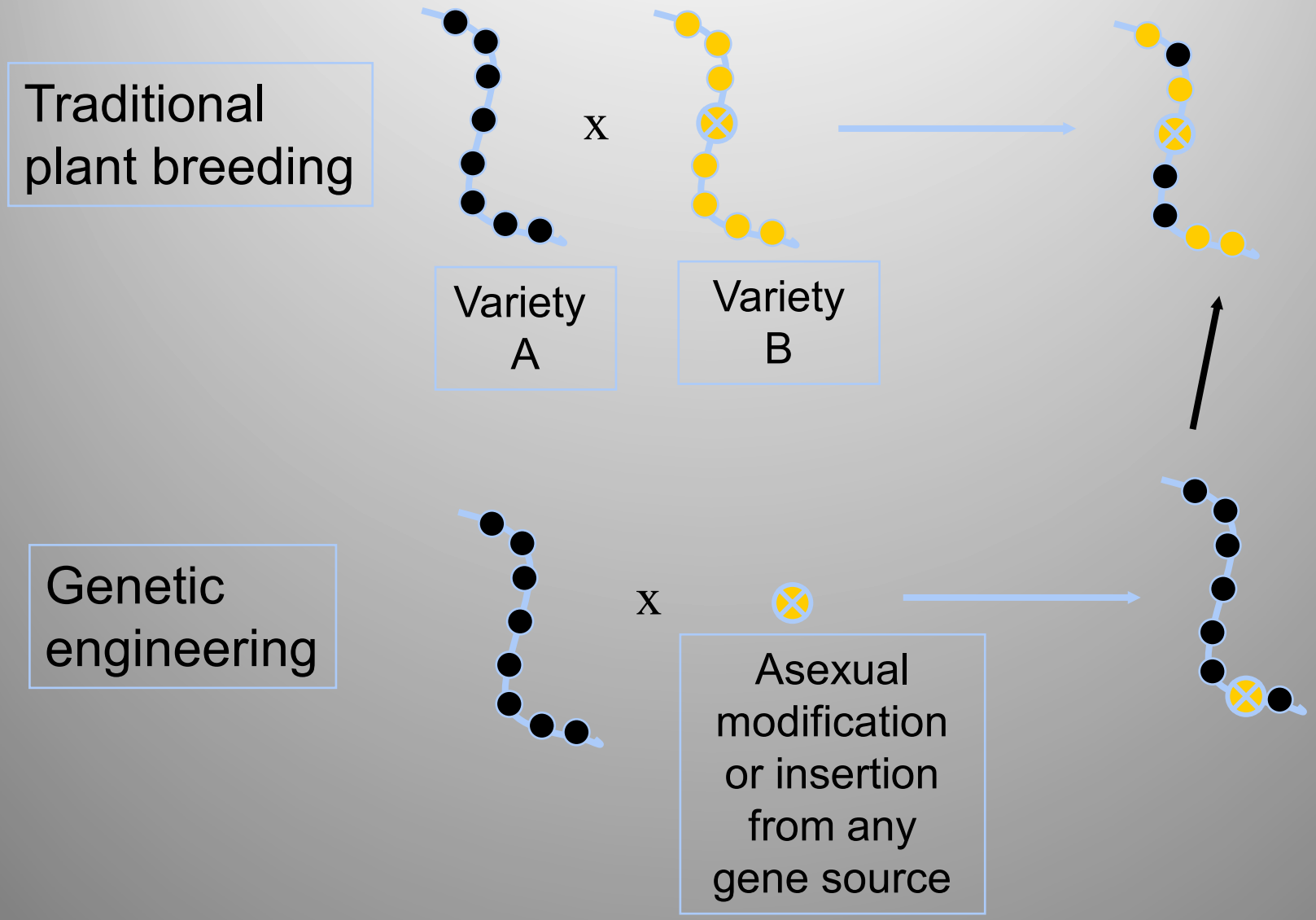








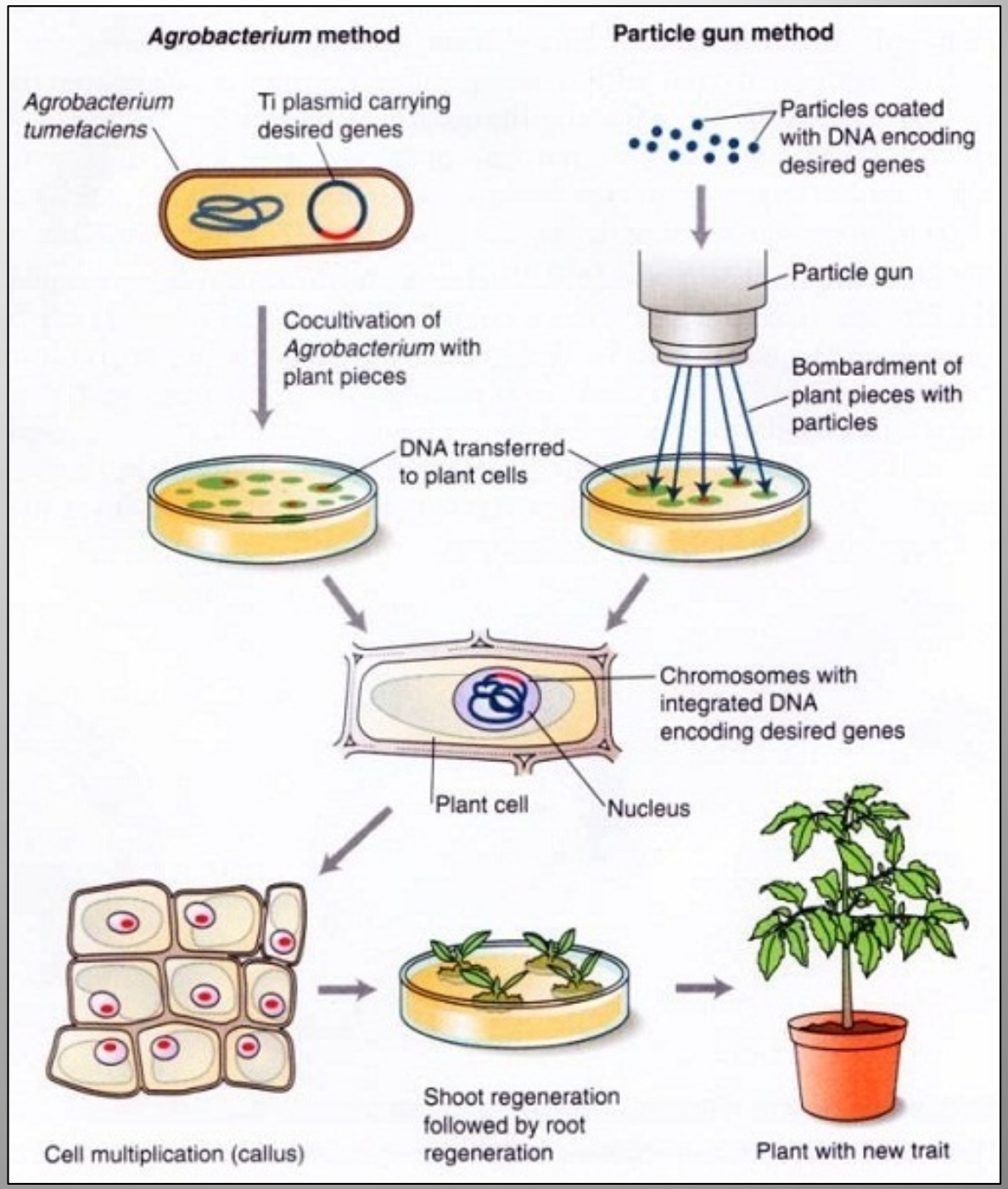
GE method (genetic engineering) defined: Asexual genetic modification



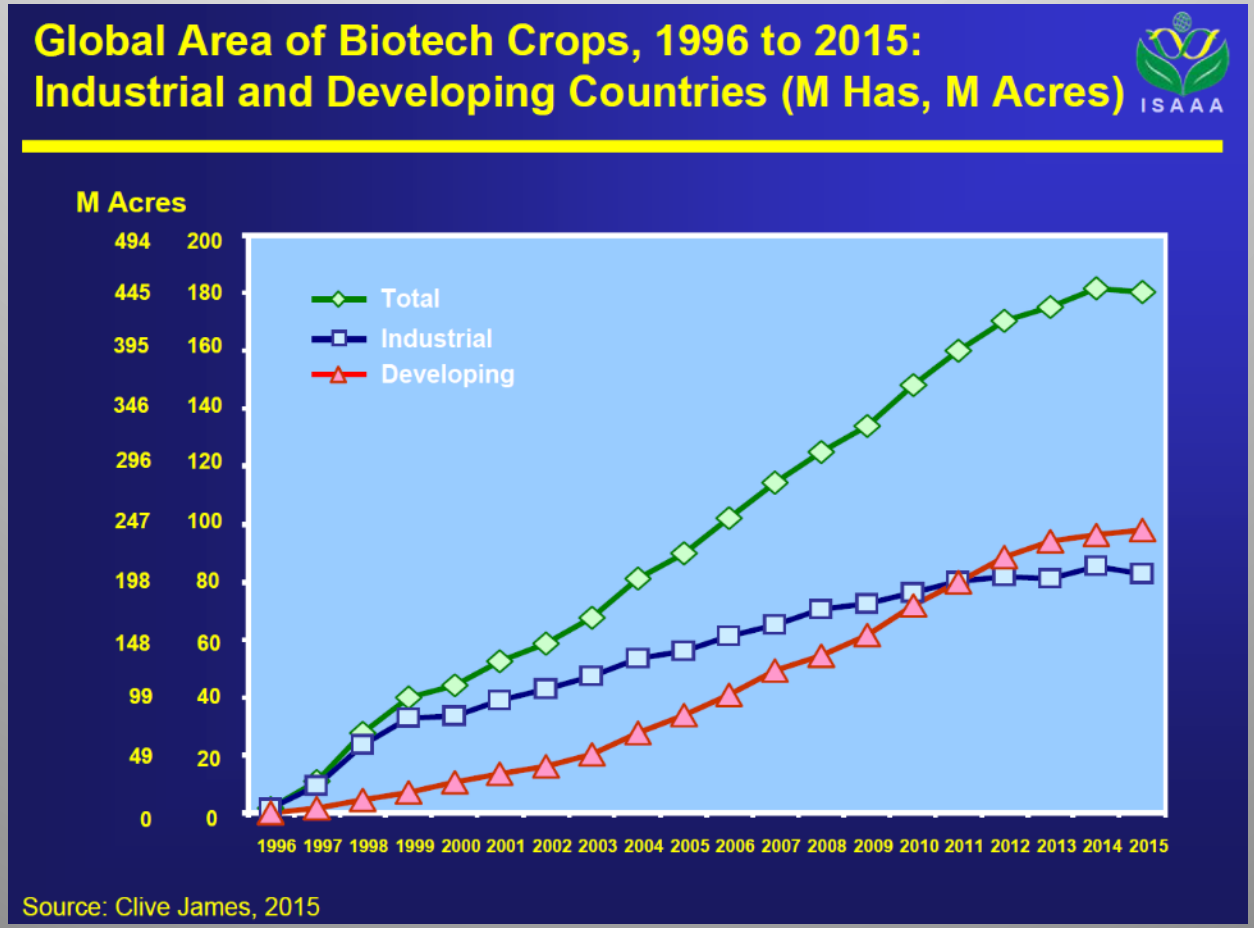
What is GE

- 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

Overview of steps to create a GE plant



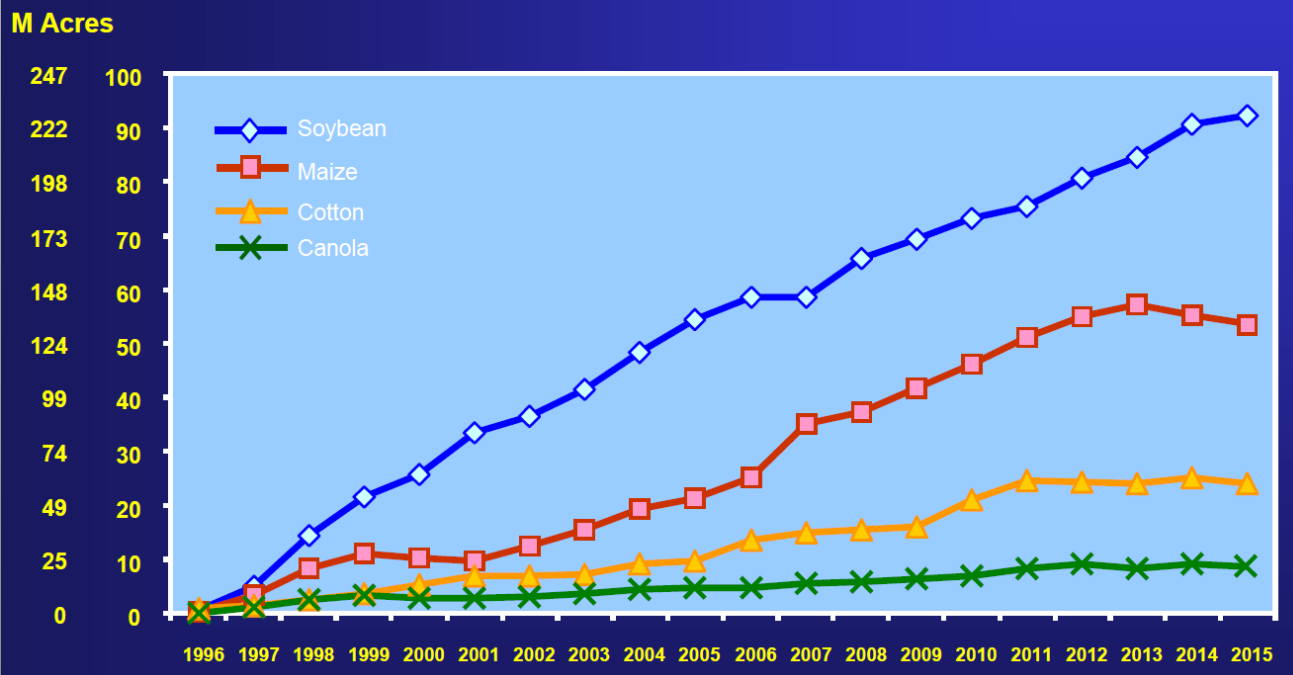
First generation herbicide and insect resistant crops were rapidly adopted by farmers, both in the developed and developing world



Four crops dominate, 8+ in USA

Global Area of Biotech Crops, 1996 to 2015: By Crop (Million Hectares, Million Acres)

ISAAA

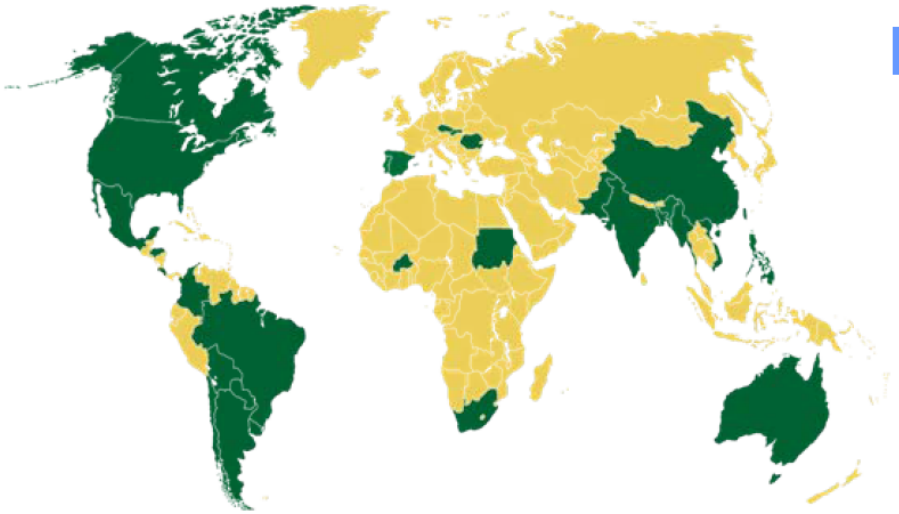


Source: Clive James, 2015

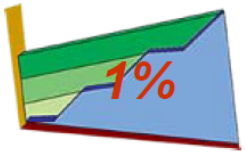
Adoption by 28 countries, but rates highly variable due to social stigma

Global Area (Million Hectares) of Biotech Crops, 2015: by Country

ISAAA



Marginal Decrease from 2014



28 countries which have adopted biotech crops

In 2015, global area of biotech crops was 179.7 million hectares, representing a marginal decrease of 1% from 2014, equivalent to 1.8 million hectares.

Source: Clive James, 2015.

Biotech Mega Countries

50,000 hectares (125,000 acres), or more

		Million Hectares
1.	USA	70.9
2.	*Brazil**	44.2
3.	*Argentina**	24.5
4.	*India**	11.6
5.	Canada	11.0
6.	*China**	3.7
7.	*Paraguay**	3.6
8.	*Pakistan**	2.9
9.	*South Africa**	2.3
10.	*Uruguay**	1.4
11.	*Bolivia**	1.1
12.	*Philippines**	0.7
13.	Australia	0.7
14.	*Burkina Faso**	0.4
15.	*Myanmar**	0.3
16.	*Mexico**	0.1
17.	Spain	0.1
18.	*Colombia**	0.1
19.	*Sudan**	0.1

Less than 50,000 hectares

*Honduras**
*Chile**
Portugal
*Vietnam**
Czech Republic
Slovakia
*Costa Rica**
*Bangladesh**
Romania

** Developing countries*

Global “meta-analysis” of early impacts: 2014

“147 original studies were included.”
“On average, GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%.”

Insect resistant crops with huge impact on economics and sustainability

Pray et al., 2002. Plant J. 31:423-430
Photo: entomologytoday.org Dominic Reisig

Non-GMO vs. insect resistant Bt cotton without pesticide use

At edges: Insect resistant eggplant a great success in Bangldesh, illegal plantings in India in news

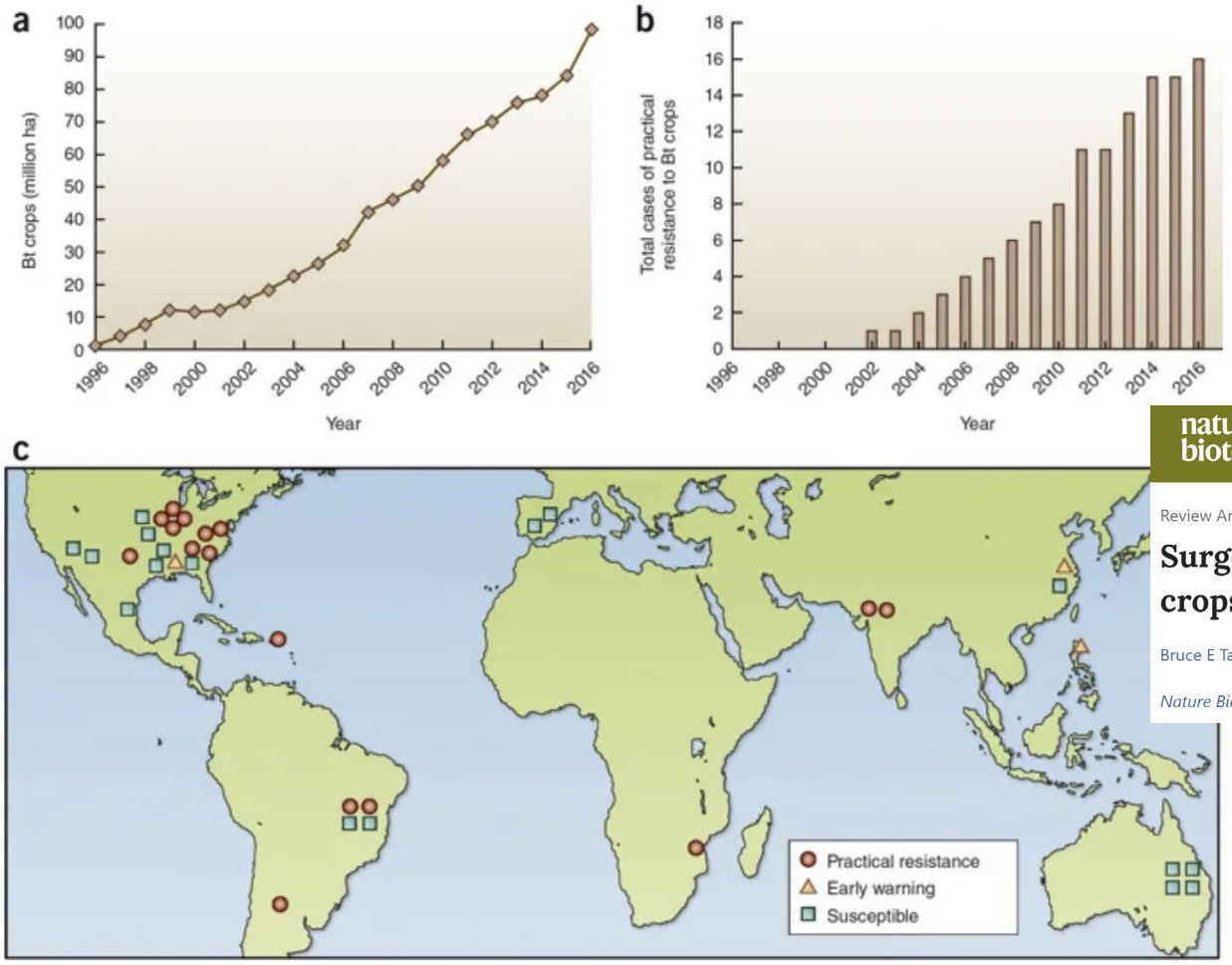
Non-Biotech

Biotech

http://www.isaaa.org/resources/publications/briefs/47/download/isaaa-brief-47-2014.pdf

Insect pest resistance growing

Figure 1: Global status of pest resistance to Bt crops.



nature biotechnology

Review Article | Published: 11 October 2017

Surge in insect resistance to transgenic crops and prospects for sustainability

Bruce E Tabashnik & Yves Carrière

Nature Biotechnology **35**, 926–935(2017) | Cite this article

Herbicide tolerant plants promote conservation tillage – With many environmental benefits thereof

Conservation Technology Information Center

- Lowers greenhouse gas emissions
- Improves soil organic matter
- Reduces erosion and fertilizer runoff into water

GMO crops have accelerated development of herbicide-resistant weeds

And motivated development of new kinds of herbicide tolerant crops

nature biotechnology

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NATURE BIOTECHNOLOGY | NEWS

Glyphosate resistance threatens Roundup hegemony

Emily Waltz

Nature Biotechnology **28**, 537–538 (2010) | doi:10.1038/nbt0610-537
Corrected online **13 October 2010**
Corrigendum (October, 2010)

PDF | Citation | Reprints | Rights & permissions | Article metrics

Weeds are becoming increasingly resistant to glyphosate, a report from the US National Academy of Sciences (NAS) released in April has found. The driving force, according to the report, is farmers' dependence on the weed killer accompanied by the widespread adoption of genetically modified (GM) herbicide-tolerant crops. Seed makers are hoping to forestall the problem by developing GM crops with 'stacked' traits that tolerate multiple herbicides. But weed scientists warn that if farmers manage these new crops in the same way as they managed their glyphosate-tolerant predecessors, weeds will simply become resistant to the new technologies.

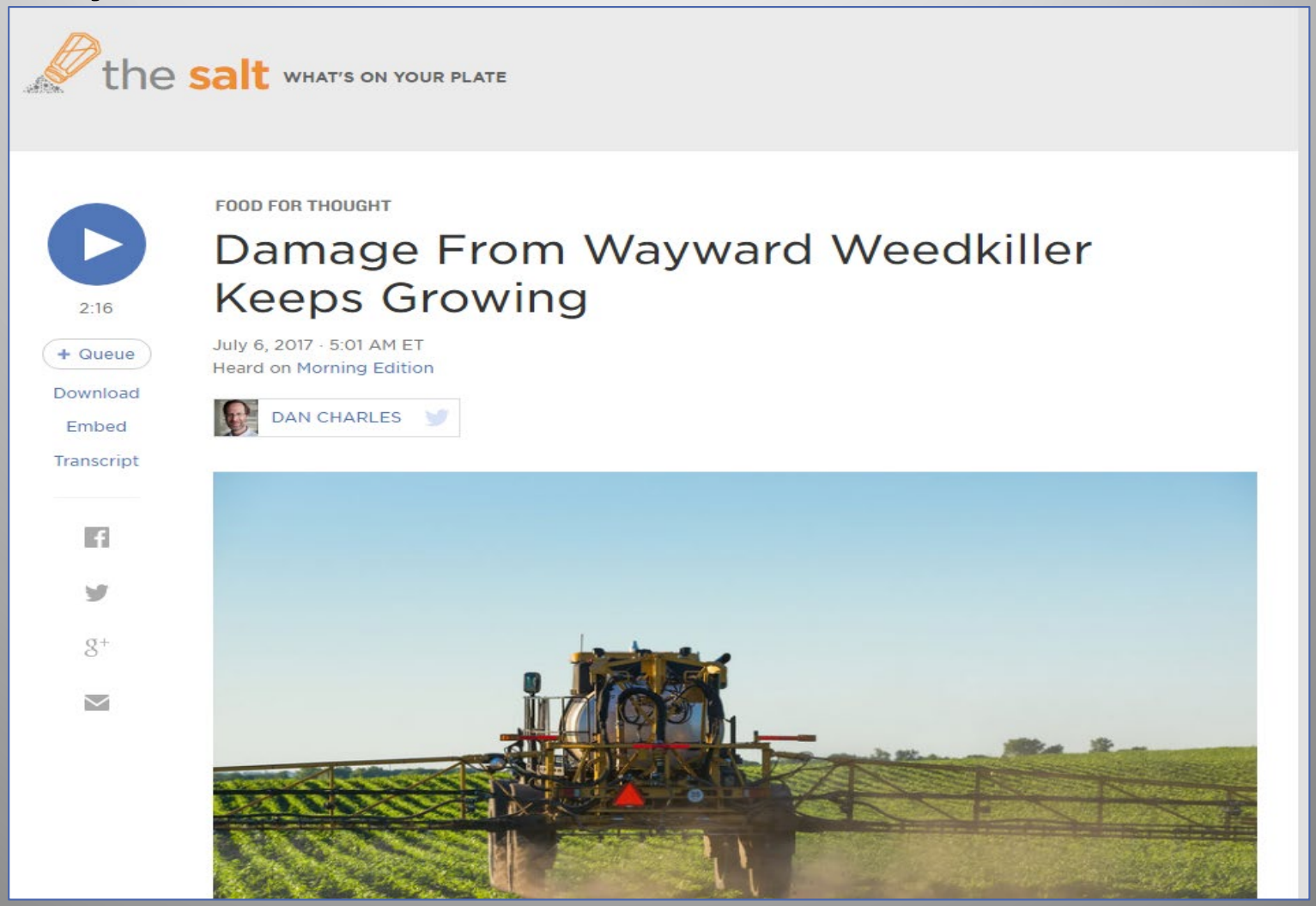
*The number of weed species evolving resistance to glyphosate

BILL BARKSDALE / AGSTOCKUSA /

The original clean fields – HR cotton

Not an uncommon sight now

Damage from growing use of dicamba resistant crops – due to chemical's volatility



Roundup tolerant bentgrass escape in Oregon

483

GMO gr
divides

8.1k
shares

Feds deregulate controversial GMO grass seed

Linn County bills itself as the grass seed capital of the world. But the thriving grass business has been divided by a controversial genetically modified grass developed by Scotts Miracle-Gro. *(Jeff Manning/The Oregonian)*

By **Jeff Manning | The Oregonian/OregonLive**
Email the author | Follow on Twitter
on January 18, 2017 at 10:00 AM, updated January 18, 2017 at 10:18 AM

The U.S. Department of Agriculture on Tuesday deregulated a genetically modified grass that some Oregon farmers and dealers say threatens the state's grass seed business.

http://www.oregonlive.com/business/index.ssf/2017/01/grass_seed_industry_fearful_ab.html

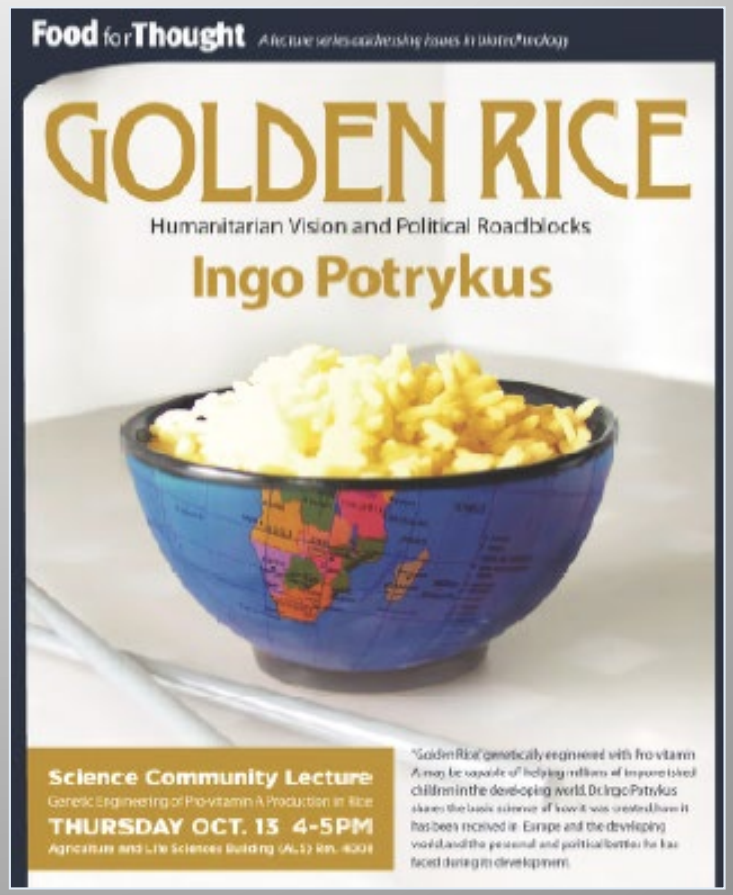
http://www.oregonlive.com/business/index.ssf/2017/01/post_248.html

“Innate” potato – Reduced browning and more – only native potato genes

One hour after cutting – Control vs. Innate

Two days after cutting – Control vs. Innate

Diverse pipeline of biofortification products = enhancement of critical vitamins or nutrients – nearly out there



More than half of the human population suffers from malnutrition!

Forest health a major and growing concern

REVIEW

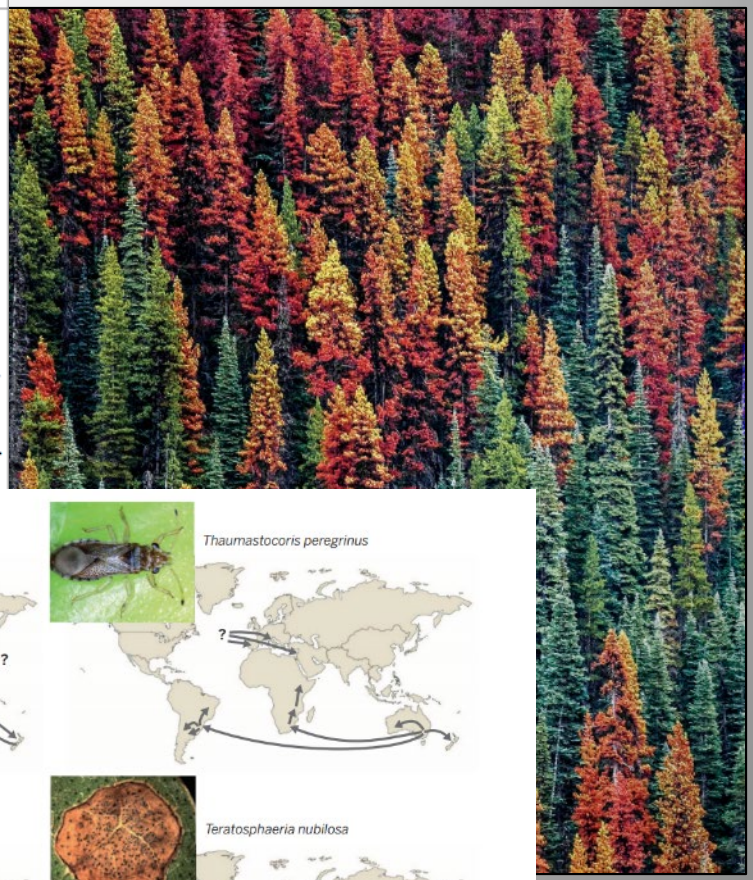
Planted forest health: The need for a global strategy

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

Several key tree genera are used in planted forests worldwide, and these represent valuable global resources. Planted forests are increasingly threatened by insects and microbial pathogens, which are introduced accidentally and/or have adapted to new host trees. Globalization has hastened tree pest emergence, despite a growing awareness of the ng of the costs, and an increased focus on the importance of and potential of planted forests, innovative solutions and a ach are needed. Mitigation strategies that are effective only in n invasions elsewhere in the world, ultimately leading to global st problems in the future should mainly focus on integrating lly, rather than single-country strategies. A global strategy to portant and urgently needed.

ems are a huge- ce, easily over- (1–3). Globally, ted to rely on y more depend

have been separated from their natural enemies. However, when plantation trees are reunited with their coevolved pests, which may be introduced accidentally, or when they encounter novel pests to which they have no resistance, substantial



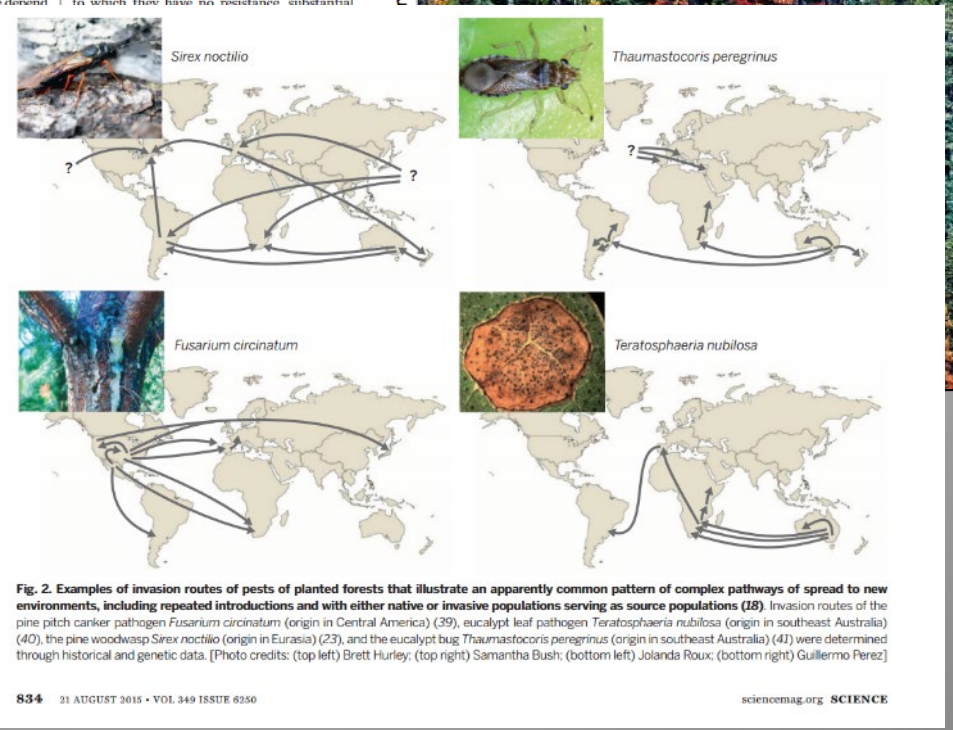


Fig. 2. Examples of invasion routes of pests of planted forests that illustrate an apparently common pattern of complex pathways of spread to new environments, including repeated introductions and with either native or invasive populations serving as source populations (18). Invasion routes of the pine 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]

American chestnut was an iconic, widespread keystone forest tree in the USA

It was extirpated as a forest tree by Chestnut Blight

1912 photo of blight in NY

Complete destruction of chestnut trees in mixed stands. Note healthy condition of trees of other species. Views along Long Island Railroad, near Richmond Hill, New York.—*Photograph by Prof. Collins.*

American Chestnut restoration – genomics and genetic engineering

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Energy & Sustainability » Scientific American Volume 310, Issue 3 — 2 :: Email :: Print

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

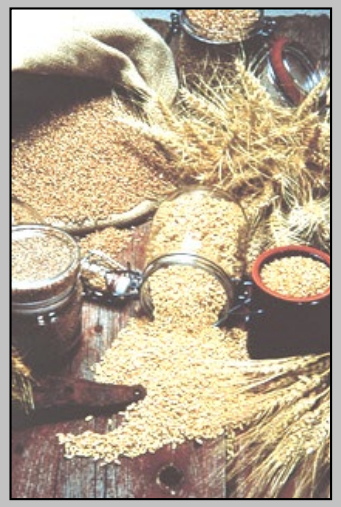
In 1876 Samuel B. Parsons received a shipment of chestnut seeds from Japan and decided to grow and sell the trees to orchards. Unbeknownst to him, his shipment likely harbored a stowaway that caused one of the greatest ecological disasters ever to befall eastern North America. The trees probably concealed spores of a pathogenic fungus, *Cryphonectria parasitica*, to which Asian chestnut trees—but not their American cousins—had evolved resistance. *C. parasitica* effectively strangles

More In This Article

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

March 2014 issue - Scientific American

Most effective
gene is oxalate oxidase
from wheat – OK?



Gene editing

- = Specific, efficient modification of native genes
- CRISPR the main method out there
- Works well everywhere!

Gene editing technology a big science deal

Science magazine names CRISPR ‘Breakthrough of the Year’

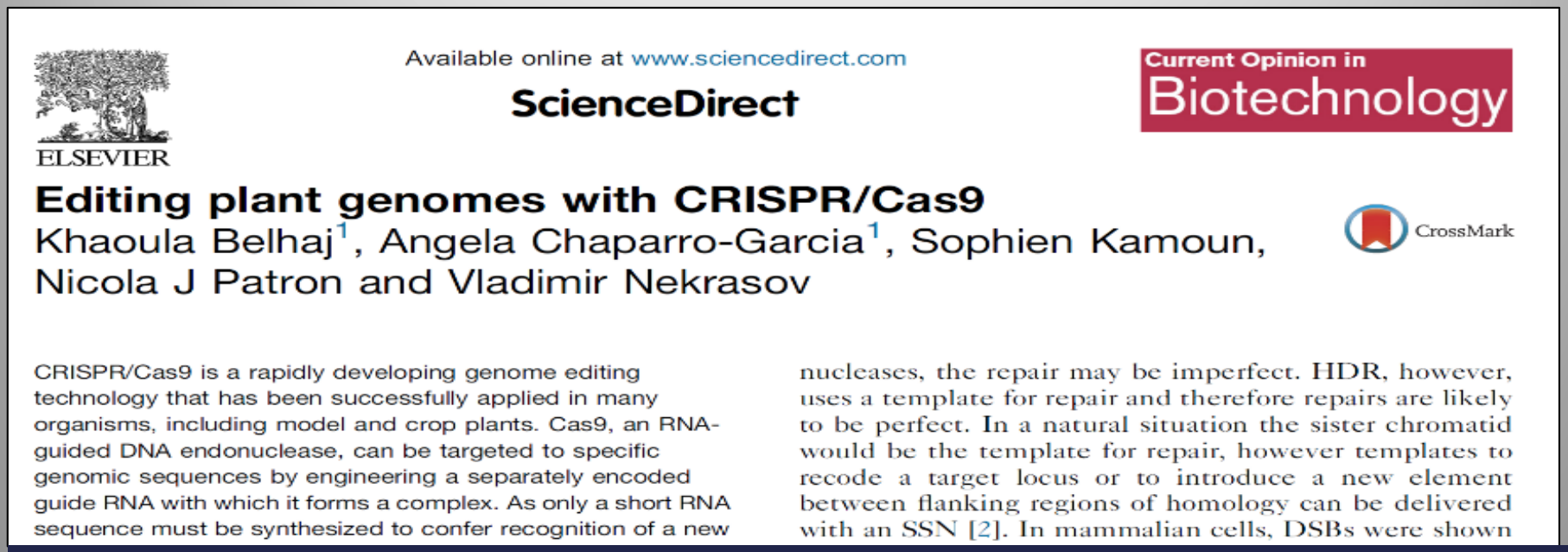
By Robert Sanders | DECEMBER 18, 2015

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

A big deal for crops ?

Ability to modify native genes efficiently -- The theoretical becomes practical



Available online at www.sciencedirect.com

ScienceDirect

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

CRISPR/Cas9 is a rapidly developing genome editing technology that has been successfully applied in many organisms, including model and crop plants. Cas9, an RNA-guided DNA endonuclease, can be targeted to specific genomic sequences by engineering a separately encoded guide RNA with which it forms a complex. As only a short RNA sequence must be synthesized to confer recognition of a new

nucleases, the repair may be imperfect. HDR, however, uses a template for repair and therefore repairs are likely to be perfect. In a natural situation the sister chromatid would be the template for repair, however templates to recode a target locus or to introduce a new element between flanking regions of homology can be delivered with an SSN [2]. In mammalian cells, DSBs were shown

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

Science journalist Carl Zimmer explains CRISPR DNA editing in 90 seconds

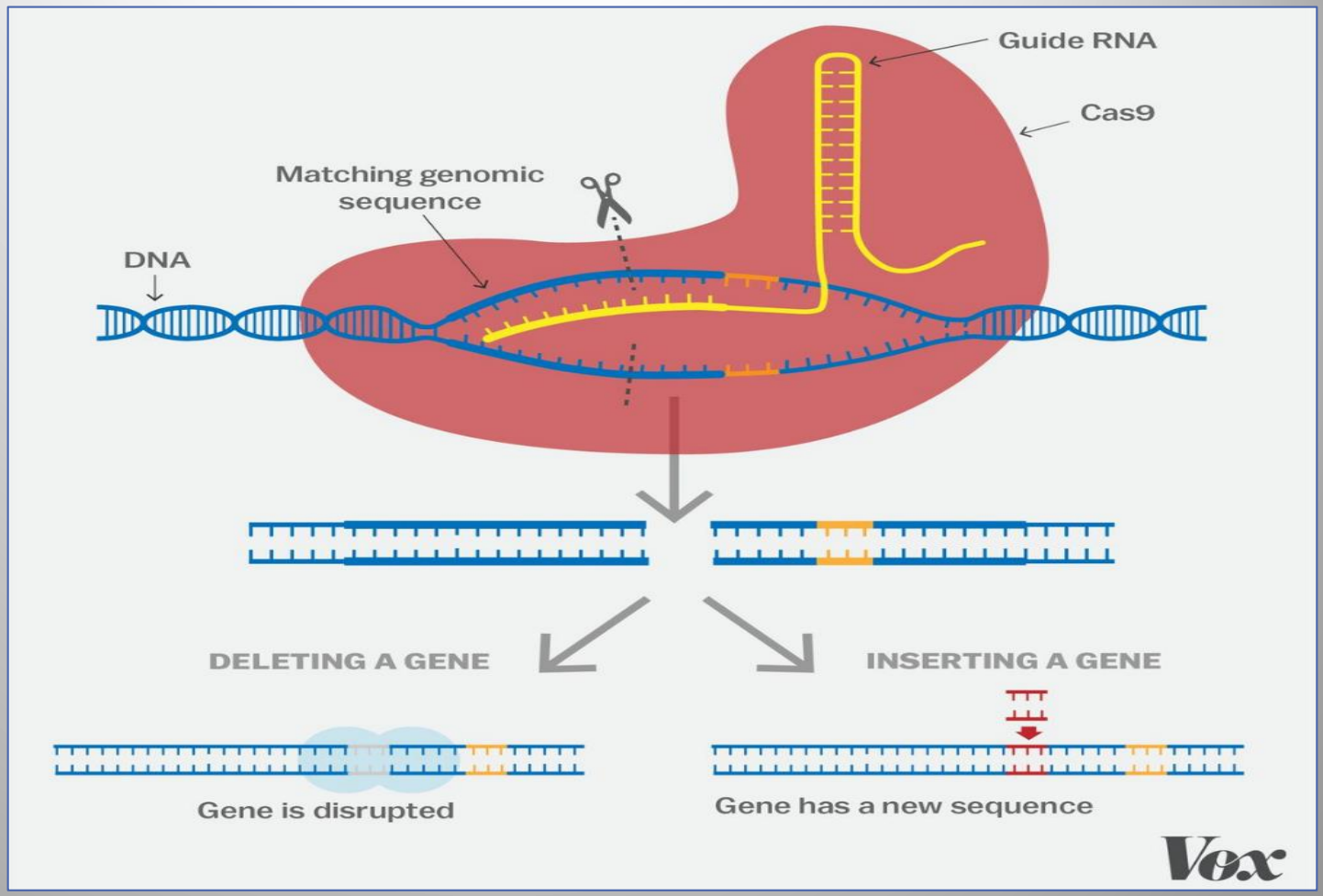
https://youtu.be/ZImVkI8QTW8

Sandman CRISPR !

https://youtu.be/k99bMtg4zRk

Overview of gene edit machinery

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



Soybean with increased oleic acid

- Its soy oil with properties of olive oil!
- Two brands, two gene-edit methods, to knock-out fatty acid desaturase genes
  - Calyxt used TALENS
  - DowDupont used CRISPR/Cas9
- Benefits to consumer and producer
  - Consumer-centric trait: Reduced saturated fats, no trans fats
  - Producer-centric trait: Improved shelf-life without need for hydrogenation

CRISPR- modified grapefruit resistant to citrus canker

Plant Biotechnology Journal

Plant Biotechnology Journal (2016), pp. 1–7 doi: 10.1111/pbi.12677

Genome editing of the disease susceptibility gene *CsLOB1* in citrus confers resistance to citrus canker

Hongge Jia[1], Yunzeng Zhang[1], Vladimir Orbović[2], Jin Xu[1], Frank F. White[3], Jeffrey B. Jones[3] and Nian Wang[1,*]

Reduced gluten wheat by mutation of dozens of genes

Plant Biotechnology Journal

aab Association of Applied Biologists · SEB Society for Experimental Biology

Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9

Susana Sánchez-León[1,#], Javier Gil-Humanes[2,*,#], Carmen V. Ozuna[1], María J. Giménez[1], Carolina Sousa[3], Daniel F. Voytas[2] and Francisco Barro[1,*]

[1]*Departamento de Mejora Genética Vegetal, Instituto de Agricultura Sostenible (IAS-CSIC), Córdoba, Spain*

[2]*Department of Genetics, Cell Biology, and Development, Center for Genome Engineering, University of Minnesota, Minneapolis, MN, USA*

60-85% reduction in gluten content

Multiplex CRISPR

62 genes targeted in pig

Science AAAS

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REPORT

Genome-wide inactivation of porcine endogenous retroviruses (PERVs)

Luhan Yang[1,2,3,*,†], Marc Güell[1,2,3,†], Dong Niu[1,4,†], Haydy George[1,†], Emal Lesha[1], Den Aach[1], Ellen Shrock[1], Weihong Xu[6], Jürgen Poci[1], Rebeca Cortazio[1], Robert A. Wilkinson[5], Jay A. Fishman[5], George Church[1,2,3,*]

+ Author Affiliations

*Corresponding author. E-mail: gchurch@genetics.med.harvard.edu (G.C.); luhan.yang@egenesisbio.com (L.Y.)

† These authors contributed equally to this work.

Science 27 Nov 2015:
Vol. 350, Issue 6264, pp. 1101-1104
DOI: 10.1126/science.aad1191

Virally cleansing the pig genome

Transplants from pigs could be a solution to a shortage of human organs for transplantation. Unfortunately, porcine endogenous retroviruses (PERVs) are rife in pigs and can be transmitted to humans, risking disease. L. Yang *et al.* integrated CRISPR-Cas into the pig cell genome, where continuous induction of the Cas9 editing enzyme resulted in the mutation of every single PERV reverse transcriptase gene. This prevented replication of all copies of PERV, viral infection, and transmission to human cells.

Science, this issue p. 1101

Abstract

The shortage of organs for transplantation is a major barrier to the treatment of organ failure. Although porcine organs are considered promising, their use has been checked by concerns about the transmission of porcine endogenous retroviruses (PERVs) to humans. Here we describe the eradication of all PERVs in a porcine kidney epithelial cell line (PK15). We first determined the PK15 PERV copy number to be 62. Using CRISPR-Cas9, we disrupted all copies of the PERV *pol* gene and demonstrated a >1000-fold reduction in PERV transmission to human cells, using our engineered cells. Our study shows that CRISPR-Cas9 multiplexability can be as high as 62 and demonstrates the possibility that PERVs can be inactivated for clinical application of porcine-to-human xenotransplantation.

Hornless cattle by gene editing

Open Season Is Seen in Gene Editing of Animals

By **AMY HARMON** NOV. 26, 2015

A calf, left, approximately the same age as the first two genetically modified calves to have their DNA edited so that they do not grow horns, right. Jenn Ackerman for The New York Times

The New York Times

Agenda

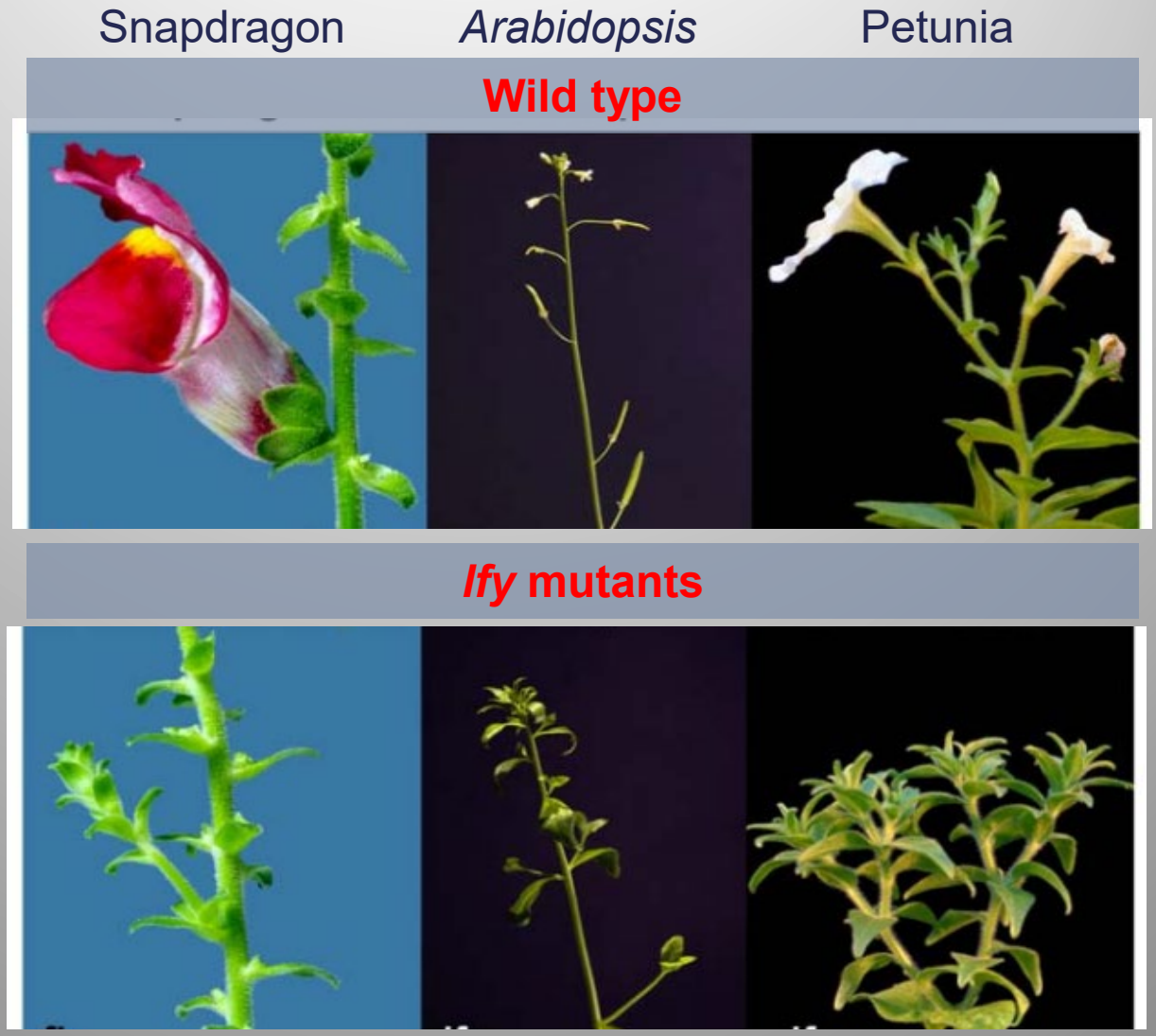
- Setting the stage for “the fuss”
- The science
- Some recent research

PhD thesis project, in part, by Estefania Elorriaga

Why use CRISPR as a tool for containment of exotic and GMO/GE trees?

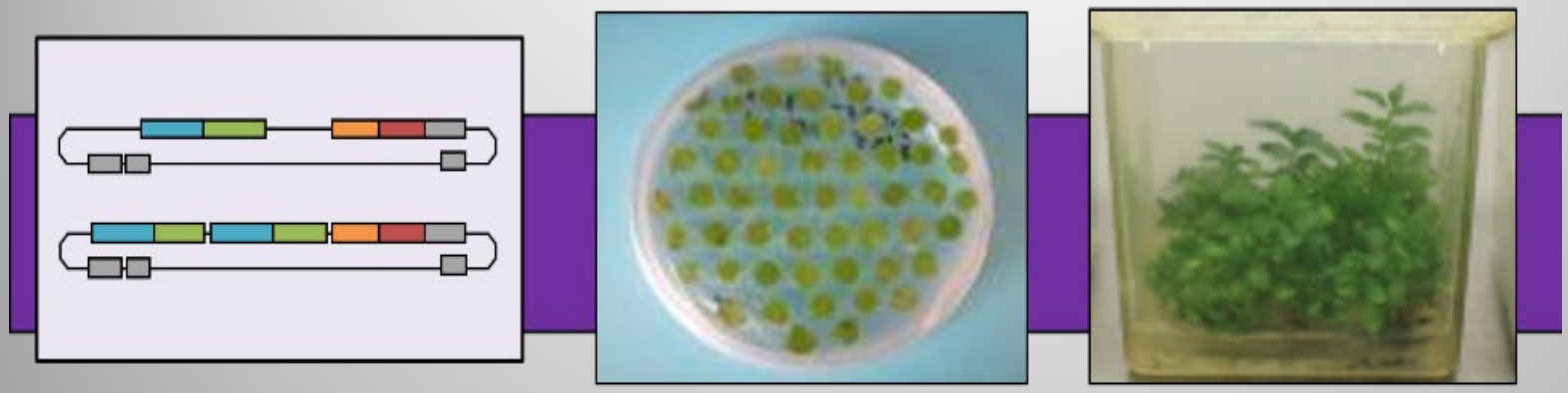
- Social, regulatory, ecological concerns – especially with trees, invasive plants
- Gene mutation/deletion the strongest and most stable form of genetic containment
- Examined in greenhouse for growth rate and flowering/sterility – flowering field trials costly, slow, controversial

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



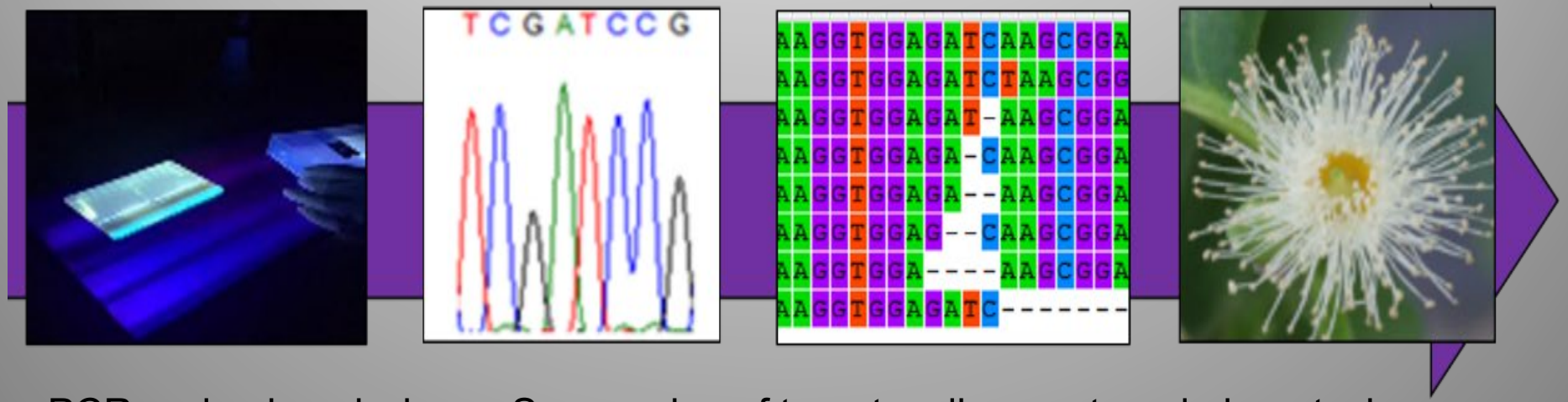
Parcy et al. 2002; Moyroud et al. 2010

CRISPR pipeline



Construct

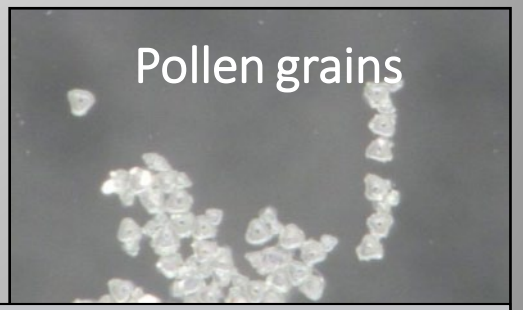
Transformation and regeneration



PCR and gel analysis (allele specific)

Sequencing of targets, alignment, and phenotyping

Early flowering *FT*-eucalypts to speed floral phenotyping



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

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revised 29 May 2015;
accepted 10 June 2015.

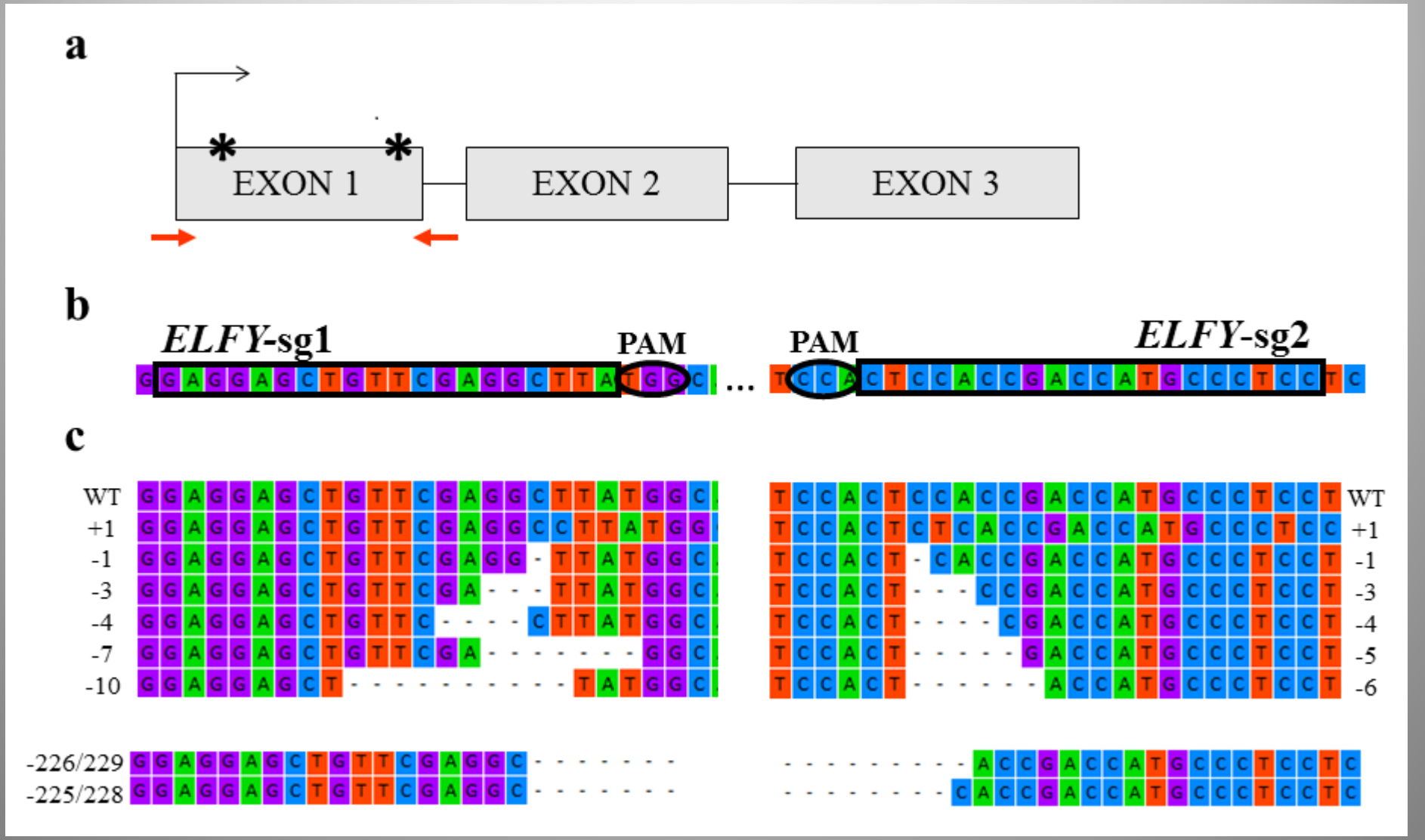
Summary

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

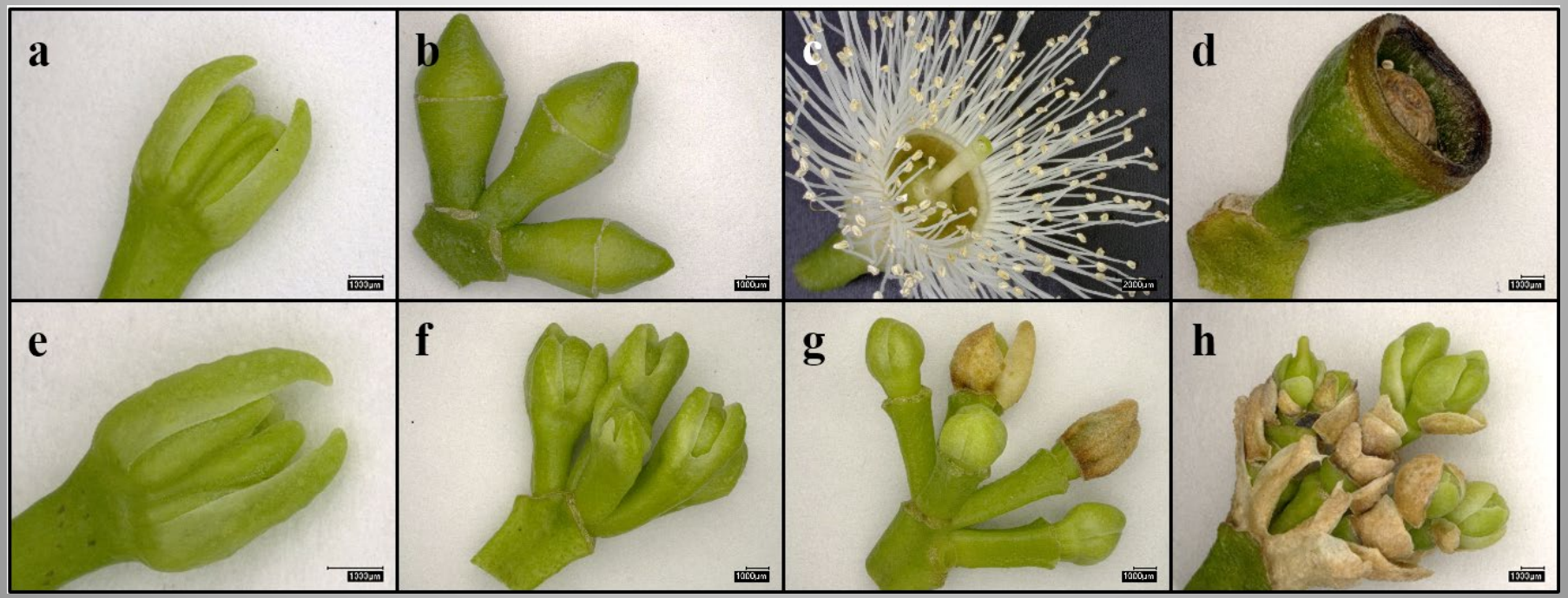
High knock-out mutation rate

Population	Total events (alleles)	Alleles modified	N° events
WT LFY-CRISPR	9 (18)	Both alleles	9 (100%)
		One allele	0 (0%)
		None	0 (0%)
***AtFT* LFY-CRISPR**	59 (118)	Both alleles	58 (98%)
		One allele	1 (2%)
		None	0 (0%)
All eucalypt	**68 (136)**	Both alleles	**67 (99%)**
		One allele	**1 (1%)**
		None	**0 (0%)**

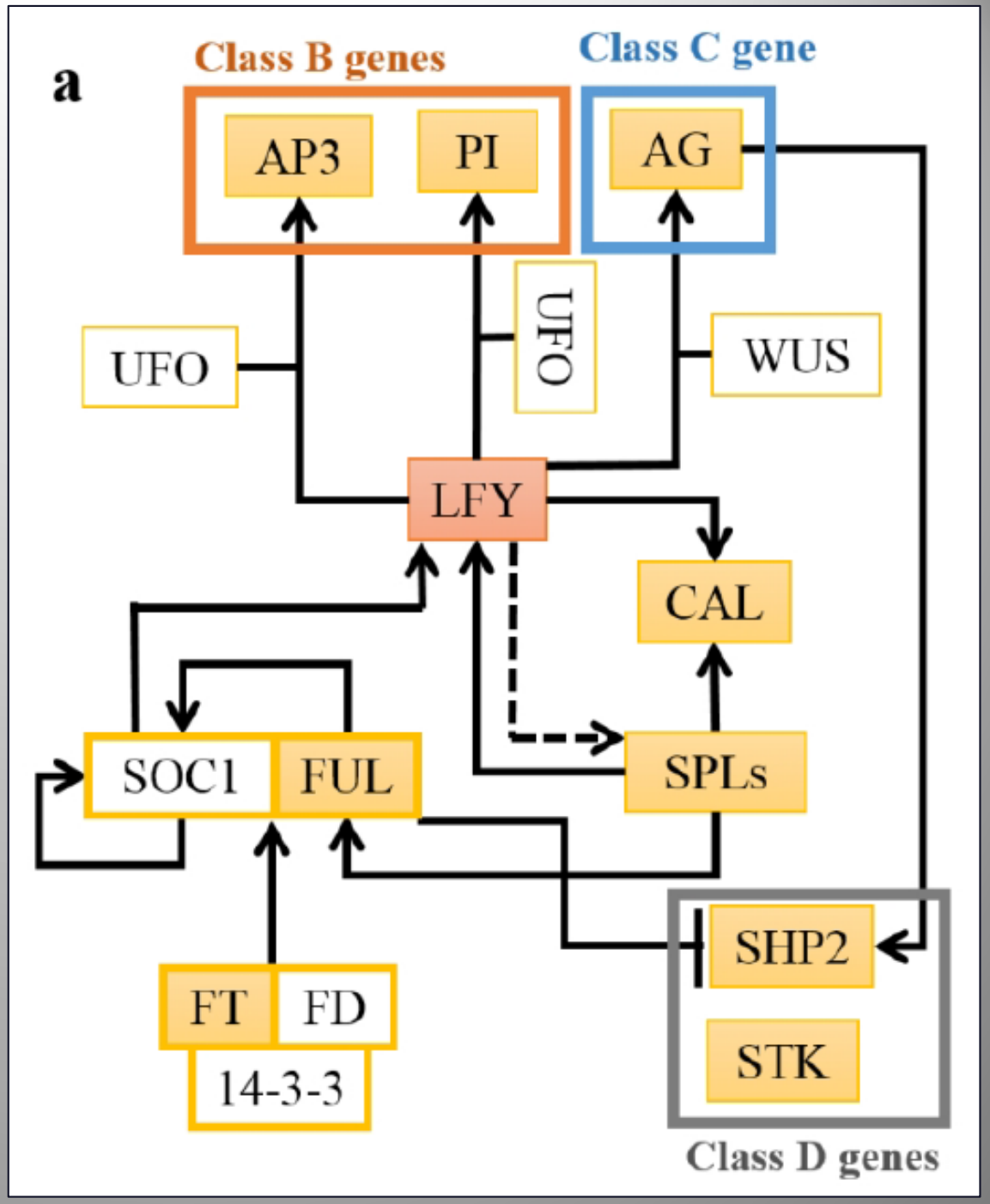
sgRNA locations, example mutations



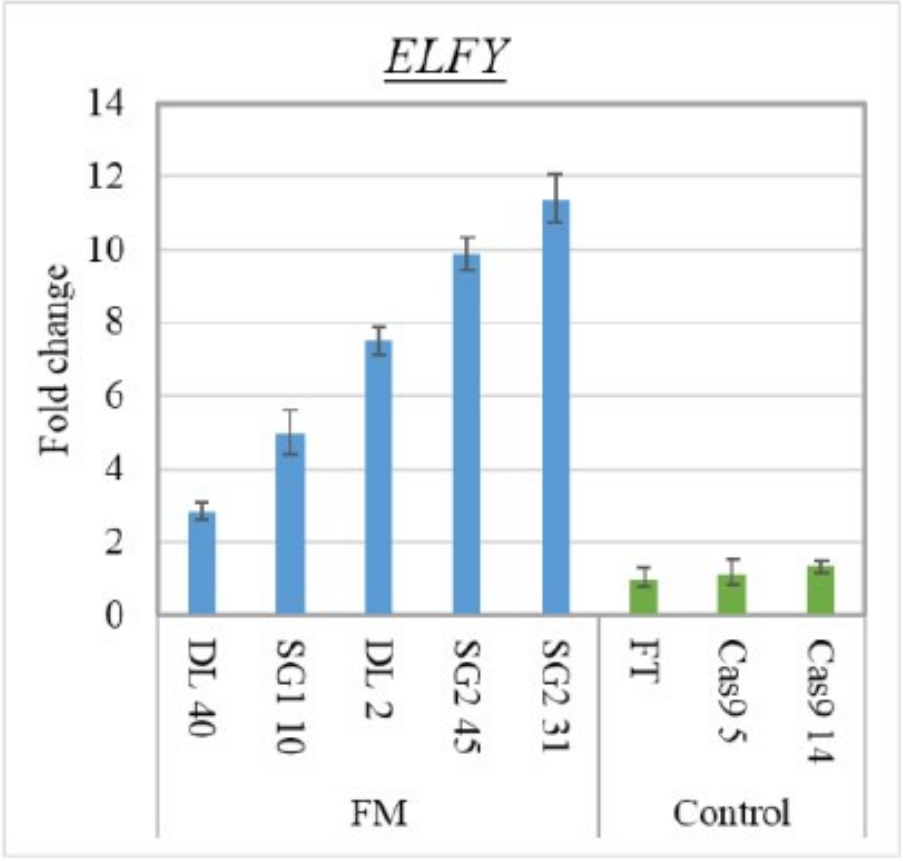
Wild-type vs. knockout stages



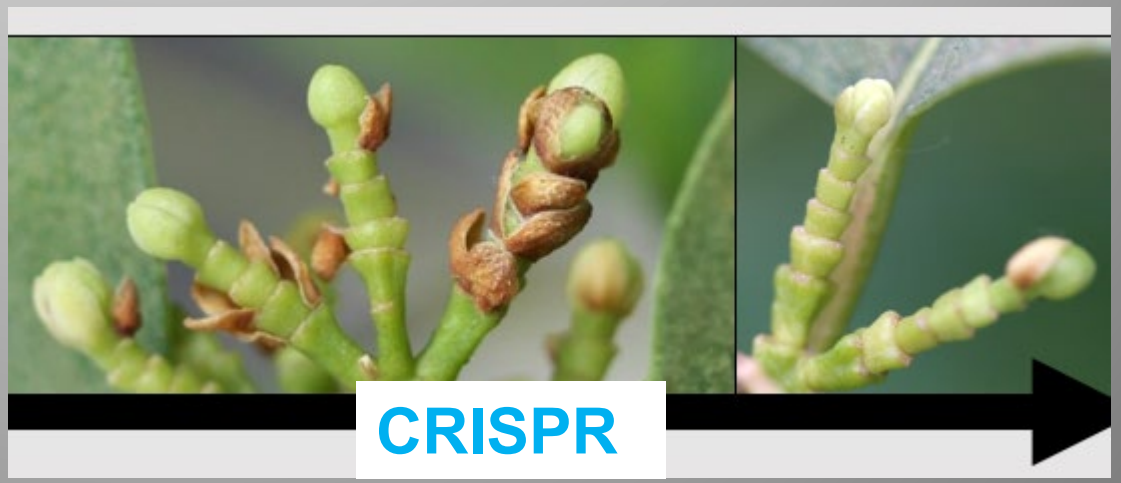
LEAFY a central gene in complex pathway to flowering



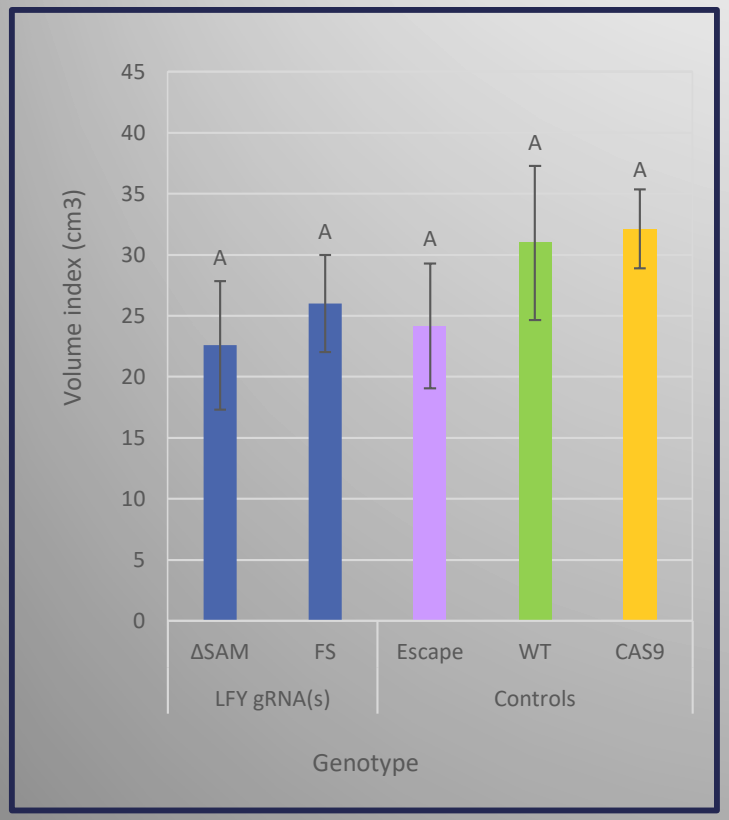
LEAFY itself is upregulated in knockout “floral” buds

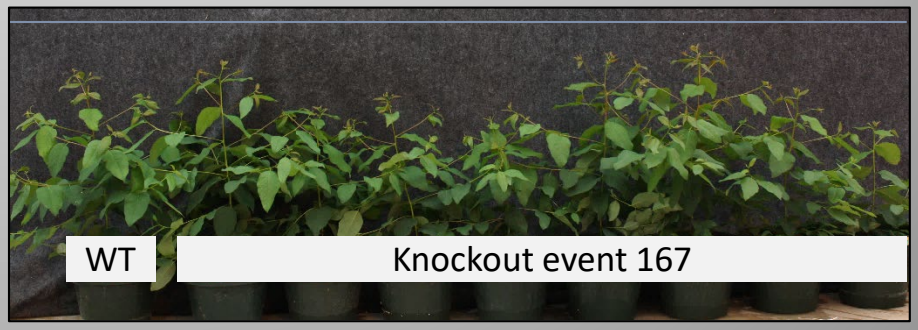


Summary view of floral shoot development in knockouts vs. wild-type



Vegetative growth and morphology in non-FT trees in greenhouse unaffected by knock-out





Other traits studied include chlorophyll density, leaf area, and leaf specific weight

Summary

- Distinctive social and “fake news” dimension of GMOs -- impede public understanding and rational uses
- Genetics key to economics and sustainability amidst growing population and climate crisis – GMOs one more, but a large, genetic tool when society allows it
  - A very few examples from ag and forestry were shown
- Severe market restrictions and regulations are major obstacles to research and application in USA and many other countries – with great life and environment-harming consequences now and in the future