Gene Editing = Genome Editing

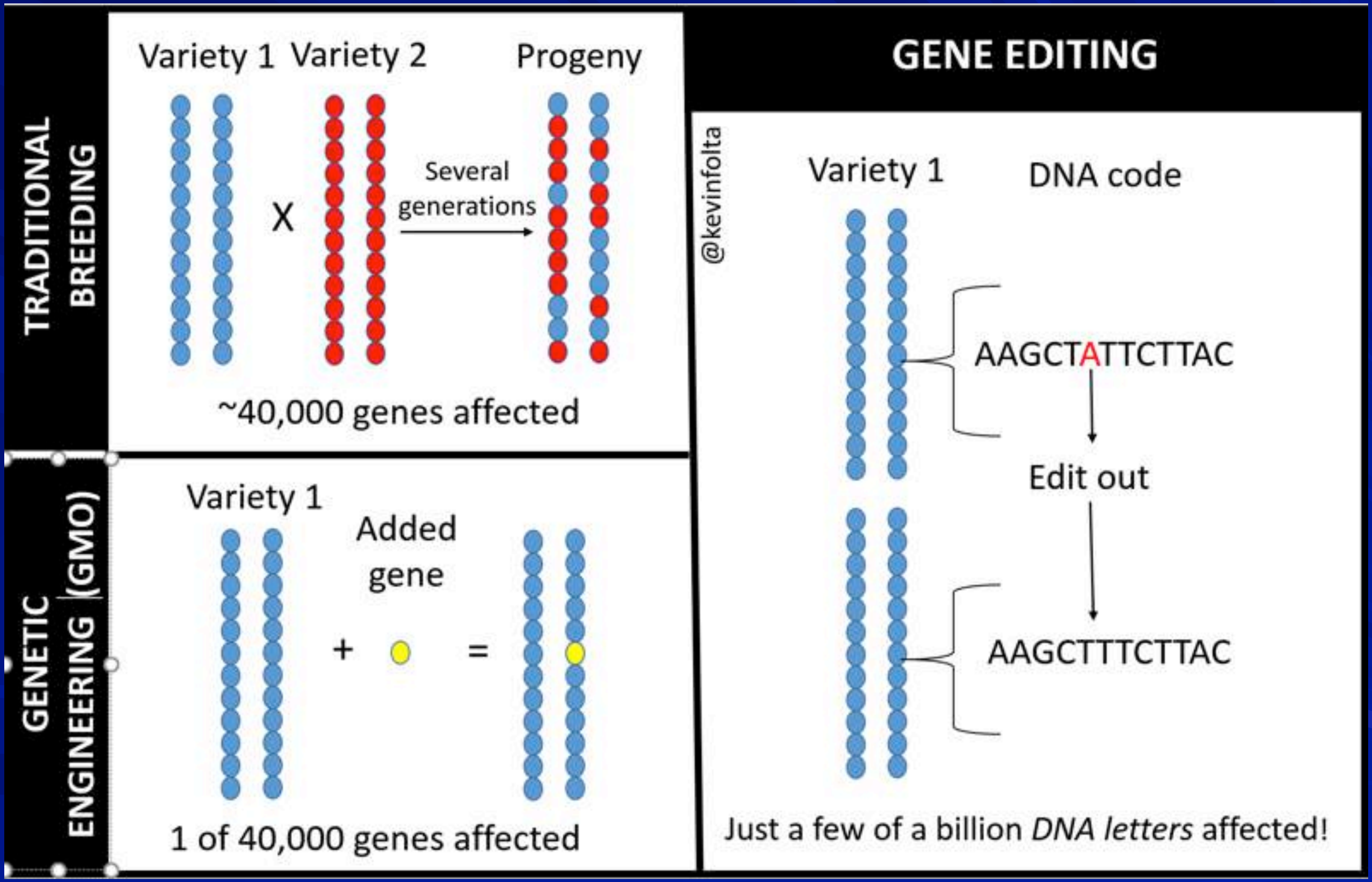
Gene Editing

Zinc Finger nucleases

TALEN

CRISPR/Cas9

The Gene Editing Revolution



TALEN based gene edits

Transcription activator like effector nuclease

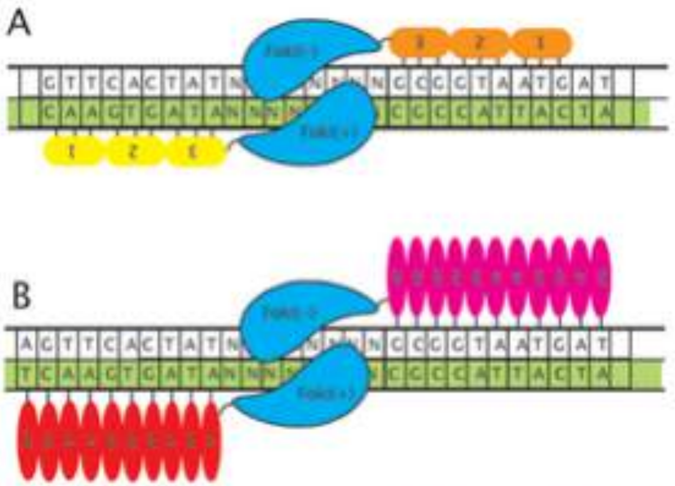
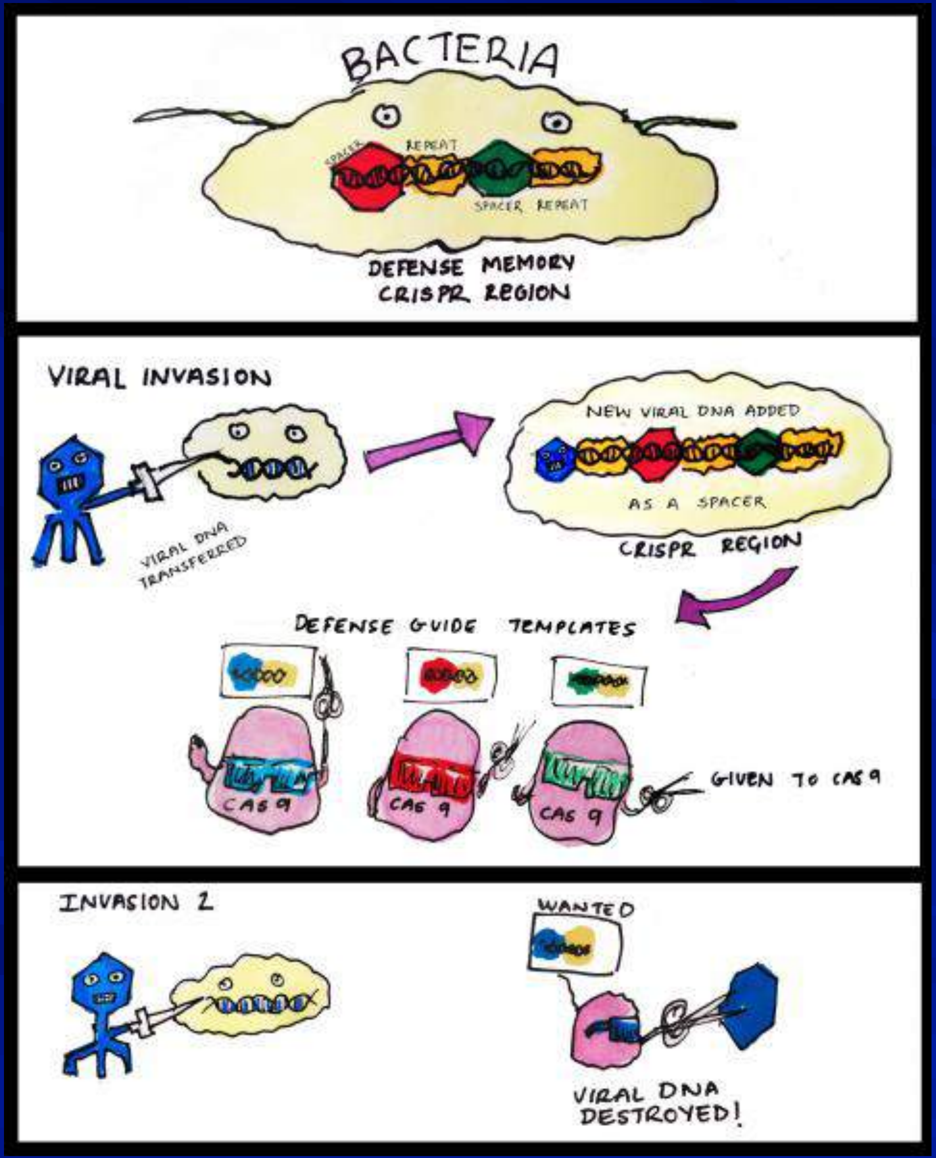


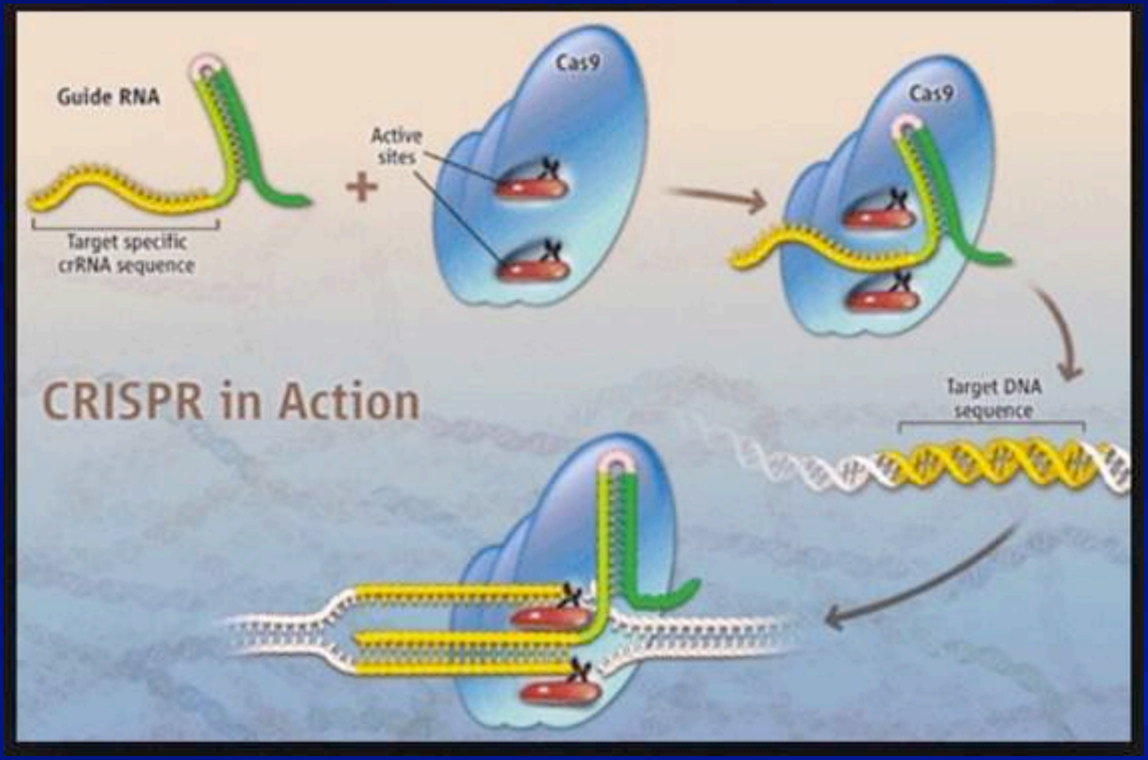
Figure 1: (A) Representation of a zinc finger (ZF) nuclease. Zinc fingers recognize triplets and the FokI nuclease operates as a dimer, cutting in the spacer region between two distinct ZF target sites. **(B)** A TAL effector nuclease (TALEN). Similar in principle to the ZF nuclease, the components of the array recognize individual nucleotides.

The bacterial adaptive immune system



Maintains clusters of repeated viral sequence

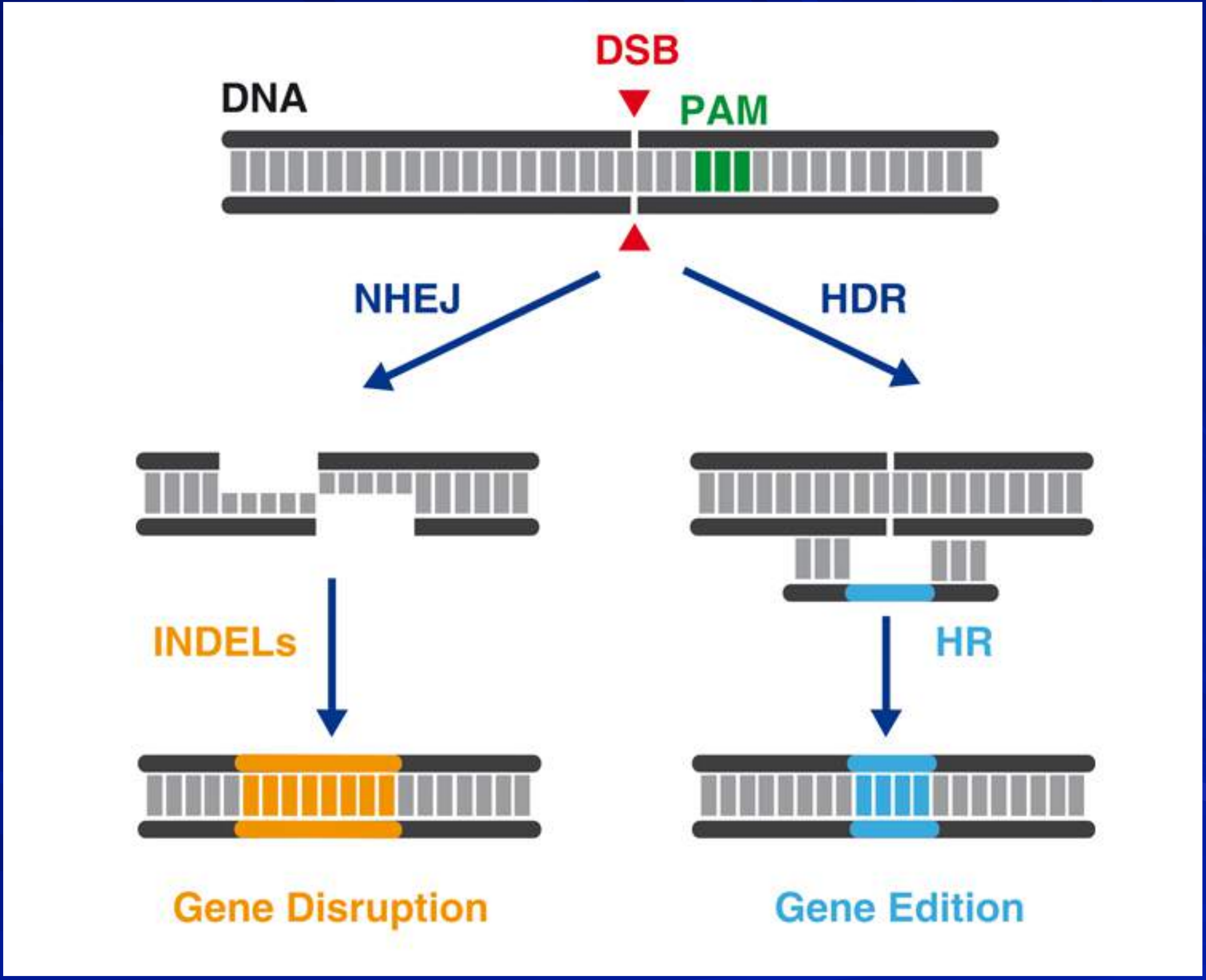
In the lab, we can supply the guide RNA sequence.



Constrained by PAM site (protospacer adjancent motif)

5'- NGG – 3'

Required for cleavage, not in bacterial spacers, so it does not self-digest!


DSB
DNA
PAM
NHEJ
HDR
INDELs
HR
Gene Disruption
Gene Edition

Products of Genetic Engineering

Add a Transgene

Natural Mutations

ACGAGTCGATCAGTCTAG

ACGAGTCGATGAGTCTAG

Gene Editing

ACGAGTCGATCAGTCTAG

ACGAGTCGATGAGTCTAG

Property	TALEN	CRISPR-Cas9
Type of recognition	Protein-DNA	RNA-DNA
Methylation sensitive?	Sensitive	Not sensitive
Off-target effects	Fewer observed off-target effects	More potential for off-target effects than TALENs & ZFNs

Regulatory– not sure what long term reg's will be, but right now the changes induced with no evidence of foreign sequence will not be regulated.

Applications

Gene Editing

HORNS!!!
Bad beef
Great milk

NO HORNS!!!
Good beef
Bad milk

Horn Gene

Horn Gene

Cross….

Mix of bad beef, bad milk production

Gene Editing

HORNS!!!
Bad beef
Great milk

NO HORNS!!!
Good beef
Bad milk

STOP

Horn Gene

Horn Gene

STOP

Horn Gene

NO HORNS!!!
Bad beef
Good milk

Gene Editing

Could these piglets become Britain's first commercially viable GM animals?

Pigs 'edited' with a warthog gene to resist African swine fever could help spawn GM animal farms in the UK

These piglets were created by using a gene-editing technique to make them resistan
Photograph: Murdo MacLeod for The Guardian

Changing domestic pigs' DNA to provide resistance to African Swine Fever

Episode 037

	Farmers
X	Consumers
	Environment
	Needy

Talking Biotech

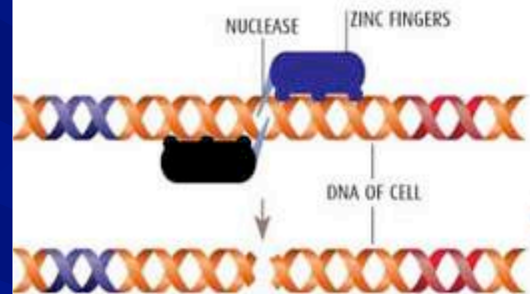
Health Implications

A Groundbreaking Gene-Editing Therapy Eliminated Cancer in Two Infants

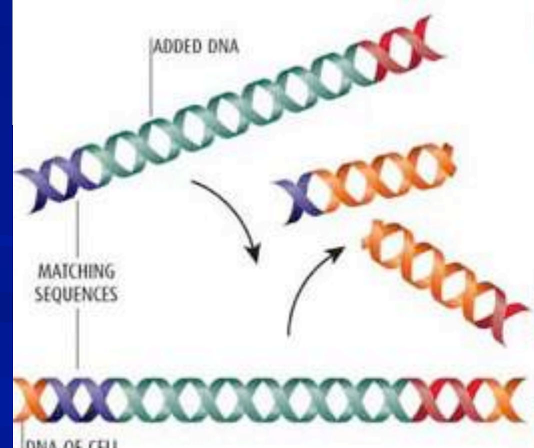
Kristen V. Brown
1/26/17 1:58pm · Filed to: SCIENCE

Targeting DNA with molecular scissors

The scissors – a combination of gene-gripping "zinc fingers" and a DNA-cutting enzyme called a nuclease – find the target sequence, latch on to it and cut it



Natural repair enzymes fix the break, but in the process sometimes chop out the broken section and replace it with a new chunk of DNA – cunningly added by engineers – in a poorly understood process called homologous recombination



Adding DNA with a recombinase

Viral enzymes called recombinases cut DNA at target site 1 and target site 2, and then join the ends together. So if you create a circular piece of DNA containing target site 1, recombinases will intergrate this DNA into the genome of any cell containing target site 2

Mechanisms of Current GE Crops

Design of Future Solutions

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www.talkingbiotechpodcast.com

UF UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

Regulatory Framework

The Coordinated Framework for the Regulation of Biotechnology was established in 1986 and describes the U.S. regulatory policy for ensuring the safety of biotechnology products, including field trials and cultivation of GE crops and safety reviews of foods derived from them (OSTP, 1986). Three regulatory agencies have jurisdiction over different aspects of GE crops (Figure 3-5):

- USDA's Animal and Plant Health Inspection Service (APHIS) regulates GE plants to control and prevent the spread of plant pests that could damage crops, plants, or trees.
- The U.S. Environmental Protection Agency (EPA) regulates the safety of pesticides and "plant-incorporated protectants" for the environment and human health.
- The U.S. Food and Drug Administration (FDA) oversees the safety of food and feed, including the review of data used to compare GE food with its conventional counterpart (FDA, 1992).

Success Stories

What are the current crops and how do they work?

What are the next generation of crops?

-Provide needed micronutrients to hungry populations
-Add virus resistance to key crops in the developing world
-Help plants grow in changing climates and weather extremes, such as heat, drought, flooding, cold.
-Plants that protect themselves from pests, cutting need for insecticides

-Why don't we use them?

Current use of GE crops

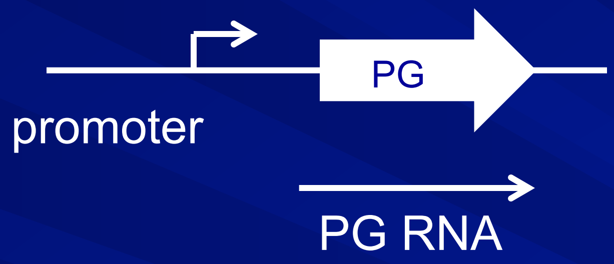
First Commercialized GE Plant / Food Product

Flavr Savr Tomato
(Calgene Inc, 1994)

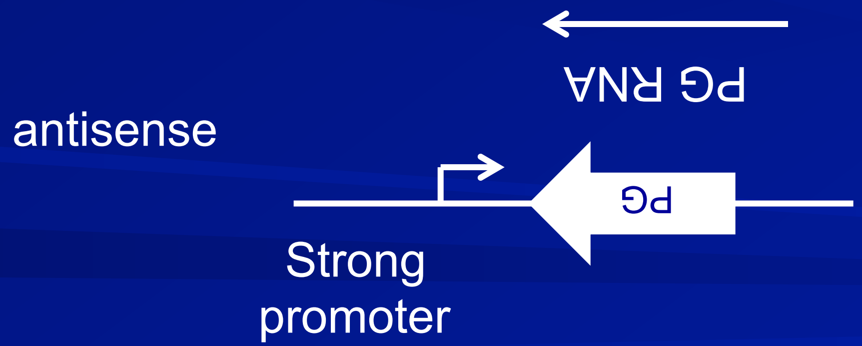
Problem: Tomatoes lose firmness as the sugars and flavors increase.

Solution: Uncouple the processes.

How did Favr Savr work?



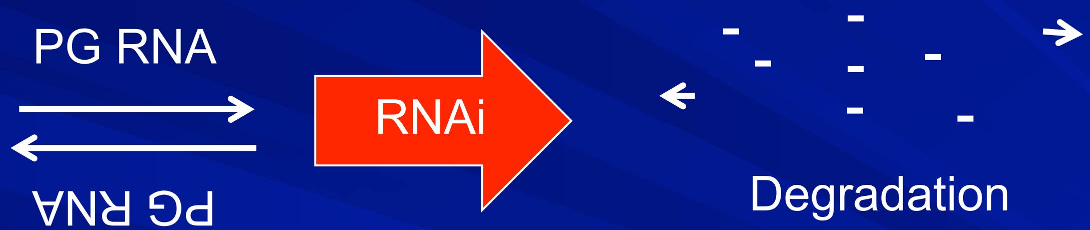
Softening is caused by an enzyme called polygalaturonase (PG).



A sequence is added that produces “antisense” PG RNA. It matches the gene sequence like a mirror. Creating “RNA interference”

How did Favr Savr work?

Softening is caused by an enzyme called polygalaturonase (PG).



A sequence is added that produces “antisense” PG RNA. It matches the gene sequence like a mirror.
Creating “RNA interference”

First Commercialized GE Plant / Food Product

Flavr Savr Tomato

Antisense RNA against the enzyme activity developed to inhibit the synthesis of the enzyme and delayed the fruit ripening of tomato by GM technology. These tomato have longer shelf life called as *"FLAVR SAVR TOMATO"*

Where did it go?

It was not much of an improvement.

The lines didn't taste great.

It was not a trait that consumers wanted to pay for.

Bt (delta endotoxin)

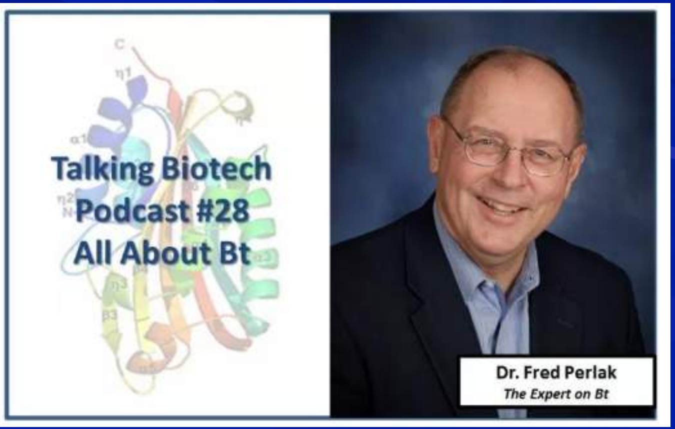
Upon spore formation *Bacillus thurengiensis* produces a protein that crystalizes, and is toxic to specific insects.

Narrow target range

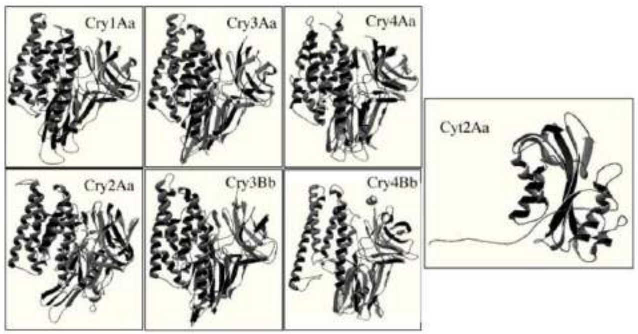
Produced as a protoxin that is proteolytically cleaved in the insect gut

Activated toxin binds to a specific receptors in midgut columnar cells, then inserts into membrane (Bravo et al., 2005).

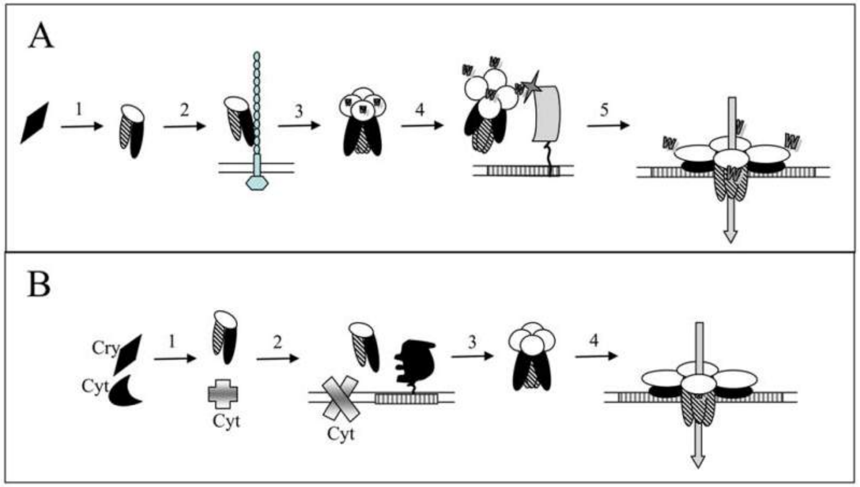
Leads to production of pores, leads to ion leakage and cell lysis where spores can germinate and infect the insect.



Bt (delta endotoxin)



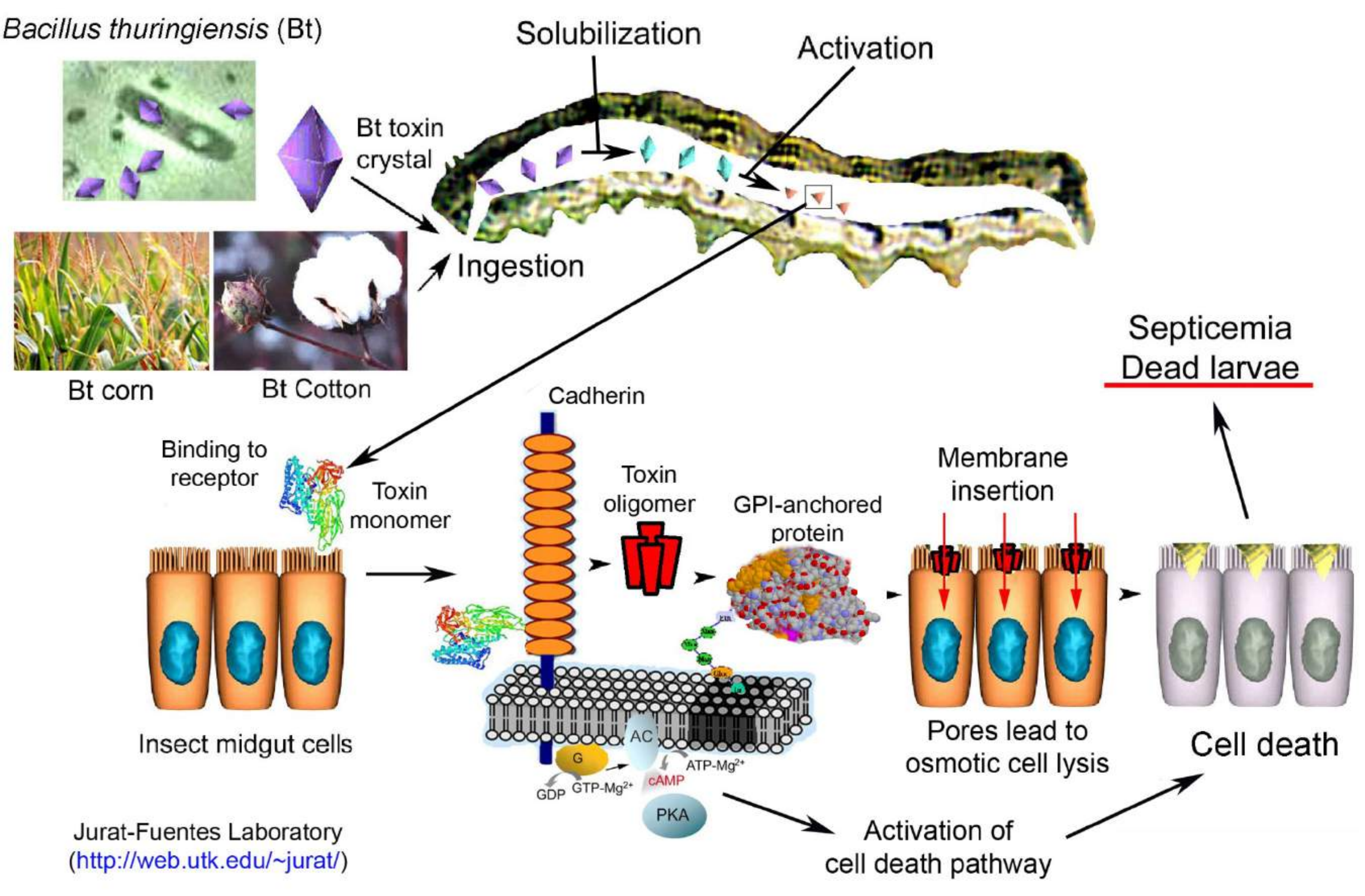
Three dimensional structures of insecticidal toxins produced by *Bacillus thuringiensis* Cry1Aa, Cry2Aa, Cry3Aa, Cry3Bb, Cry4Aa, Cry4Bb and Cyt2A.



Model of the mode of action of Cry and Cyt toxins. Panel A, sequential interaction of Cry toxins with different receptor molecules in lepidopteran larvae. (1) Solubilization and activation of the toxin; (2). Binding of monomeric Cry toxin to the first receptor (CADR or GCR), conformational change is induced in the toxin and α-helix 1 is cleaved; (3) Oligomer formation; (4) Binding of oligomeric toxin to second receptor (GPI-APN or GPI-ALP), a conformational change occurs and a molten globule state of the toxin is induced; (5) insertion of the oligomeric toxin into lipid rafts and pore formation. Panel B, role of Cyt and Cry toxins in the intoxication of dipteran larvae. (1) Cry and Cyt toxins are solubilized and activated; (2) Cyt toxin inserts into the membrane and Cry toxin binds to receptors located in the membrane (ALP or Cyt toxin); (3) oligomerization of the Cry toxin is induced; (4) oligomer is inserted into the membrane resulting in pore formation.

Different proteins target receptors of different pests.

How Bt Works



Bacillus thuringiensis (Bt)
Bt toxin crystal
Solubilization
Activation
Ingestion
Bt corn
Bt Cotton
Septicemia
Dead larvae
Cadherin
Binding to receptor
Toxin monomer
Toxin oligomer
GPI-anchored protein
Membrane insertion
Insect midgut cells
AC
G
GDP
GTP-Mg2+
ATP-Mg2+
cAMP
PKA
Pores lead to osmotic cell lysis
Cell death
Activation of cell death pathway
Jurat-Fuentes Laboratory
(http://web.utk.edu/~jurat/)

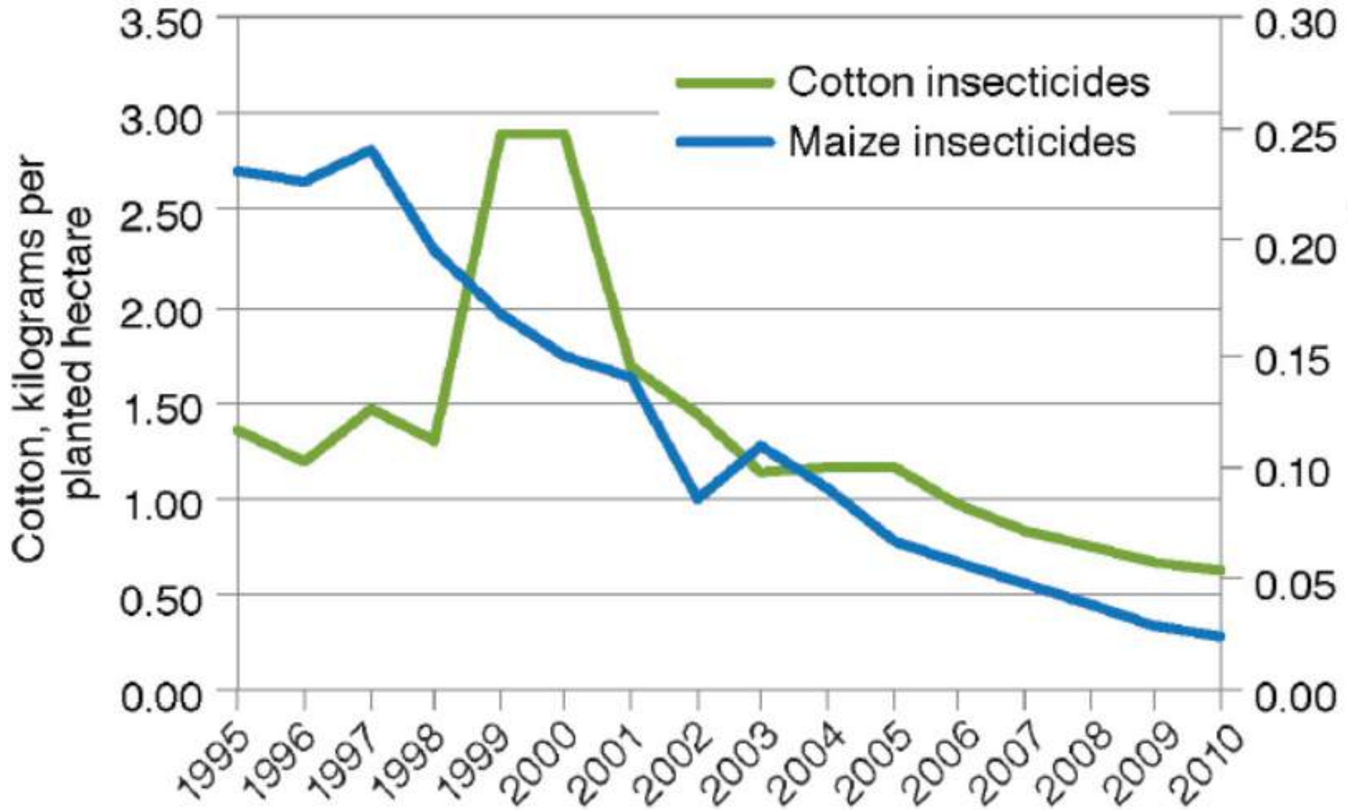


FIGURE 4-4 Rates of insecticide application for maize and cotton in the United States from 1995 to 2010.
SOURCE: Fernandez-Cornejo et al. (2014).

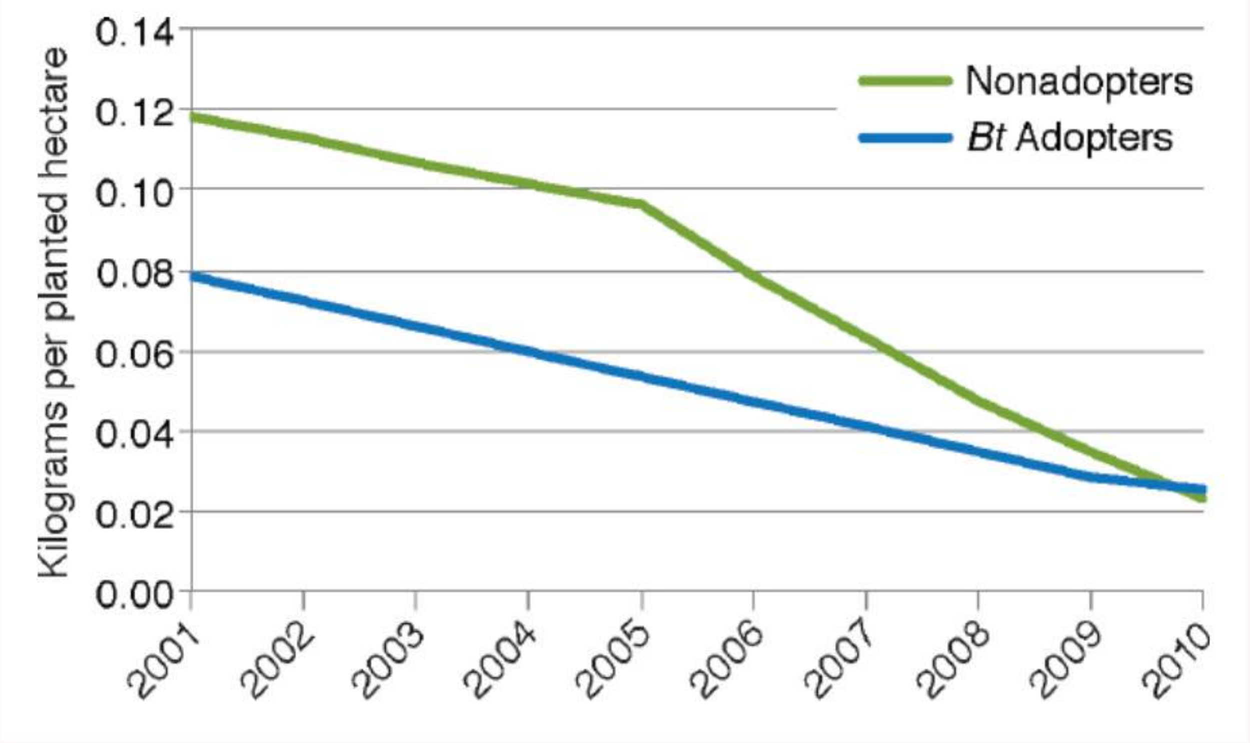


FIGURE 4-5 Rates of insecticide application by adopters and nonadopters of *Bt* maize in the United States from 2001 to 2010. SOURCE: Fernandez-Cornejo et al. (2014).

Major Crops

Corn

comparison of corn with disease and Bt corn.(Photo by Biotech info center)

Eggplant

Courtesy DM Hautea

Bt (delta endotoxin)

Advantages

Eliminates need for broad spectrum insecticide

Currently in: Cotton, maize, eggplant (Bangladesh)

Disadvantages

Requires refugia to slow resistance
Development of resistance (observed)

Herbicide Resistance

- Tolerance to herbicide application
- Glyphosate, glufosinate (dicamba, 2,4-D)

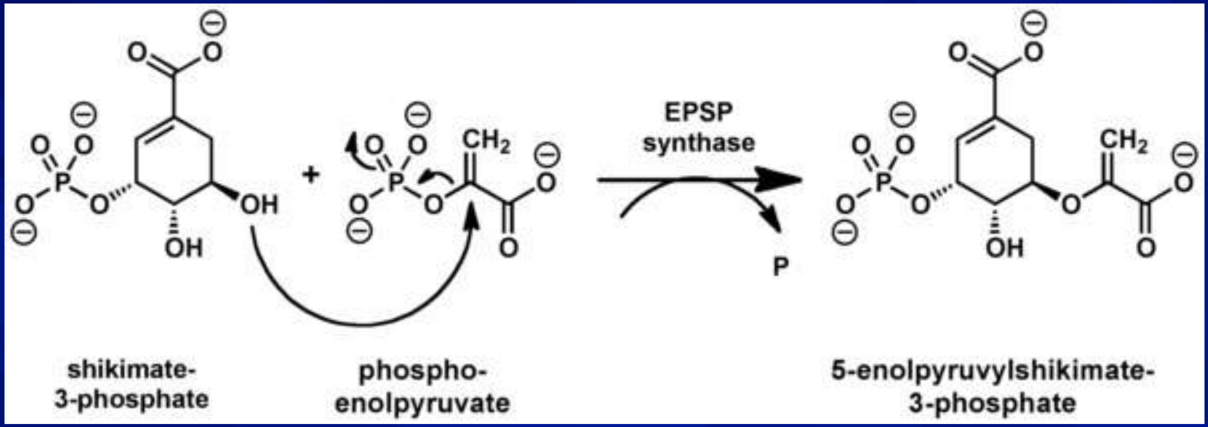
Glyphosate-Resistant Products

A gene is inserted that allows plants to survive in the presence of the herbicide. Farmers can spray to kill non-transgenic plants.

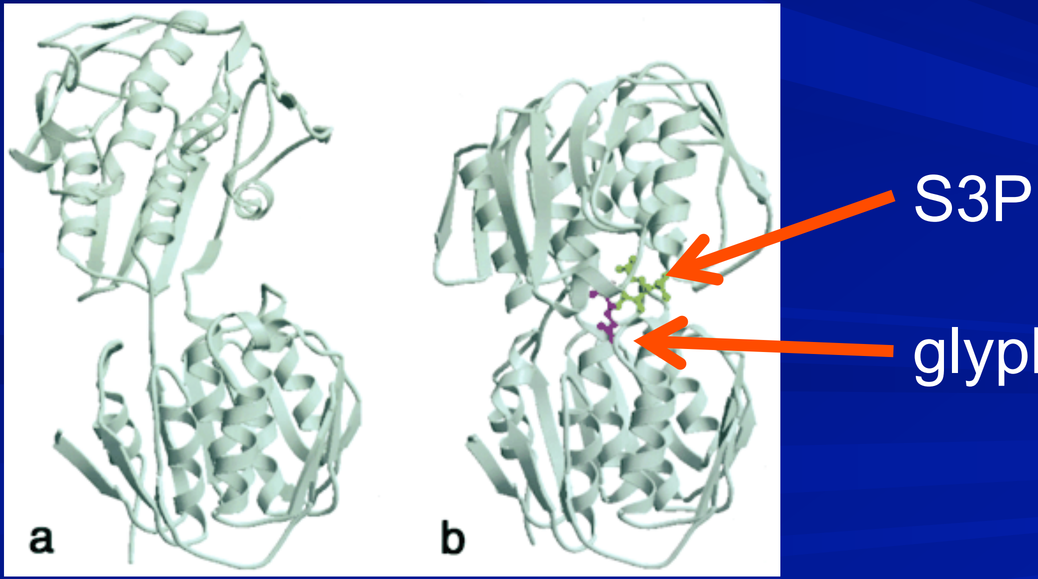
Glyphosate-Resistant (Roundup Ready) Products

One of the most widely used herbicides

Mechanism of action



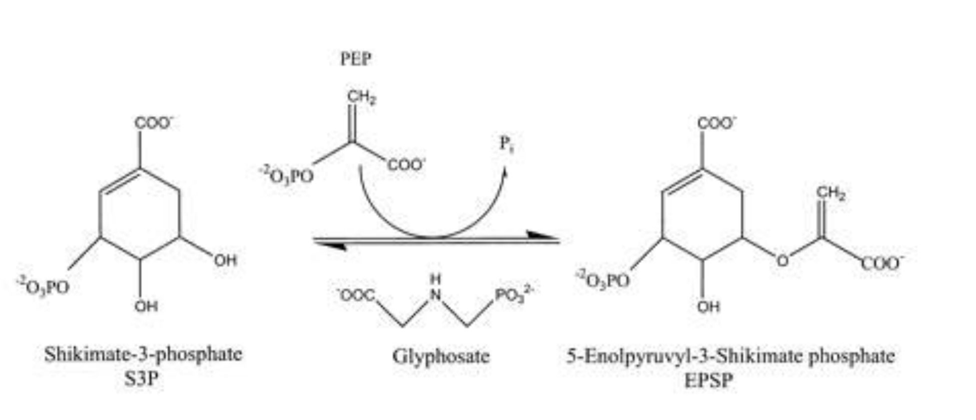
Aromatic
Amino
Acids



A bacterial EPSPS enzyme does not bind glyphosate

Herbicide Resistance

- Aromatic amino acid synthesis



Depends on the activity of 5-enolpyruvylshikimate phosphate synthase (EPSPS)

Typically binds phosphoenol pyruvate

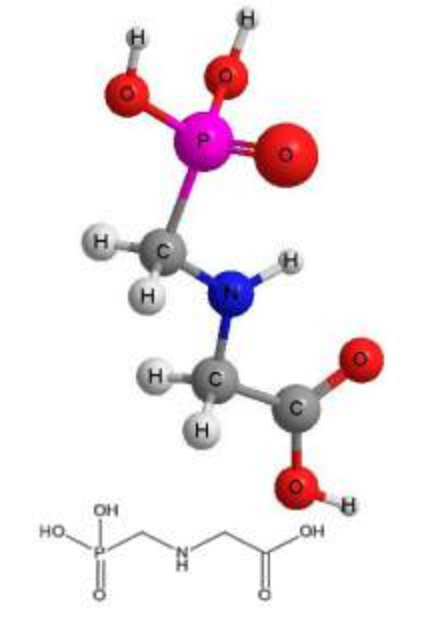
Competitively inhibits enzyme activity by binding into the active site.

What is Glyphosate?

Non-selective herbicide. Kills all plants.

Pathway not present in animals

Acute toxicity is low (4320 -10,000 mg/kg)



TOXICITY CLASSIFICATION - GLYPHOSATE

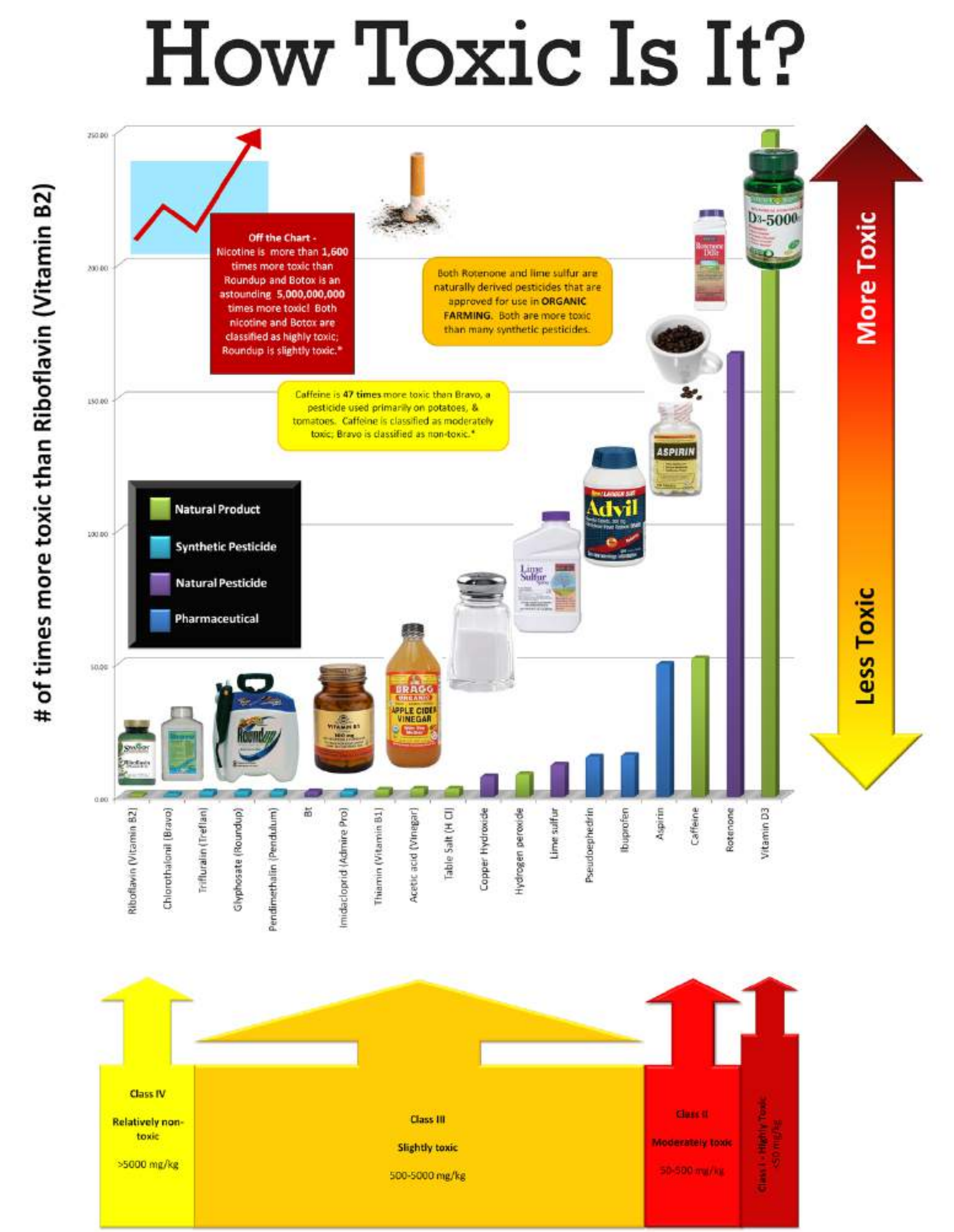
	High Toxicity	Moderate Toxicity	Low Toxicity	Very Low Toxicity
Acute Oral LD_{50}	Up to and including 50 mg/kg (≤ 50 mg/kg)	Greater than 50 through 500 mg/kg (> 50 – 500 mg/kg)	Greater than 500 through 5000 mg/kg (> 500 – 5000 mg/kg)	Greater than 5000 mg/kg (> 5000 mg/kg)
Inhalation LC_{50}	Up to and including 0.05 mg/L (≤ 0.05 mg/L) (aerosol)	Greater than 0.05 through 0.5 mg/L (>0.05 – 0.5 mg/L)	Greater than 0.5 through 2.0 mg/L (> 0.5 – 2.0 mg/L)	Greater than 2.0 mg/L (> 2.0 mg/L) (dust)
Dermal LD_{50}	Up to and including 200 mg/kg (≤ 200 mg/kg)	Greater than 200 through 2000 mg/kg (> 200 - 2000 mg/kg)	Greater than 2000 through 5000 mg/kg (>2000 – 5000 mg/kg)	Greater than 5000 mg/kg (> 5000 mg/kg)
Primary Eye Irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8 – 21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Primary Skin Irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or erythema)

The highlighted boxes relfect the values in the "Acute Toxicity" section of this fact sheet. Modeled after the U.S. Environmental Protection Agency, Office of Pesticide Programs, Label Review Manual, Chapter 7: Precautionary Labeling. http://www.epa.gov/oppfead1/labeling/lrm/chap-07.pdf

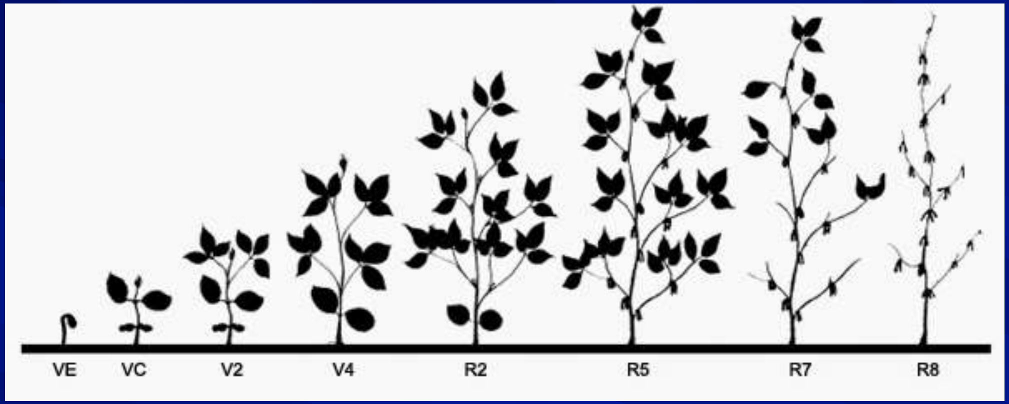
Relatively low acute toxicity.

IARC classified as a “probable carcinogen”

Classified as no evidence of carcinogenicity by many other international health organizations



Glyphosate-Resistant (Roundup Ready) Products



The herbicide is applied to the field at the first-trifoliate leaf stage.

Amount ~750 ml/acre (25 fl. ounces)

Herbicide Resistance

Resistance is conferred by CP4 EPSPS

Installed into:

Corn, soy, cotton, sugar beet, canola, alfalfa, others

EVOLVED RESISTANCE

Nine mechanisms of herbicide resistance have been identified, some weeds extremely problematic

Herbicide Resistance

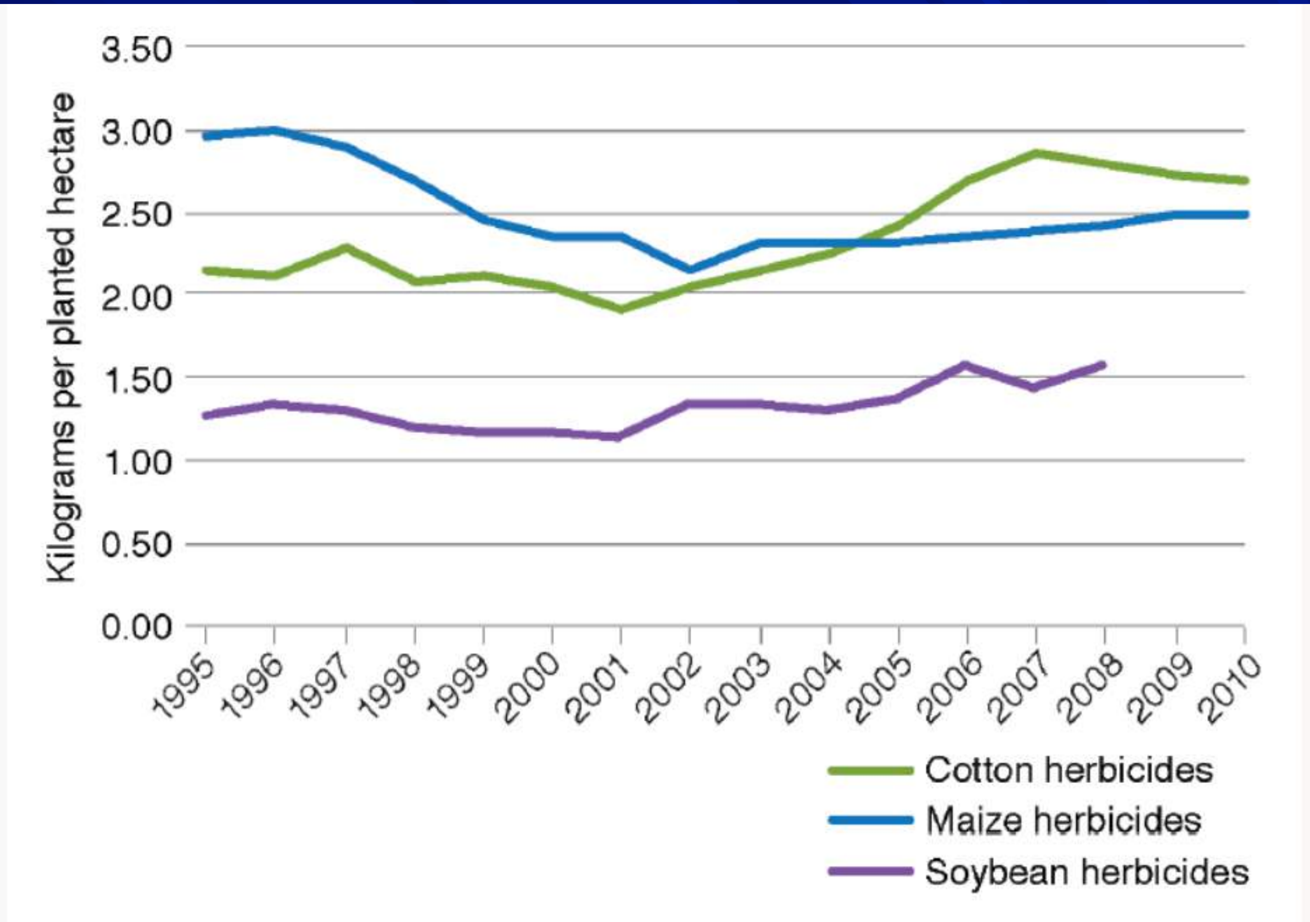
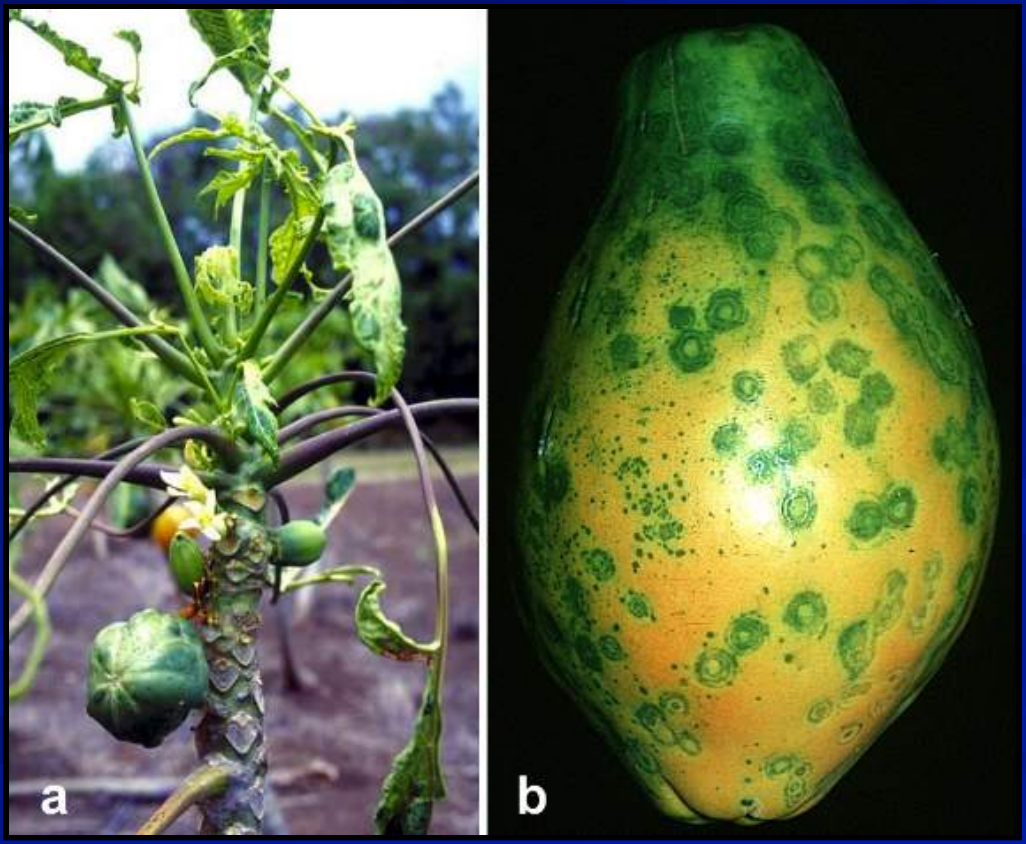


FIGURE 4-8 Herbicide use in cotton, maize, and soybean in the United States, 1995–2010.
SOURCE: Fernandez-Cornejo et al. (2014).

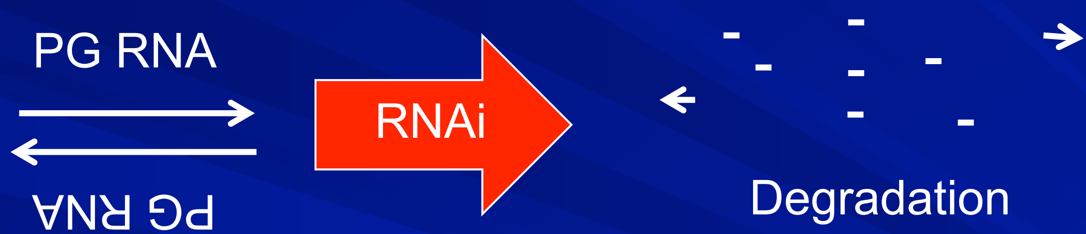
Papaya Ringspot Virus

Good example of RNAi suppression



How to Beat a Virus (like shutting off a gene)

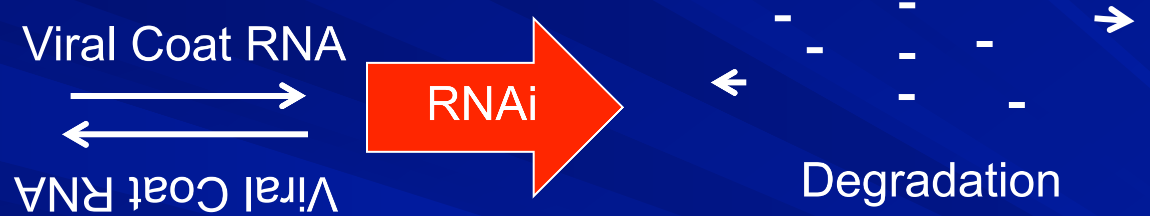
Softening is caused by an enzyme called polygalaturonase (PG).



A sequence is added that produces “antisense” PG RNA. It matches the gene sequence like a mirror. Creating “RNA interference”

How to Beat Papaya Ringspot Virus

The virus produces proteins encoded in several genes. One is a viral coat protein.

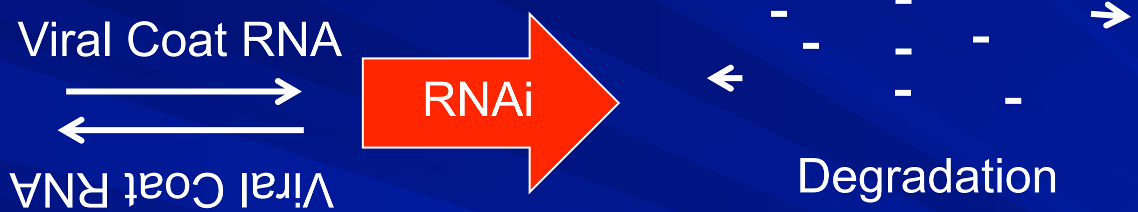


An “antisense” sequence for the viral coat protein can be installed that will effectively “cancel out” the viral RNA.

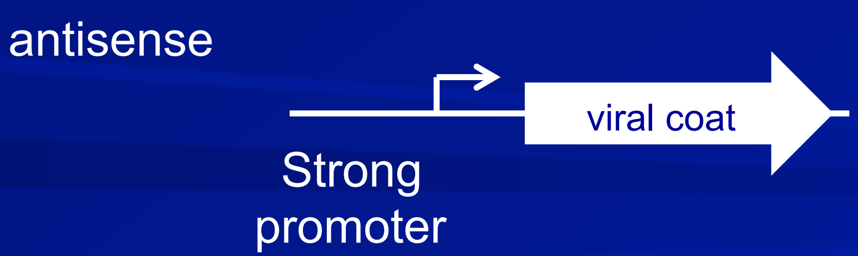
Again, “RNA interference”

NOT EXACTLY CORRECT.

The virus produces proteins encoded in several genes. One is a viral coat protein.



RNA interference can be triggered also by overexpression of the gene.

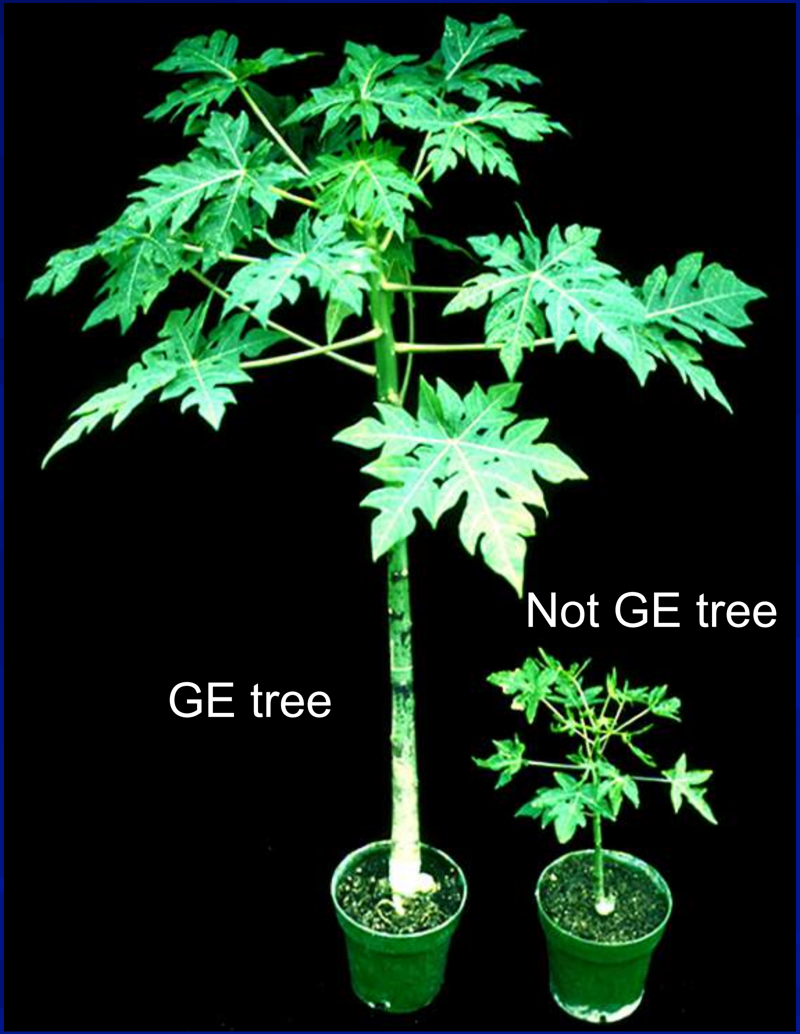


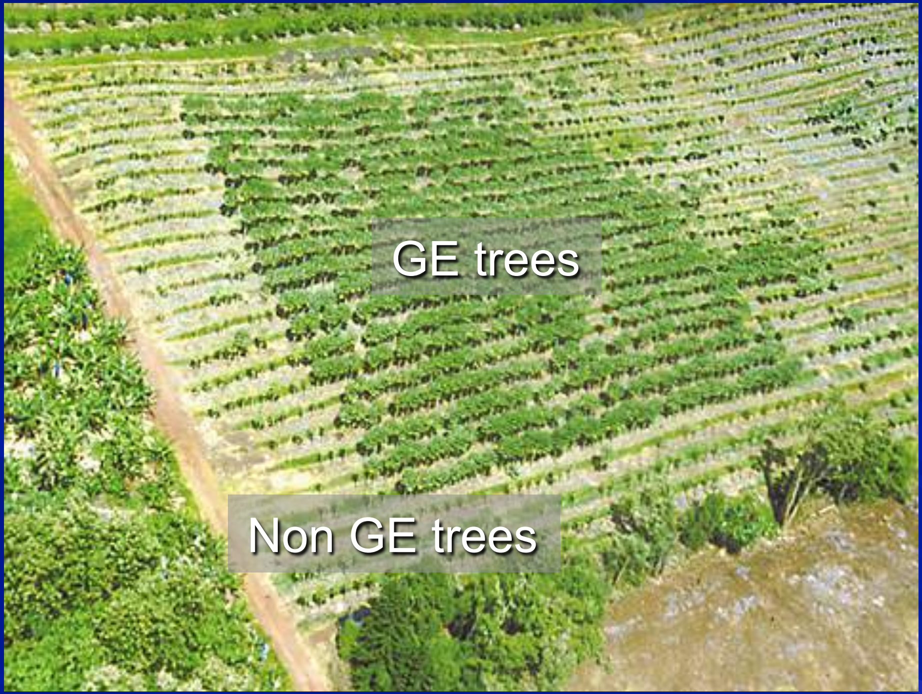
Again, “RNA interference”

Talking Biotech

Episode 26

Both Trees Virus Infected






R. Taylor Raborn @rtraborn 5h
If you had 'Ashton Kutcher weighs in on CRISPR' on your 2017 Bingo card, please come claim your prize.
ashton kutcher @aplusk
We will look back at crispr as one of the most significant advances of human kind.

Biofortification

Worldwide deficiencies in vitamin A, iron, protein, zinc, some B vitamins.

Add genes associated with biosynthesis to food staples, which tend to be carbohydrate rich and nutrient poor.

Biofortification

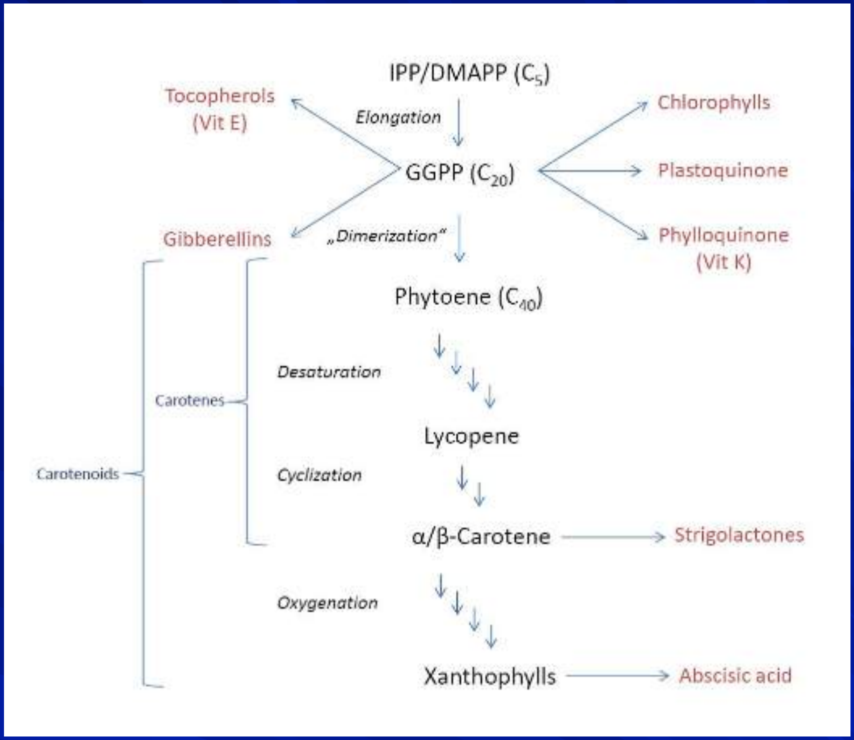
Beta Carotene

-Requires mechanisms for production and deposition, typically lipid associated.

-Potatoes, grains, cassava, taro– starchy and not amenable to carotenoid deposition

-GGPP not limiting

-Could be done with addition of two transgenes (Ye et al., 2000)

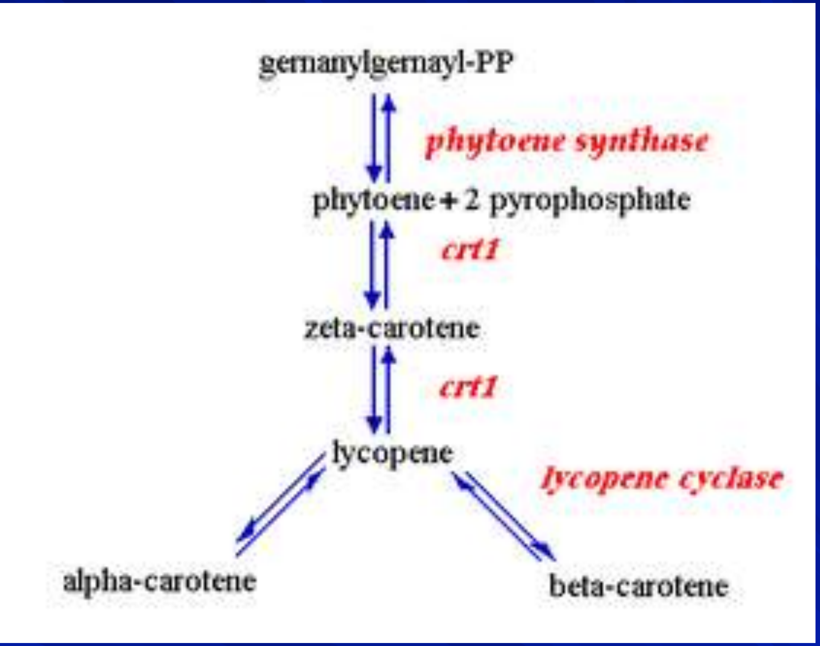


Cazzonelli, 2011

Biofortification

Beta Carotene

- Need to add phytoene synthase (PSY)
- Carotene desaturase (CRT1; bacterial, vs multiple plant ones, Romer 2000)
- Gets to Lycopene

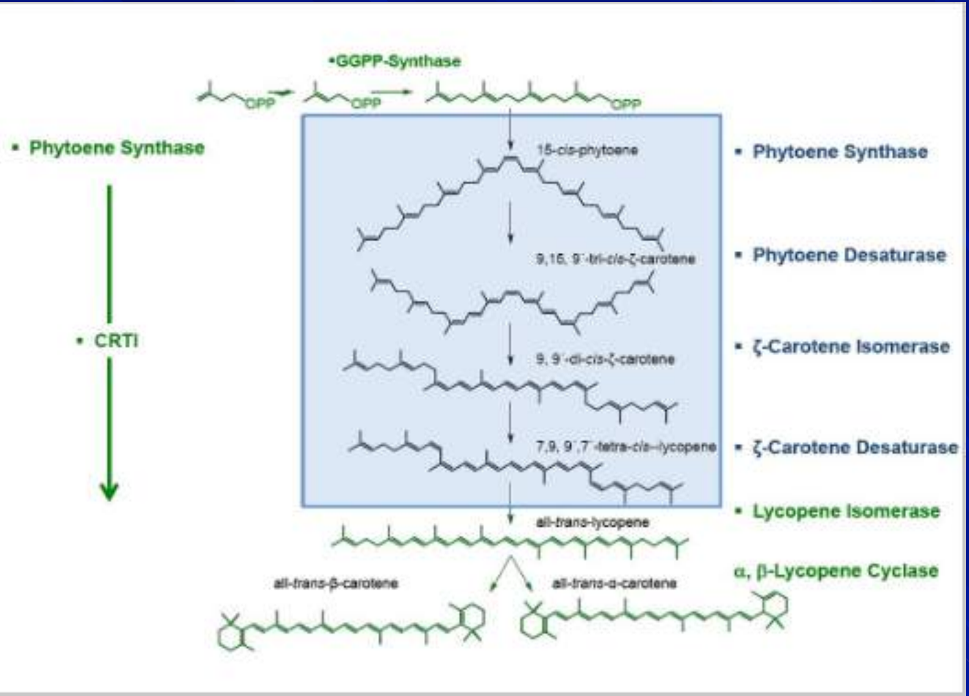


- WAIT… rice is not red. Alpha and beta carotene are observed and also xanthophylls like lutein and zeaxanthin.

Biofortification

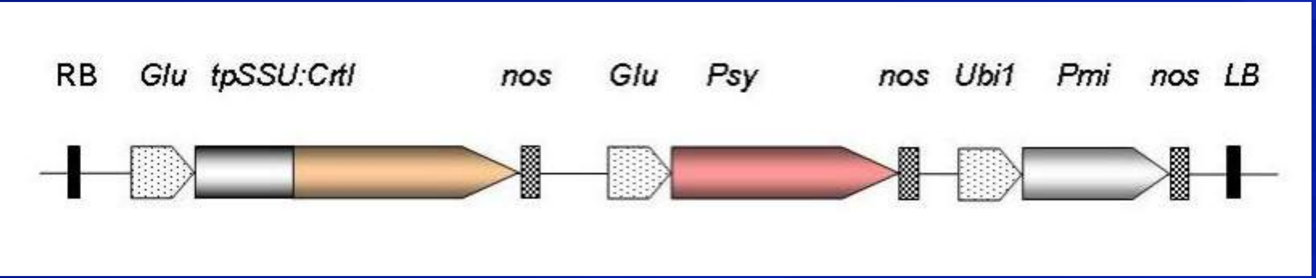
Beta Carotene

Addition of PSY and CRT1 allows lycopene to be available for downstream reactions where the enzymes are still present.



Schaub et al, 2005

First-generation transgene cassette



Biofortification

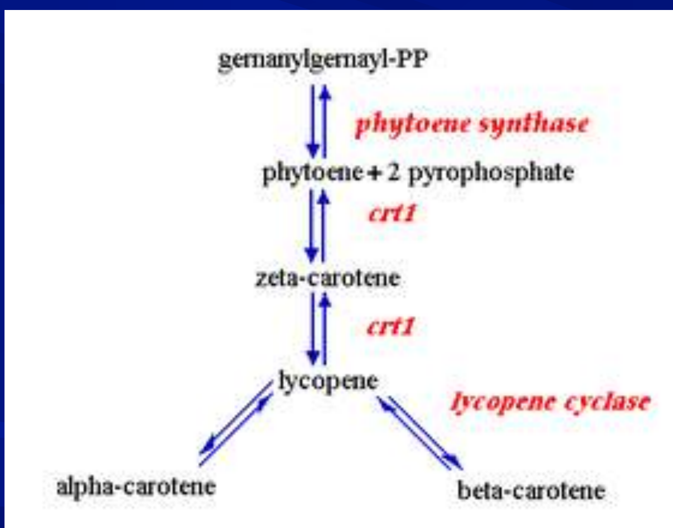
Beta Carotene

GR1 – *Psy* from *Narcissis pseudonarcissis*

GR2 – from maize

Golden Rice

Opposition to golden rice cost $2 billion to farmers in developing countries and 1.4 million human years – Wesseler et al., 2014



- 250,000-500,000 children go blind each year
- Half of them die within 12 months of losing their sight
- 1.9-2.7 million deaths per year may be due to VAD
- Impoverished families cannot afford vitamin A-rich food sources
- Supplementation is expensive and limited in effectiveness

	Farmers
	Consumers
	Environment
X	**Needy**

East African Highland Banana

15-33% of children in Uganda suffer from VAD.

Bananas have low pro-vitamin A
1-4 ug/g dw

Fe'i bananas of New Guinea have up to 340 ug/g dw

Bananas are hard to breed by sexual crossing.

Used PSY from maize, Fe'i banana, and bacterial *Crt1*

Talking Biotech

Biofortification

Beta Carotene

Cassava

Natural variation for beta carotene accumulation in cassava

Table 1. Yellow-orange root cassava accessions evaluated of the Cassava Genebank, maintained by Embrapa Cassava and Tropical Fruits

Identification number	Root color*	Common name	Brazilian State of origin
BGM 1667	Yellow	Mandioquinha	Pará
BGM 1708	Deep yellow	Jabuti- IM 957	Amazonas
BGM 1757	Deep yellow	Rosa	Maranhão
BGM 1668	Yellow	Cacau amarelo	Pará
BGM 1456	Pinkish	Vermelha	Mato Grosso
BGM 1702	Yellow	Peixe Boi – IM 929	Amazonas
BGM 1666	Yellow	Manteiga	Pará
BGM 1669	Yellow	Amarela	Pará
BGM 1692	Deep yellow	Aipim Dendê	Bahia
BGM 1700	Deep yellow	Varejão – IM 924	Amazonas
BGM 1701	Deep yellow	Sem nome – IM 928	Amazonas
BGM 1703	Deep yellow	Olho verde	Amazonas
BGM 1704	Deep yellow	Caniço – IM 936	Amazonas
BGM 1706	Deep yellow	Amarelinha	Amazonas
BGM 1709	Yellow	Canela de Velho – IM 958	Amazonas
BGM 1711	Yellow	Arani – IM 962	Amazonas
BGM 1722	Deep yellow	João Velho (Abóbora)	Maranhão
BGM 1740	Deep yellow	Branca	Maranhão
BGM 1745	Deep yellow	Carema Branca	Maranhão
BGM 1751	Deep yellow	-	Maranhão
BGM 1752	Deep yellow	Girau	Maranhão
BGM 1776	Deep yellow	Seis Meses	Maranhão
BGM 1782	Deep yellow	Carga de Burro	Maranhão
BGM 1783	Deep yellow	Verdinha	Maranhão
BGM 1787	Yellow	Aparecida 1157	Maranhão
BGM 1795	Yellow	Mucurona 1165	Maranhão
BGM 0019	Deep yellow	Xingu	Pará
BGM 0021	Yellow	Cachimbo	Pará
BGM 0456	Pinkish	Cenoura Rosada	Bahia
BGM 1153	Deep yellow	Klainasik	Amazonas

* Root color according to the scale proposed by Echeverry et al. (2001)

Ferreira et al., 2008

Table 2. Cassava genotypes with the highest concentration of carotene (fresh weight basis) in the roots

Genotype	Common Name	Origin	Root color	Carotene (mg/100 g)
MBRA 516	Olho Verde	Manaus - Brazil	Deep yellow	2.55
MCOL 2285	Furetsikae	Guainia - Col.	Orange	2.40
MCOL 2109	Manaca	Amazonas - Col.	Orange	2.14
MCOL 2295	Bitsurikae	Guainia - Col.	Orange	2.12
MBRA 481	Baixota	Manaus - Brazil	Orange	2.04

Iglesias 1997

Biofortification

Beta Carotene

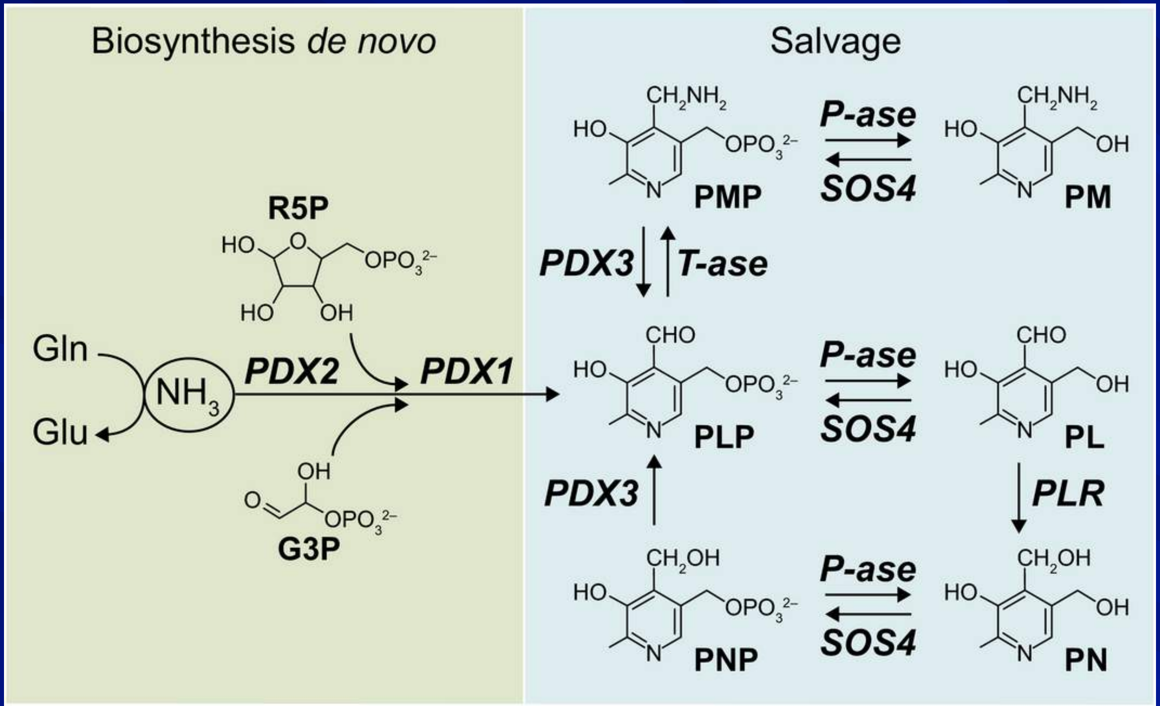
HarvestPlus
@HarvestPlus

Thanks to Dr. Elizabeth Parkes, a HarvestPlus cassava breeder at @IITA_CGIAR, Nigeria, for a great presentation on Vitamin A Biofortification of Cassava! Cassava has grown to be one of the most popular staple crops in the developing world, with huge biofortificaiton potential

4:56pm - 28 Nov 2017 - Twitter Web Client

9 RETWEETS 22 LIKES

Vitamin B_6 Deficiency in USA (24%) and Africa (~65%)



PDX2, PDX1 overexpression

-increased PLP in Arabidopsis

- Increased PLP in cassava (Li et al., 2015)

Medicine is not health care, food is health care: plant metabolic engineering, diet and human health

New Phytologist
Volume 216, Issue 3, pages 699-719, 10 AUG 2017 DOI: 10.1111/nph.14730
http://onlinelibrary.wiley.com/doi/10.1111/nph.14730/full#nph14730-fig-0003

Talking Biotech

Folate Vitamin B_9

Deficiency in USA (24%) and Africa (~65%)

Expression of limiting enzymes can increase folate up to 100x.
(Storozhenko *et al*., **2007**)

Ascorbate Vitamin C

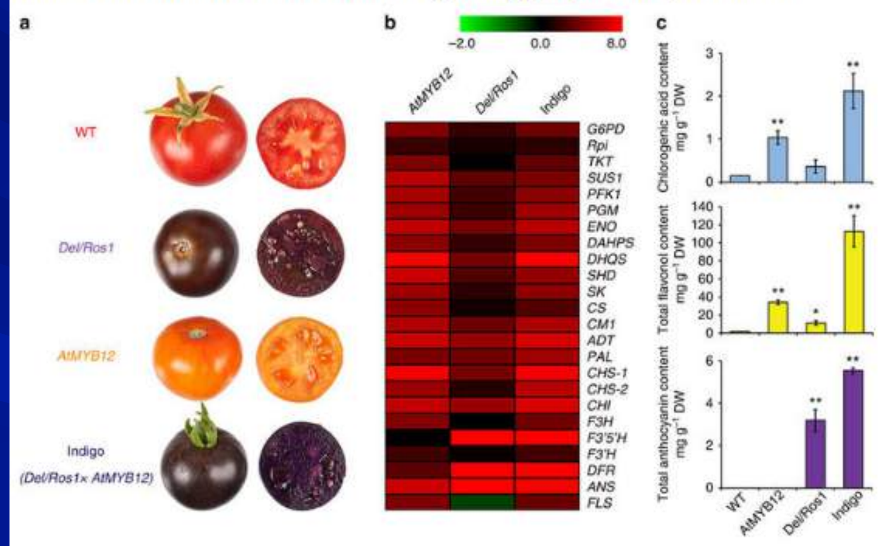
Vitamin E

All increased with metabolic engineering, at least in models.

High anthocyanin tomatoes

Overexpression of MYB12 with other transcription factors

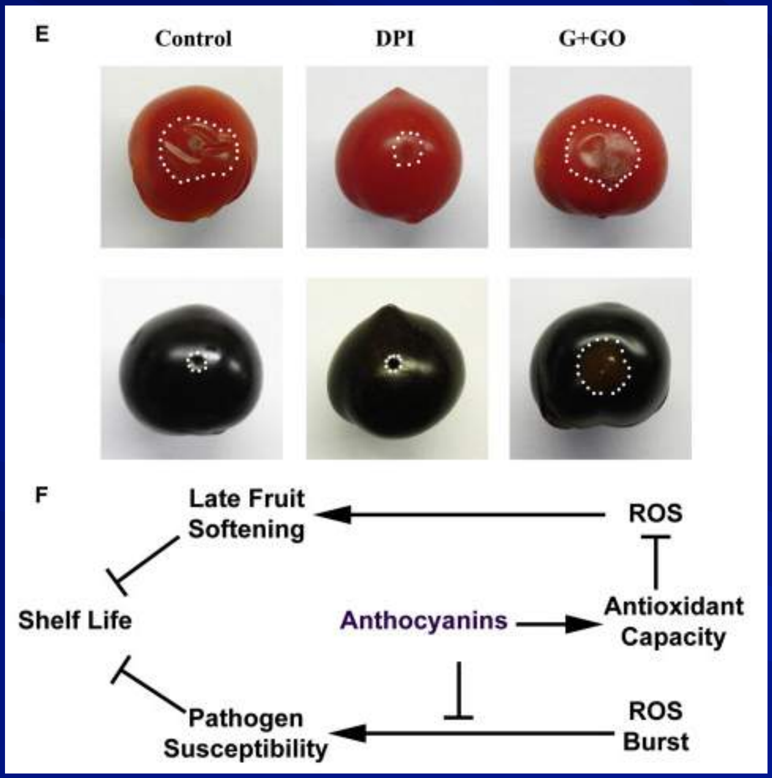
Figure 5: Co-expression of AtMYB12 with other transcription factors in tomato fruit enhances phenylpropanoid production.



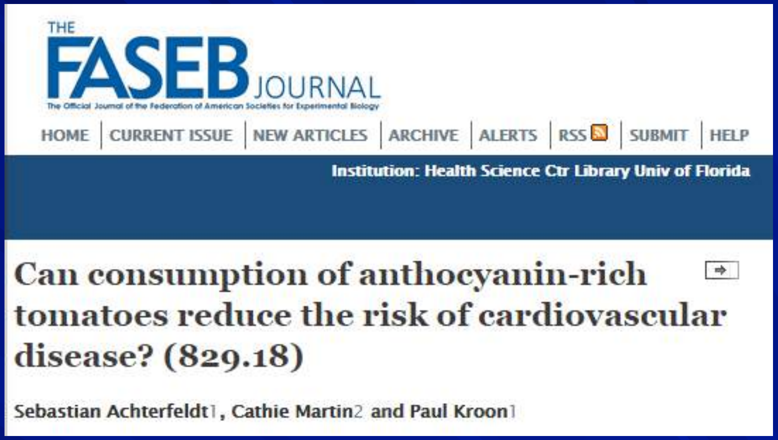
Talking Biotech

In 2018

Improved postharvest quality



Zhang 2013


THE FASEB JOURNAL
The Official Journal of the Federation of American Societies for Experimental Biology
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Institution: Health Science Ctr Library Univ of Florida

Can consumption of anthocyanin-rich tomatoes reduce the risk of cardiovascular disease? (829.18)

Sebastian Achterfeldt[1], Cathie Martin[2] and Paul Kroon[1]

Talking Biotech

In 2018

Elimination of Anti-nutrients and Allergens

Corn that limits aflatoxin accumulation

PROBLEM: Chronic exposure to aflatoxin in the developing world.

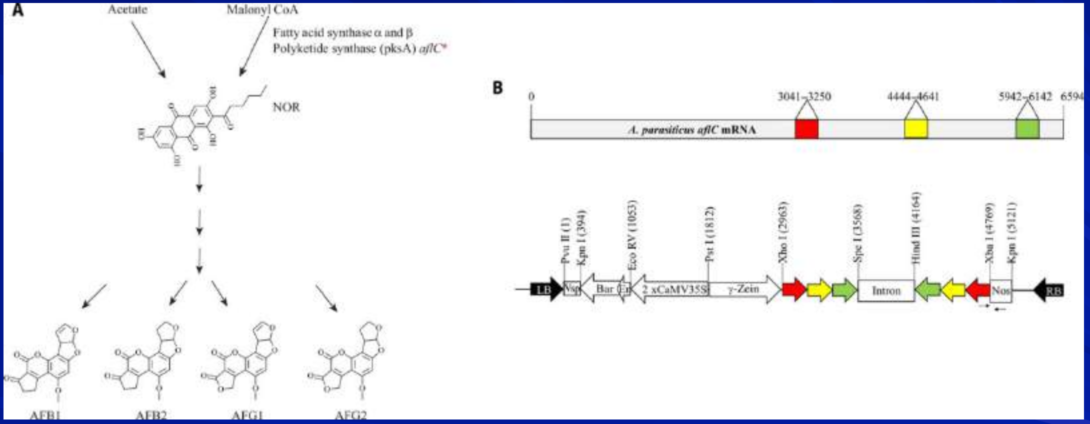
RESEARCH ARTICLE | AGRICULTURE

Aflatoxin-free transgenic maize using host-induced gene silencing

Dhiraj Thakare[1], Jianwei Zhang[1,2], Rod A. Wing[1,2], Peter J. Cotty[3] and Monica A. Schmidt[1,*]
+ See all authors and affiliations

Science Advances 10 Mar 2017:
Vol. 3, no. 3, e1602382
DOI: 10.1126/sciadv.1602382

Figure 1. Aspergillus ear rot symptoms on corn ear (left) and growth of *Aspergillus flavus* in artificial culture (right).



Also accomplished in ground nut (peanut)

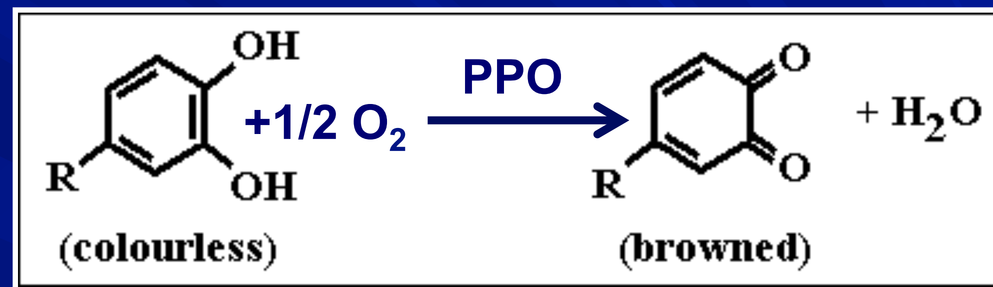
Talking Biotech

74 corn; 114 peanut

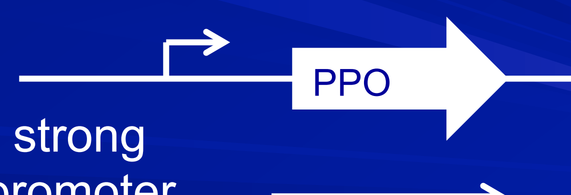
Non-Browning Fruit

Non Browning Apples

Silencing a gene that leads to discoloration



Overexpression of PPO leads to suppression

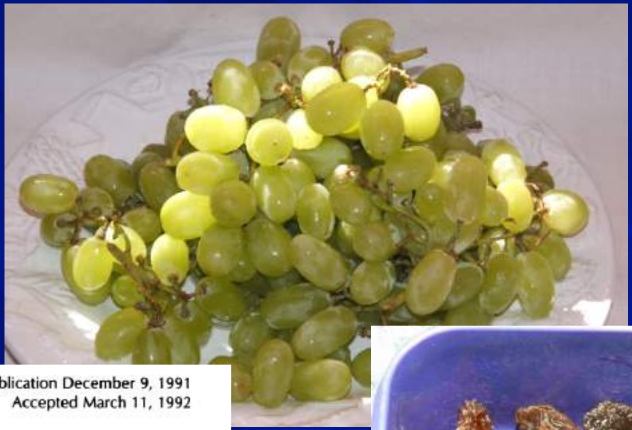


Talking Biotech

PPO Suppression- Anything New?

Bruce's Sport

1962 'Sultana' grape



Plant Physiol. (1992) 99, 1619–1625
0032-0889/92/99/1619/07/$01.00/0

Received for publication December 9, 1991
Accepted March 11, 1992

Aberrant Processing of Polyphenol Oxidase in a Variegated Grapevine Mutant[1]

Anne H. Rathjen and Simon P. Robinson*

Commonwealth Scientific and Industrial Research Organization Division of Horticulture, GPO Box 350, Adelaide, South Australia 5001 Australia

ABSTRACT

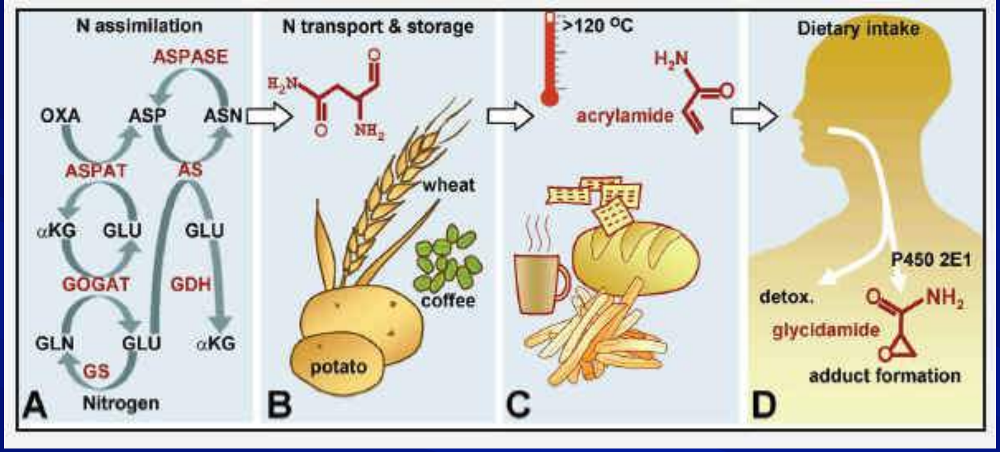
Bruce's Sport is a mutant grapevine (*Vitis vinifera* L.) with green and white variegated fruit derived from the Sultana variety. The white regions of tissue have decreased polyphenol oxidase (PPO) activity resulting in a reduced capacity for browning. Active PPO from Sultana grapes was purified and had an apparent molecular weight of 40,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Western blots indicated that mature Sultana grapes contained a single 40-kilodalton PPO, and young Sultana berries also had small quantities of a 60-kilodalton protein. Bruce's Sport grapes had much less of the 40-kilodalton PPO and greater amounts of the 60-kilodalton band. Protease digestion of Bruce's Sport extracts decreased the proportion of the 60-kilodalton protein and increased the 40-kilodalton band. A cDNA clone of grape PPO was used to probe a northern blot of Sultana and Bruce's Sport RNA and hybridized to a 2.2-kilobase transcript in both grapevines. The level of PPO mRNA was high in the early stages of berry development but then declined. The results suggest that in grapevine the active 40-kilodalton form of PPO is synthesized as a precursor protein of at least 60 kilodaltons, and normal processing is interrupted in Bruce's Sport resulting in the accumulation of the 60-kilodalton inactive preform of PPO.

vacuole and thus browning only occurs when plant cells are damaged.

The molecular mass of PPO has not yet been clearly established. Gel filtration of the PPO of *Mucuna pruriens* determined its molecular mass to be 90 kD (28). The protein was found to be a dimer, denaturation revealing two subunits of 42 kD. Partially denaturing gels stained for PPO activity revealed a 40-kD PPO in *Vicia faba*, the same size as the denatured purified protein (26). The PPOs purified from olive, sago palm, and spinach also consisted of a single subunit of 40 to 42 kD (2, 14, 23). Western blots probed with a polyclonal antibody raised against purified broad bean PPO indicated that a 43- to 45-kD band was present in broad bean, bush bean, lettuce, mung bean, soybean, spinach, and tobacco (8). *In vitro* translation of leaf mRNA isolated from each of these species produced a protein of approximately 45 kD (4), resulting in the suggestion that in a range of plant tissues PPO is synthesized as a 45-kD protein without a transit sequence.

Although PPO has been extensively studied in grapes (*Vitis vinifera* L.), much of the work has been concerned with browning during juice or wine production (29). The existence

Low Acrylamide, non Browning Potatoes



Antisense Asp synthase in the tuber

Antisense PPO

Antisense acid invertase

Addition of R gene from wild potato to confer resistance to Late Blight

Low Acrylamide, non Browning Potatoes

Antisense Asp synthase in the tuber – lower acrylamide

Antisense PPO- marketable yield, postharvest improvement

Antisense acid invertase – improved cold storage

Addition of R gene from wild potato to confer resistance to Late Blight – good for organic and conventional growers, gene from *Solanum venturii*

Disease Resistance

Bacterial Wilt in Bananas

>70% of carbohydrate calories for some areas

GM trials in Uganda

Transgenic Res
DOI 10.1007/s11248-011-9574-y

ORIGINAL PAPER

Transgenic banana expressing *Pflp* gene confers enhanced resistance to Xanthomonas wilt disease

B. Namukwaya · L. Tripathi · J. N. Tripathi · G. Arinaitwe · S. B. Mukasa · W. K. Tushemereirwe

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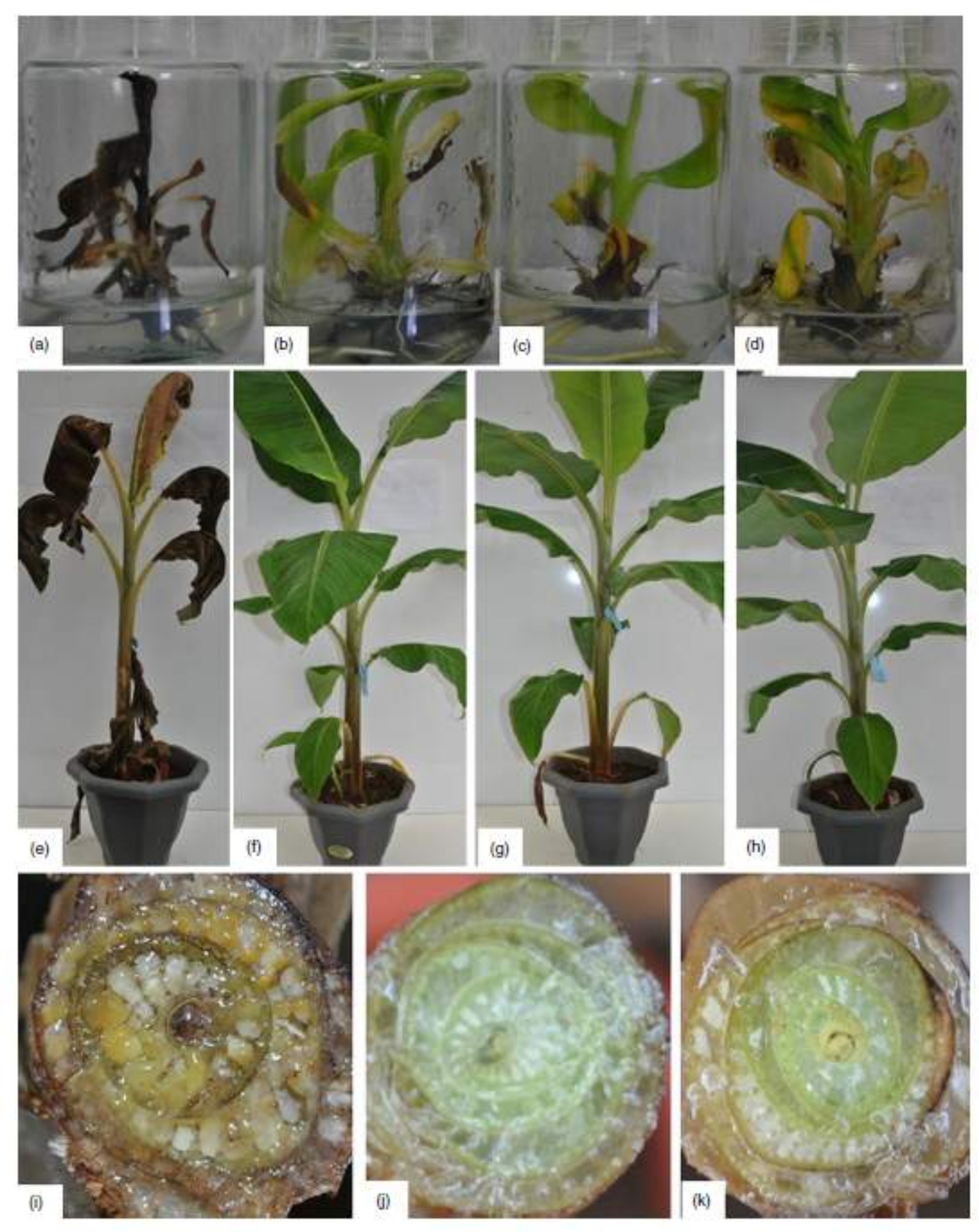
NARO
IITA
Research to Nourish Africa
Confined Field Trial (CFT) of GM Cooking Bananas for Resistance to Bacterial Wilt
UNCST Permit No: A 529
For Research Purposes Only
Authorized Personnel Only
Planted: 28/02/2017
Site: NARL - Kawanda
THE REPUBLIC OF UGANDA
Supported by: GOU and USAID
USAID
FROM THE AMERICAN PEOPLE



Over expression of rice Xa21 gene

Tripathi et al, 2014

Multiple solutions for banana

Resistance Technology/Target Gene	Origin	Target Organism	Crop	Mode of Action	Resistance		References
					Green house	Field	
Bacterial Disease Resistance							
Hrap	Sweet pepper	*X. campestris pv. musacearum*	Banana	Hypersensitivity Response	Full	Full	Tripathi et al. (2010, 2014a)
Pflp	Sweet pepper	*X. campestris pv. musacearum*	Banana	Hypersensitivity Response	Full	Full	Namukwaya et al. (2012); Tripathi et al. (2014a)
Xa21	Rice	*X. campestris pv. musacearum*	Banana	Pathogen Recognition	Full	–	Tripathi et al. (2014b)

Tripathi et al, 2017

BS2 Tomato

A pepper gene in tomato eases bacterial wilt.

#15

Figure 3. Photographs of non-transgenic and Bs2-transgenic VF36 lines in field trials. Top. Plants of the non-transformed VF36 line. Bottom. Plants of the transgenic VF36 line containing the 35S:Bs2 gene. Balm, FL, Spring 2008 Trial.
doi:10.1371/journal.pone.0042036.g003

Grapes resistant to Pierce's Disease

Anti-Microbial Peptides
Target *Xylella* wilts
(Pierce's Disease)

Stopping Citrus Greening

Bacterial disease, spread by an insect

100% lethal, >90% of groves are infected

Lower yields, mis-shapen fruits

Lower juice quality

$10 Billion lost

~3000 jobs

Can't put industry on hold

Stopping Citrus Greening

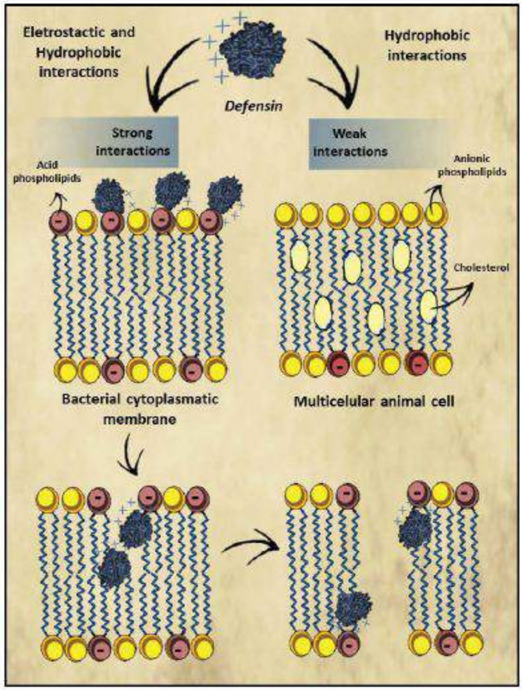


Figure 3: The biological mechanism of APs. Initially the electrostatic attraction and the transmembrane bioelectric field pull the defensin towards and into the pathogen membrane. Then the pathogen membrane is depolarized and pores are formed with subsequent loss of cytoplasmic contents.

Spinach defensin

Lytic peptides

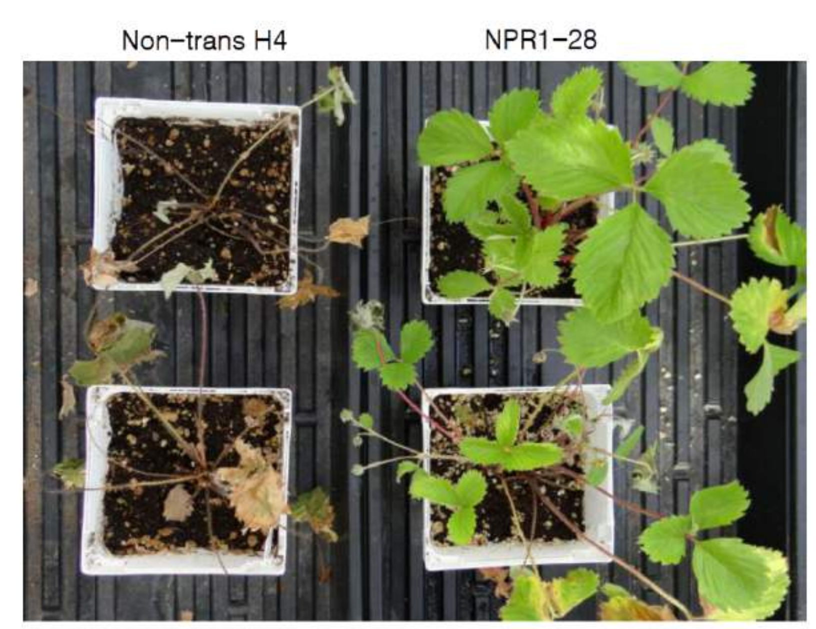
Many show promise

Earliest deregulation is 2019

Citrus Tristeza Virus

Talking Biotech

Fungicide-Free Strawberries?



Silva et al., 2015

Overexpression of *Npr1*

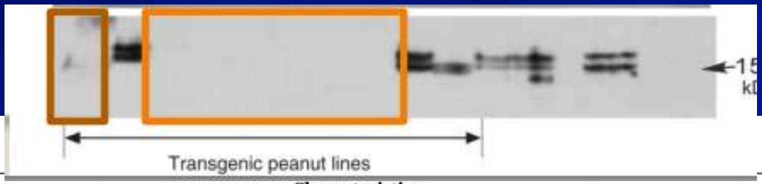
Provides excellent resistance

Some yield loss.

Allergy Suppression

Hypoallergenic Peanuts

Peanut – RNAi suppression Ara h2

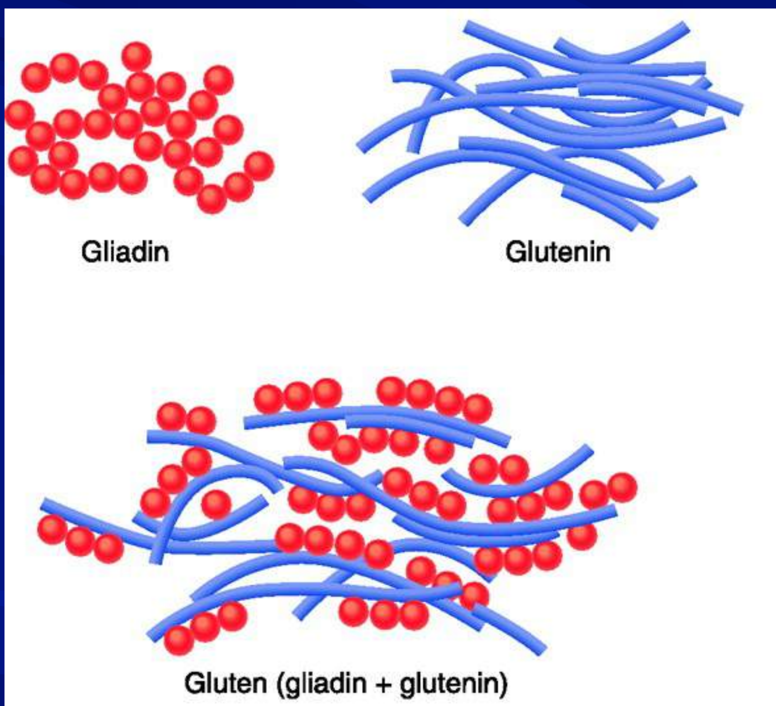


Characteristics of Peanut Allergens

Allergen	Molecular Mass	Characteristics
Ara h 1	63 k-Da	Member of vicilin family of seed storage proteins, a 7S globulin
Ara h 2	17–19 k-Da	Member of conglutin family of seed storage proteins, a 2S albumin
Ara h 3	14–45 k-Da, processed from 64 k-Da protein	Member of glycinin family of seed storage proteins; heteromultimeric protein formed from differently proteoltically processed products of the same gene, an 11S globulin
Ara h 4	37 k-Da	Isoform of Ara h 3
Ara h 5	15 k-Da	Member of profilin family of G-actin-binding proteins
Ara h 6	15 k-Da	Member of conglutin family of seed storage proteins, a 2S albumin
Ara h 7	17 k-Da	Member of conglutin family of seed storage proteins, a 2S albumin
Ara h 8	16 k-Da	Homologous to major birch pollen allergen, Bet v 1 and other pathogenesis-related proteins
Ara h 9	9.8 k-Da	Lipid transfer protein
Ara h 10	16 k-Da	Oleosin seed storage protein
Ara h 11	14 k-Da	Oleosin seed storage protein

Celiac Safe Wheat

Gene editing the epitopes of glutennin and gilladin



New Scientist

NEWS & TECHNOLOGY 26 September 2017

Genetically modified wheat used to make coeliac-friendly bread

Loafs of bread

Daily bread: if gluten's not for you

Superstock

By Michael Le Page

People forced to avoid gluten could soon have their bread (and cake) and eat it. Now there are strains of wheat that do not produce the forms of gluten that trigger a dangerous immune reaction in as many as 1 in 100 people.

Because the new strains still contain some kinds of gluten, though, the wheat can still be used to bake bread. "It's regarded as being pretty good, certainly better than anything on the gluten-free shelves," says Jan Chojecki of PBL-Ventures in the UK, who is working with investors in North America to market products made with this wheat.

Gluten is the general term for all the proteins in wheat and related cereals. During baking, these proteins link up to form elastic chains, which is what holds breads and cakes together as they rise.

Viral Resistance

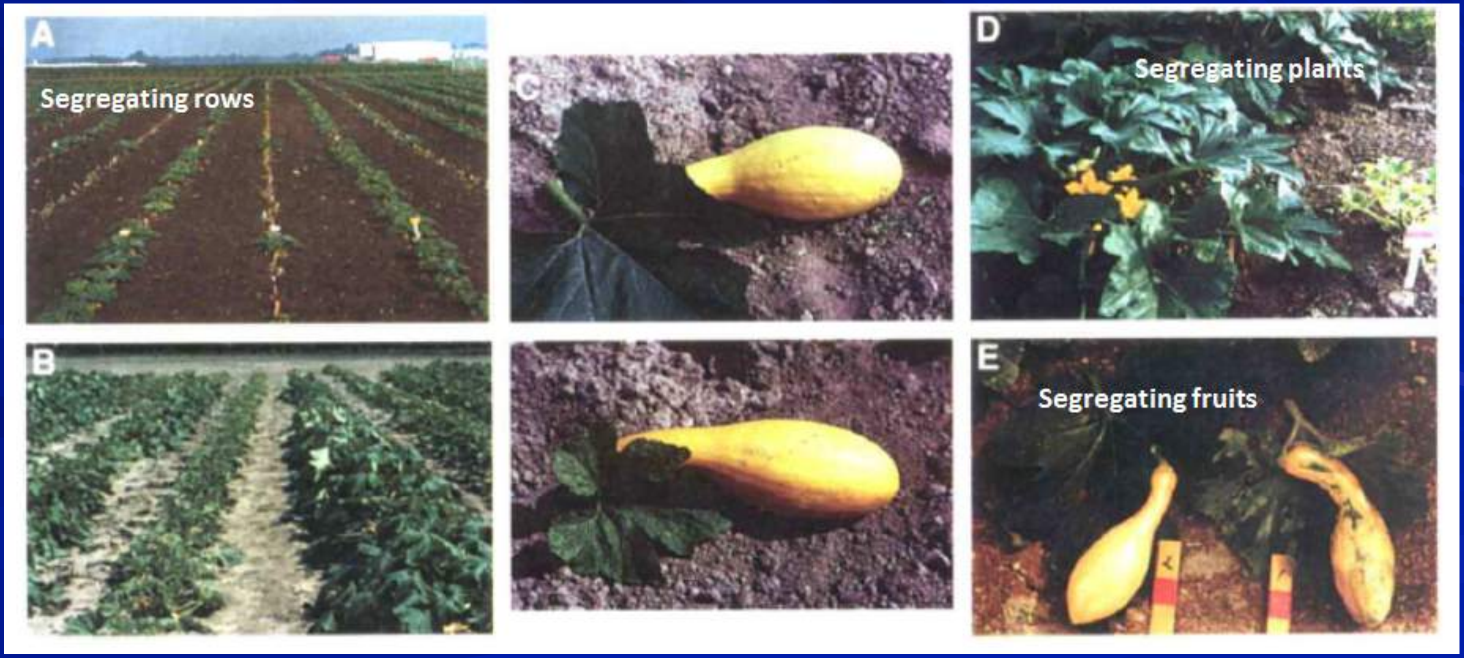
Viruses are spread by insect vectors and threaten many crops worldwide.

Transgenic strategies have been very successful in imparting durable resistance

Plum Pox Virus (Sharka Disease)

Deregulated in the USA

Virus resistant squash



Cassava

1 billion people depend on cassava

50 million tons lost to virus.

Cassava Brown Streak Virus

Cassava Mosaic Virus

Insect vectored

Cassava Mosaic Disease:
A Curse to Food Security in Sub-Saharan Africa

Olufemi J. Alabi,
(corresponding author: olufemi_alabi@wsu.edu)
Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350;

P. Lava Kumar,
International Institute of Tropical Agriculture, Oyo Road, P.M.B. 5320, Ibadan, Nigeria; and

Rayapati A. Naidu,
Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350

Plant Biotechnology Journal Open Access aab SEB Society for Experimental Biology
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Open Access

Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes

Peng Zhang, Hervé Vanderschuren, Johannes Fütterer, Wilhelm Gruissem

First published: 13 May 2005 Full publication history

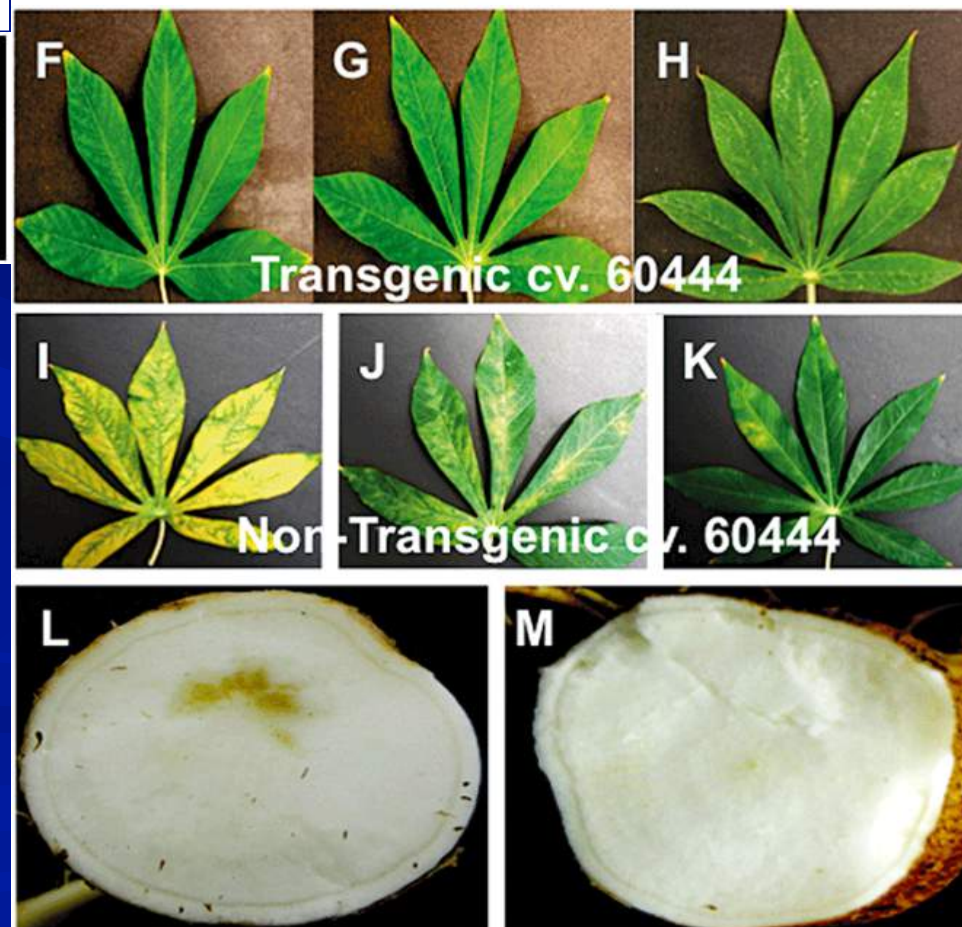
Solutions from RNAi

Molecular Plant Pathology BSPP
Explore this journal >

RNAi-mediated resistance to *Cassava brown streak Uganda virus* in transgenic cassava

JITENDER S. YADAV, EMMANUEL OGWOK, HENRY WAGABA, BASAVAPRABHU L. PATIL, BASAVARAJ BAGEWADI, TITUS ALICAI, ELIANA GAITAN-SOLIS, NIGEL J. TAYLOR, CLAUDE M. FAUQUET

First published: 17 February 2011 Full publication history



Oil Composition

Shifting profiles by silencing Fatty Acid Desaturase genes (multiple alleles).

Done by RNAi and TALEN. TALEN-based will hit the market in 2018.

Typical Fatty Acid Composition

	C16:0 Palmitic Acid	C18:0 Stearic Acid	C18:1 Oleic Acid	C18:2 Linoleic Acid	C18:3 Linolenic Acid
Plenish® High Oleic Soybean Oil	6.5	4	75	7	2.5
Commodity Soybean Oil	11	4	22	55	8

Note: Fatty acid profiles may vary slightly.

High oleic acid, improved nutrition profile and stability
0 g trans fat

	Hybrids (cross between two non-clonal plants)	Polyploids (whole genomes duplicated or added)	Mutation breeding (Chemical or radiation induced damage to DNA)	Crossing Species Barriers (interspecific crosses)	Transgenics (rDNA method to add a gene- "GMO")	Cisgenics (rDNA method to add a gene)
Examples in common foods	Almost everything	Strawberries, wheat, bananas, brassicas, others	Some bananas, pears, apples, rice, yams, mint, others	Pluots, tangelos, some apples, rice, wheat	Much corn, canola, soybeans, cotton, papaya	Coming soon.
Transfers genes from one species to another	Yes, sometimes	Yes, often	No	By definition	Yes	No
Occurs in nature	Yes	Yes	Yes, transposon movement, mutation from environment	Yes, rare, seldom fertile	Yes. Agrobacterium, other horiz. trans.	N/A
Human intervention	Yes, for crop improvement	Can be induced chemically to improve crops	Yes, to introduce variation for crop improvement	Yes, for crop improvement	Yes, for precision crop improvement	Yes, for precision crop improvement
Number of genes affected	10K to >300K, depending on species	10K to >800K	No way to assess	10-300K	1-3	1-3, usually 1
Know what genes moved or affected do	No.	No	No	No	Yes	Yes
Know where affected genes are in genome	No	No	No	No	Yes	Yes
Plant patentable	Yes	Yes	Yes	Yes	Yes	Yes
Documented adversity	Yes	??	???	Yes	No	No
Environmental assessment	No	No	No	No	Yes	Will see.
Organic acceptable	Yes	Yes	Yes	Yes	No	No
Time for new variety	5-30 years	>5 years	>5 years	5-30 years	<5 years	<5 years
Demanding label	No	No	No	No	Yes	Will see.

INSECT CONTROL II

“Sterile Insect” technique

Mutagenized adults with radiation and chemicals

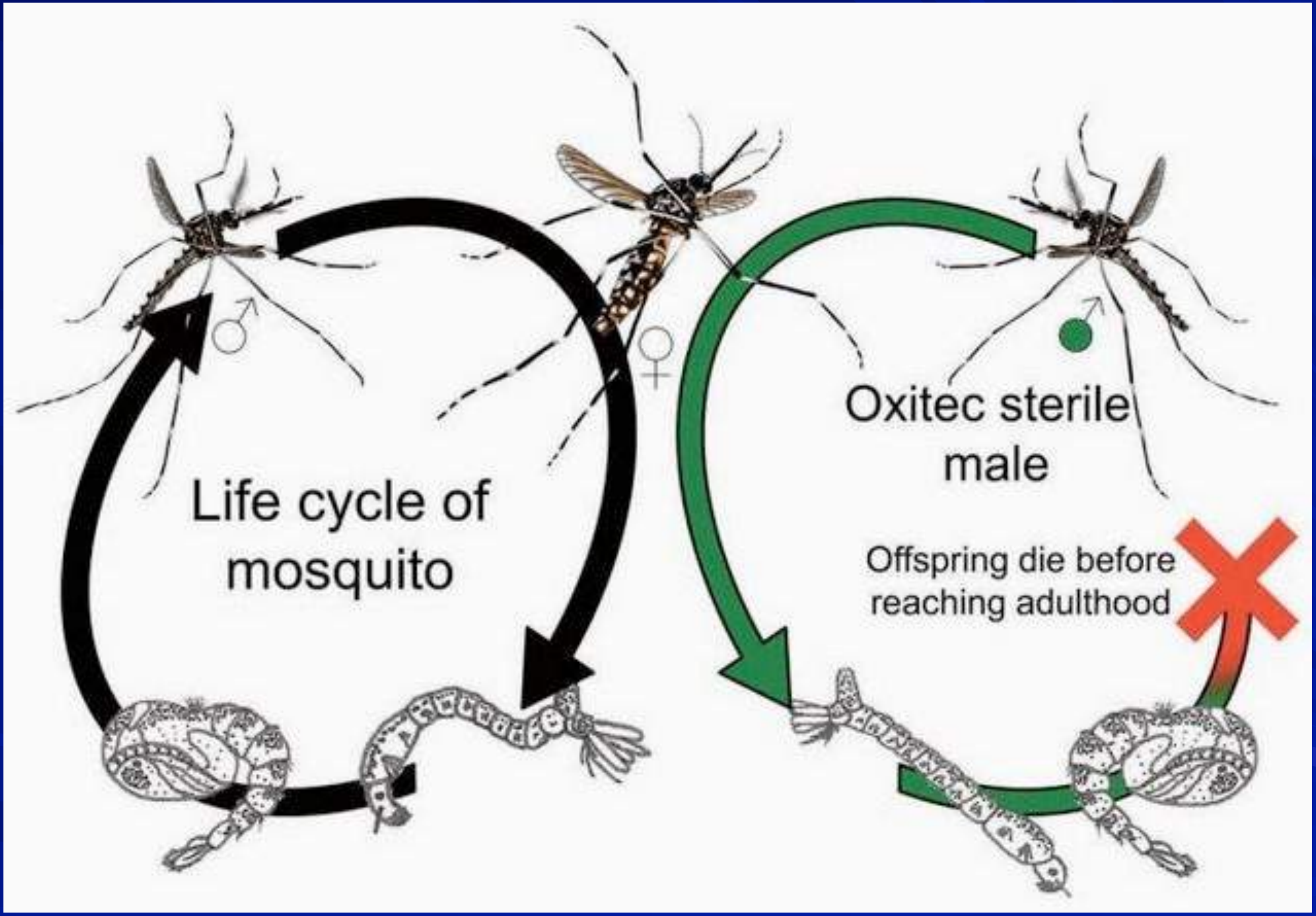
When released would produce damaged gametes that would suppress populations

New technique expresses a gene that when expressed, kills the larvae.

Suppressed in larval stage with a tetracycline-repressible promoter.

Being used on mosqutioes, diamonback moth, and fall army worm

INSECT CONTROL II



COMMUNICATION

The Collateral Effects Stall Application of Innovative Products or Practices

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Consumers are reacting to information

Pesticides

Antibiotics

Hormones

Fertilizer

Gluten

Neonics

GMO

Dihyrogen monoxide

BPA

MSG

How do we build trust as scientists and ag producers?

Educate them!
Show them the data!
Provide citations!
Teach the facts!


BOOKS FEBRUARY 27, 2017 ISSUE

WHY FACTS DON'T CHANGE OUR MINDS

New discoveries about the human mind show the limitations of reason.

By Elizabeth Kolbert

Facts do not matter without trust.

"I've learned that people will forget what you said, people will forget what you did, but people will never forget how you made them feel."

- Maya Angelou

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Solution

Our agricultural producers, scientists, ag industry, must be part of the conversation.

Stop talking to each other- and talk to non-traditional audiences.

Not so simple

Scientists, industry professionals, agricultural producers, etc.

Are not trained to do this
Don't want to do this
Make mistakes when they do this

Not so simple

There are two major barriers blocking good communication:

How humans process information
We trust people that are like us

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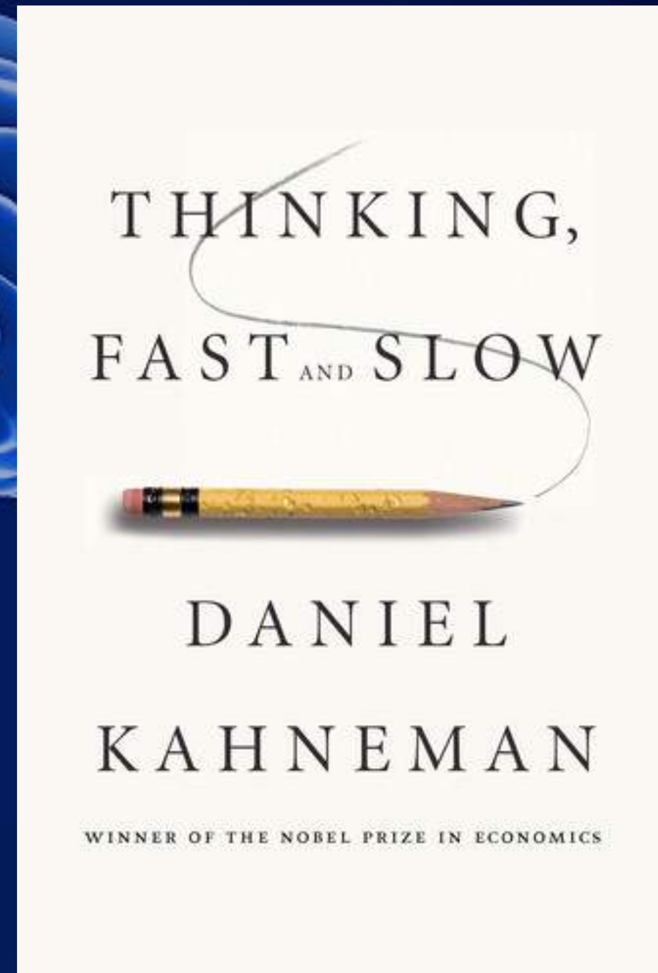
How we process information

System 1 – Emotional, irrational, reactive

System 2 – Logical, strategic, calculating



We Trust People Like Us

We group with others of similar worldview and interests.

They adopt and defend similar premises, even if incorrect.

Use groups as identity to anchor deeply held beliefs

We create trusted groups

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To Establish Trust You Have to Override the Reactive Brain and Build Trust

Establish that you are not a threat

Listening, showing empathy
Affirming shared values and dreams

Building rapport


How do we win the trust of someone that is emotional and irrational?
UF IFAS
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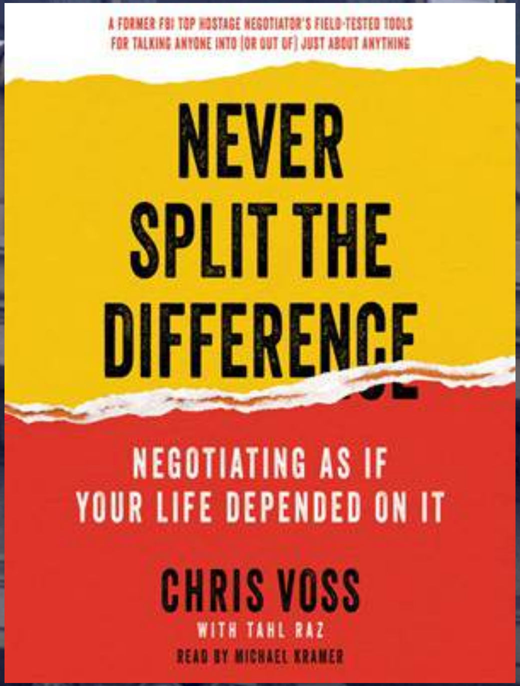
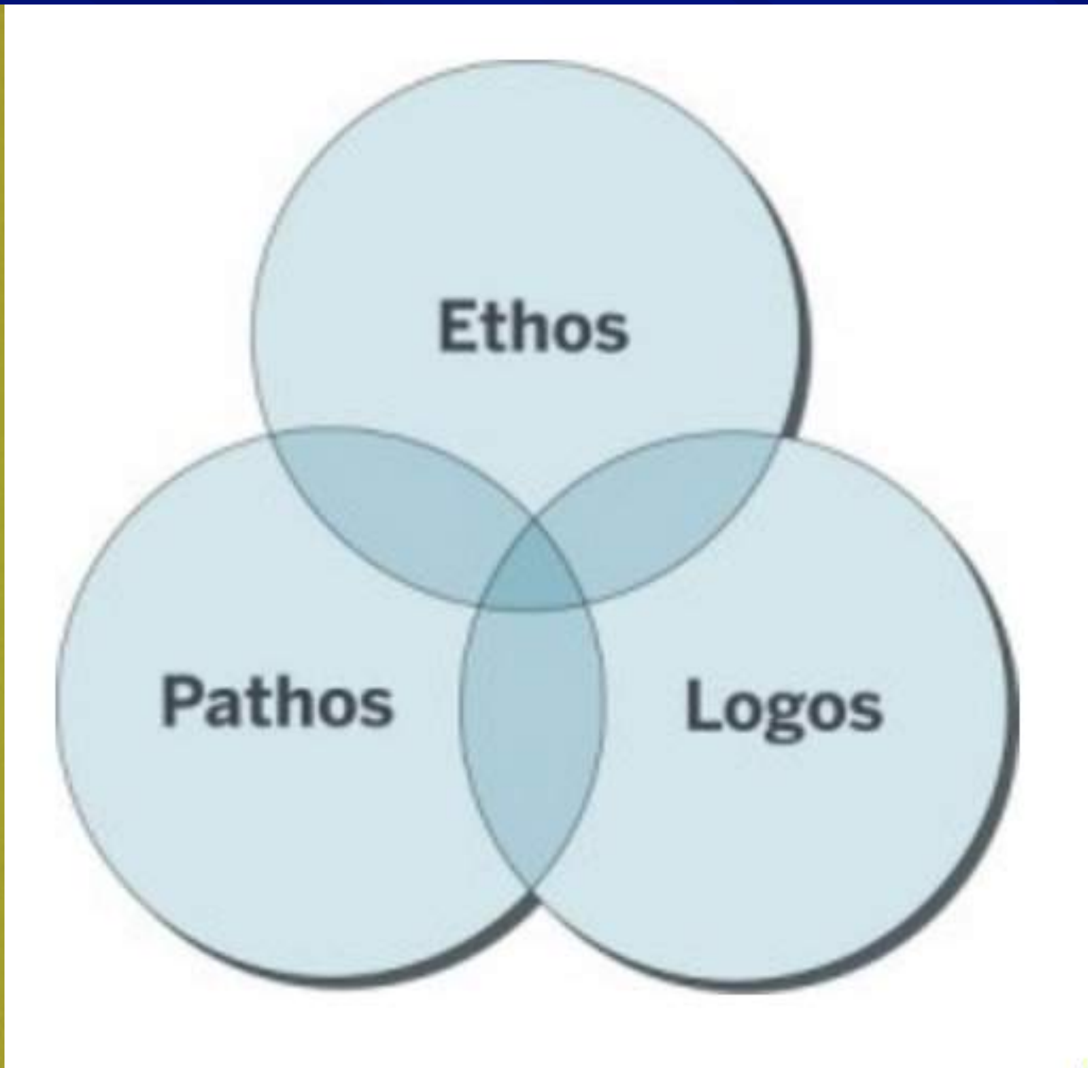
Active Listening

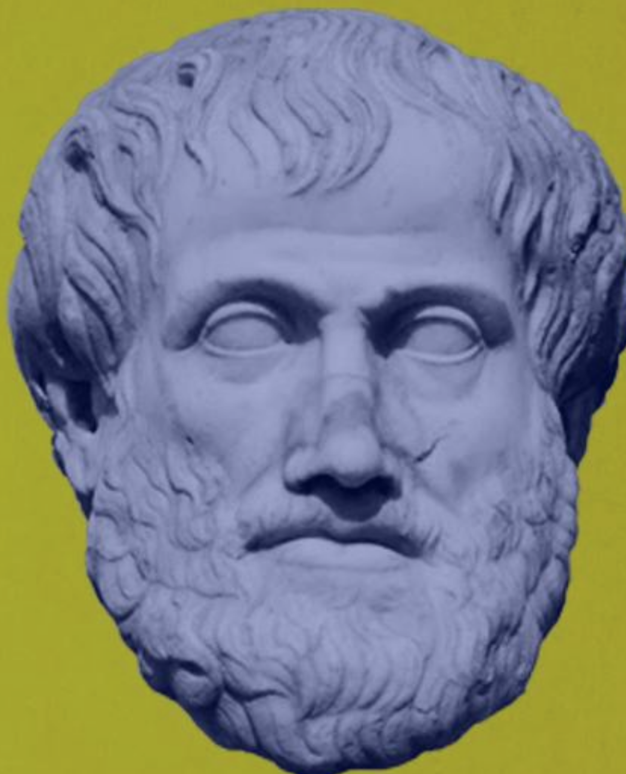
Intellectual Charity

Provide a sense of fairness and control of the conversation



Lead With Your Ethics.





What are our mutual concerns?

Contact.

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Talking Biotech

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iTunes, Stitcher, Player FM

All funding and reimbursements are provided on

www.kevinfolta.com/transparency

“Don’t tell me it can’t be done,
tell me what needs to be done
and help me do it.”