

Plant Biotechnology

Plant Tissue Culture

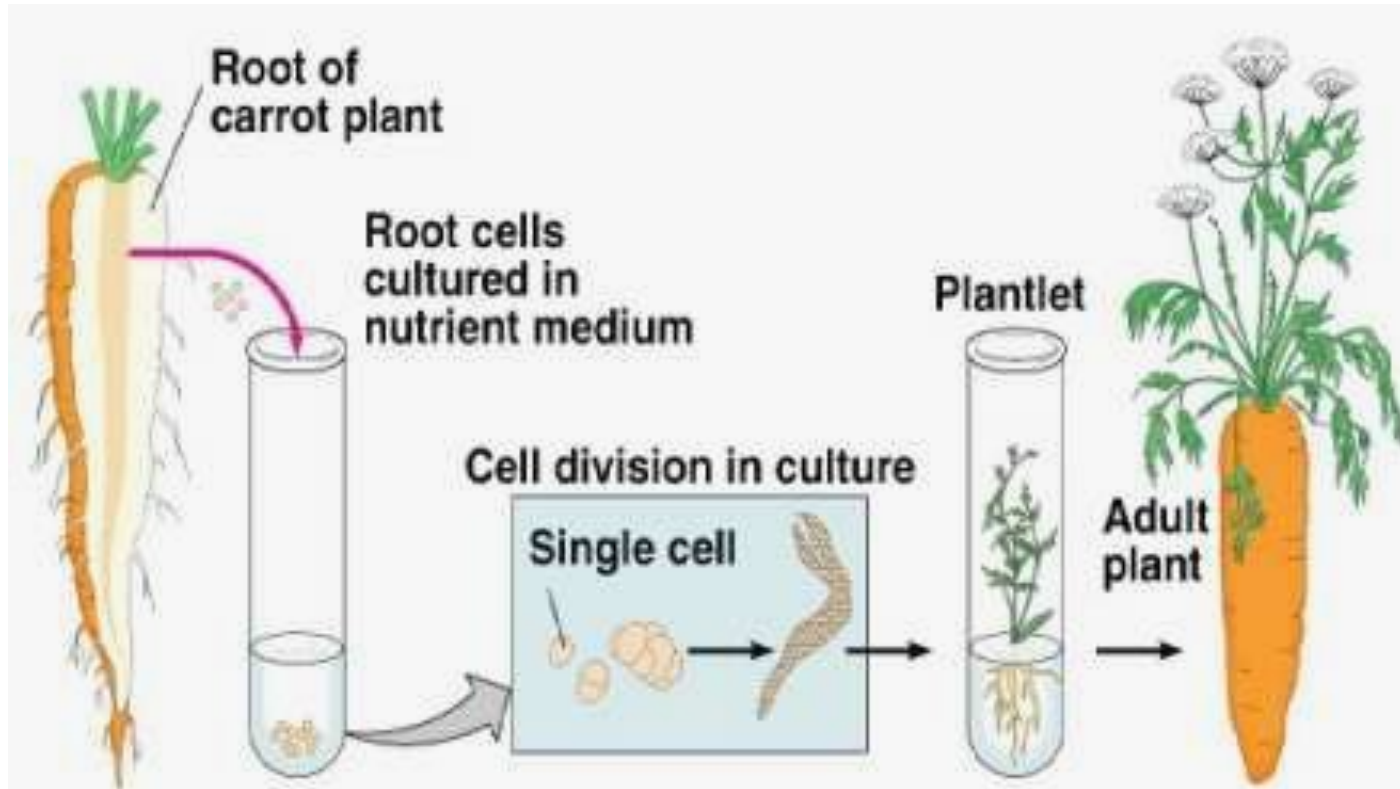
Plant cells differ from animals cells in that they are **totipotent**

A totipotent cell is one that can develop into specialized cell types & regenerate an entire organism

Tissue culture of plants and the regeneration of complete plants from cells has been done since 1930s

This allows large-scale **clonal** propagation of plants

Plant Cloning



Micropropagation



Seedlings, each from an individual cell

Callus, undifferentiated mass of plant cells

How Do They Engineer Plants?

Methods of producing transgenic plants



Plant Genetic Engineering

Ti plasmid of *Agrobacterium tumefaciens*

A. tumefaciens is a soil microbe that induces **crown gall**

Crown gall is a 'cancerous' mass which forms at the site of infection in plants

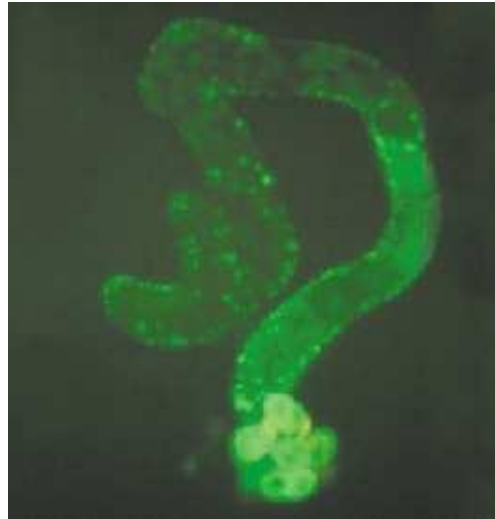


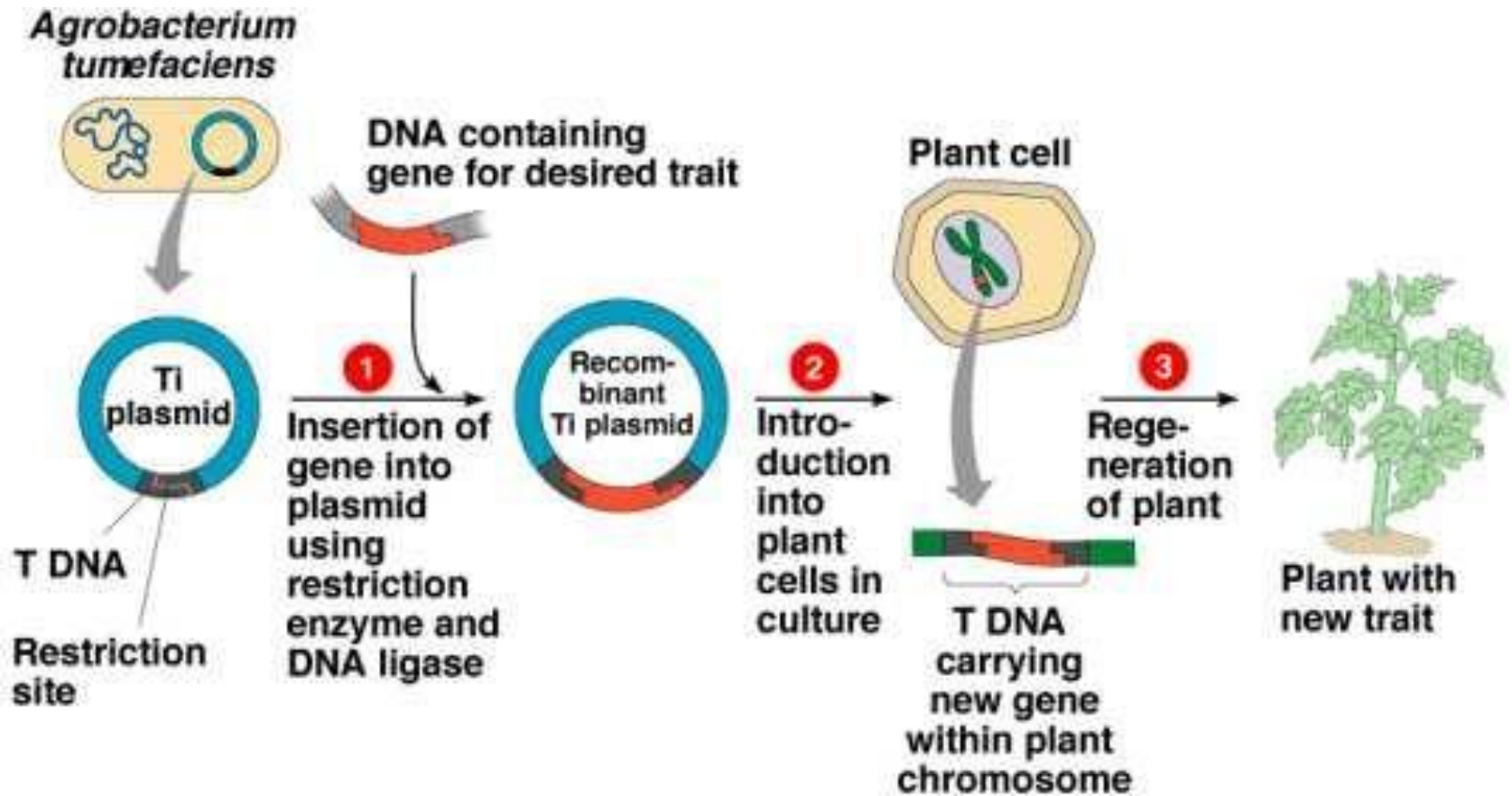
T-DNA portion of the Ti plasmid contains genes responsible for the disease

T-DNA becomes incorporated into the genome of the plant

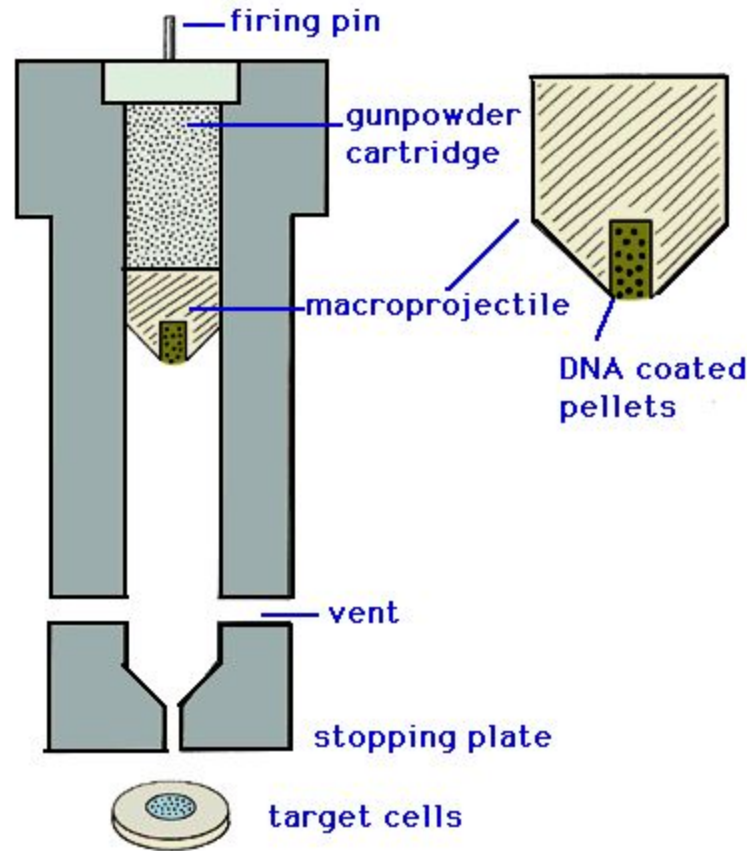
Part of the T-DNA may be replaced with a foreign gene and used to incorporate this gene into the plant's genome

A marker is also added to determine which cells have the recombinant gene





Biolistics (Biological Ballistics)



Useful for engineering corn, rice, wheat, barley, & other crops

Helios Gene Gun



Uses an adjustable burst low-pressure helium to sweep DNA- or RNA-coated gold pellets from the inner wall of a small plastic cartridge directly onto a target

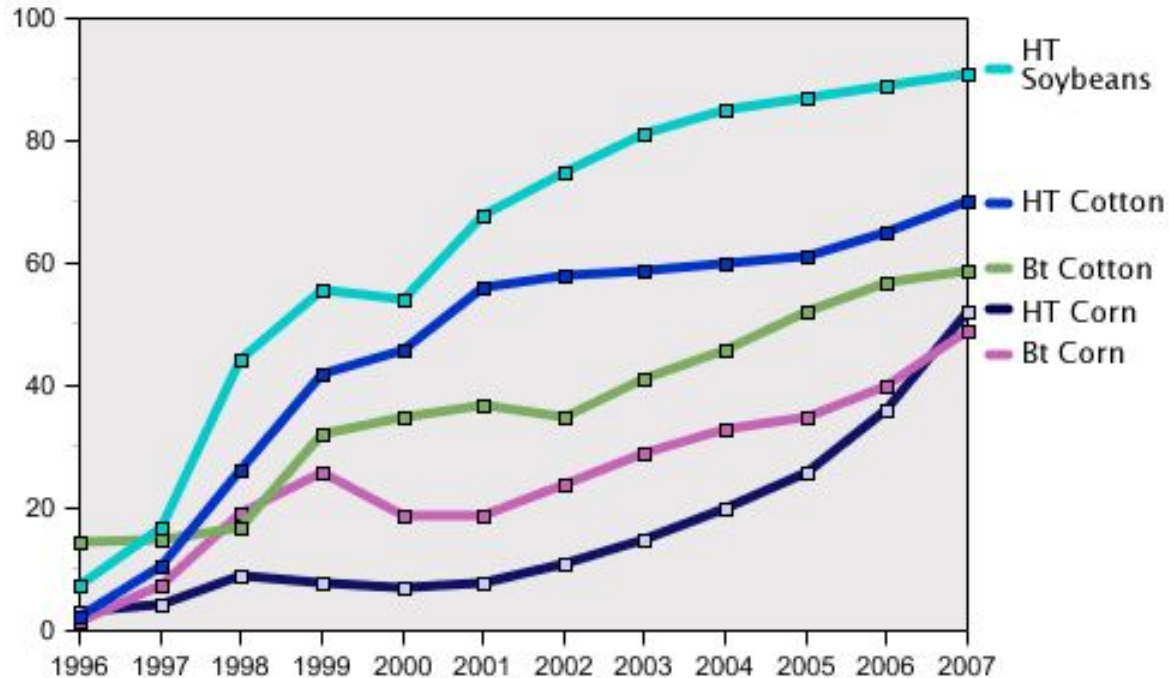
Genetically Engineered Plants

- Herbicide tolerance
- Insect resistance
- Crop improvements
- Functional foods
- Plants as bioreactors
- Biofuels
- Timber improvements
- Bioremediation

Genetically Modified (GM) Crops

Adoption of genetically engineered crops grows steadily in the U.S.

Percent of acres



Note: Data for each crop category include varieties with both HT and Bt (stacked) traits.

Source: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-07 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Currently 215 million acres of GM crops grown worldwide

How Much of What We Eat Is GM?

>60% of the foods we purchase have GM ingredients

95% of canola is biotech herbicide-tolerant

50% of corn is biotech herbicide-tolerant

35% of corn is biotech insect-resistant

61% of cotton is biotech herbicide-tolerant

52% of cotton is biotech insect-resistant

93% of soybean is biotech herbicide-tolerant

(2005 Data, Source :GM Crops: The First 10 Years -- Global Socio-economic and Environmental Impacts; PG Economics Limited)

Who Produces GM Food?

BASF Inc.

Aventis CropScience

Bayer CropScience

Syngenta Seed Inc.

Pioneer Hi-Breed International Inc.

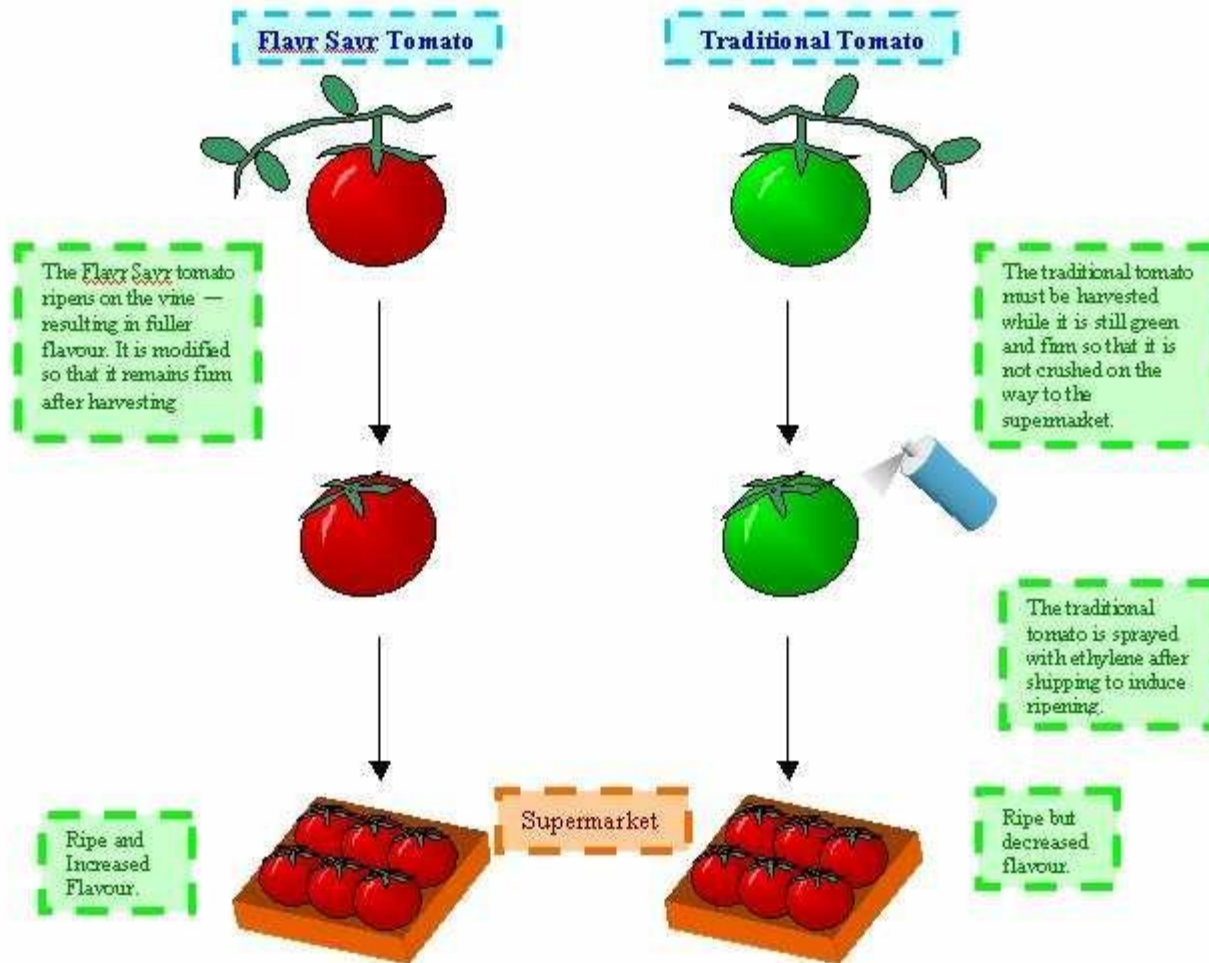
Dow Agroscience LLC

Monsanto Company

FLAVR SAVR, The First GM Food

The first GM food was the **FLAVR SAVR** tomato

Introduced in 1994 it had delayed ripening characteristics



Fruit softens because polygalacturonase degrades pectin

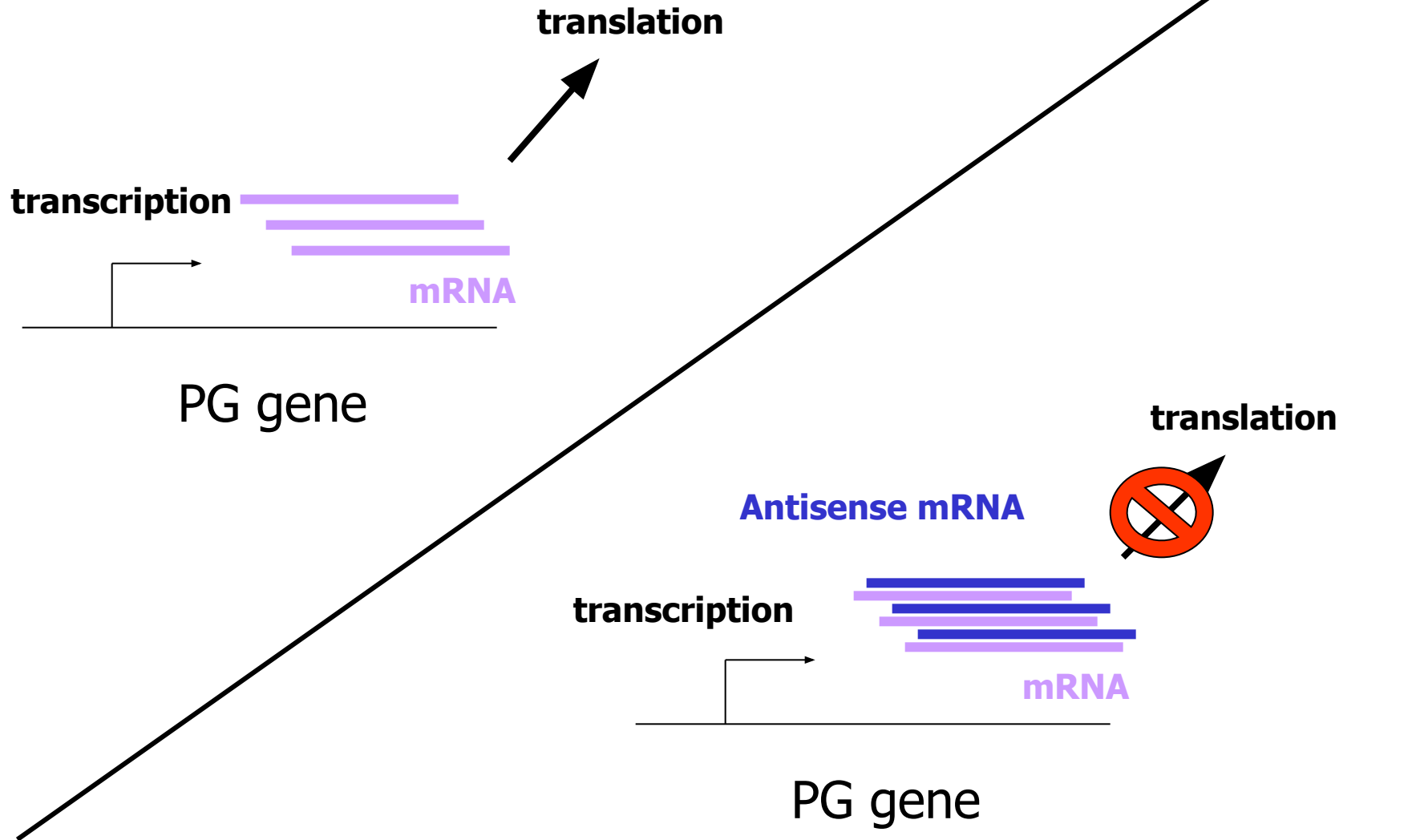
Antisense technology was used to turn off (**silence**) the polygalacturonase (PG) gene

Gene encoding antisense RNA was inserted into tomato cells

The antisense RNA finds the normal RNA and hybridizes

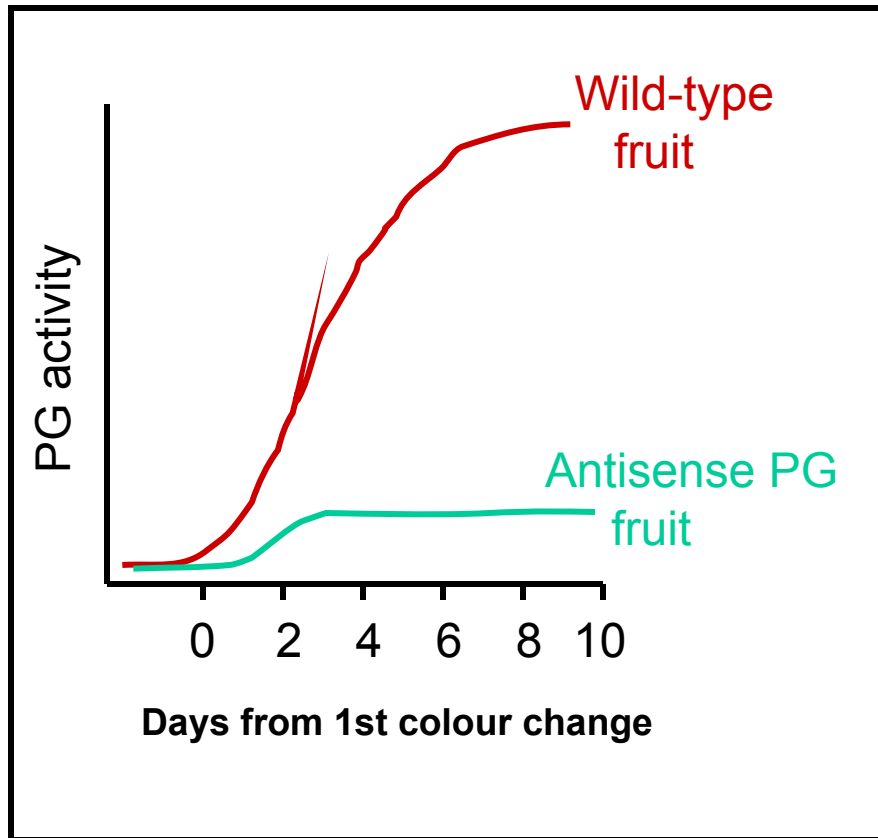
The cell then degrades this complex, preventing the normal RNA from being translated

Antisense Technology



Altering Fruit Ripening with Antisense RNA

Polygalacturonase (PG) is an enzyme that breaks down pectin in ripening fruit walls



Plants with an antisense PG transgene produce less PG. Walls soften more slowly

Many genes manipulated in the same way to answer basic questions:

- what is the role of hormones in ripening?
- what do particular enzymes do in fruit walls?



Most GM tomatoes were used only in canned puree
By 1997 FlavrSavr was no longer marketed

Current technologies aid the farmer not the consumer

- Herbicide Resistance
- Virus Resistance
- Insect Resistance

Herbicide Tolerance

Methods used to promote crop growth also promote weeds

Weeds often outgrow crops and reduce farm output

Even though there are about 100 chemical herbicides, weeds still reduce crop productivity by ~12%

Problem is that many herbicides kill both crops & weeds

This has led to the creation of herbicide tolerant crops

Herbicide Tolerance

Herbicides are used for weed control



**Soybean with no
herbicides**

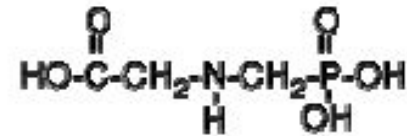
**Weeds
drastically
reduce
crop
yield
and quality**



**Soybean after
herbicides**

Roundup - Glyphosphate

Herbicide – chemical structure:



Mode of action: blocks synthesis of certain amino acids
(aromatic amino acids produced by the shikimic acid pathway)

→ Toxic to most plants, but not to animals

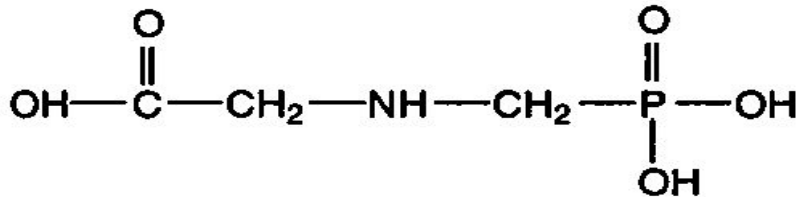
Note: can still be toxic to animals, not just the active chemical
but other components of the formulation

Monsanto Chemical Company – major moneymaker – while
under patent protection

Non-selective herbicides (Roundup Ultra and)

Roundup® (chemical name: glyphosate)

GLYPHOSATE (Roundup®)



N-(phosphonomethyl)glycine
(isopropylamine salt)

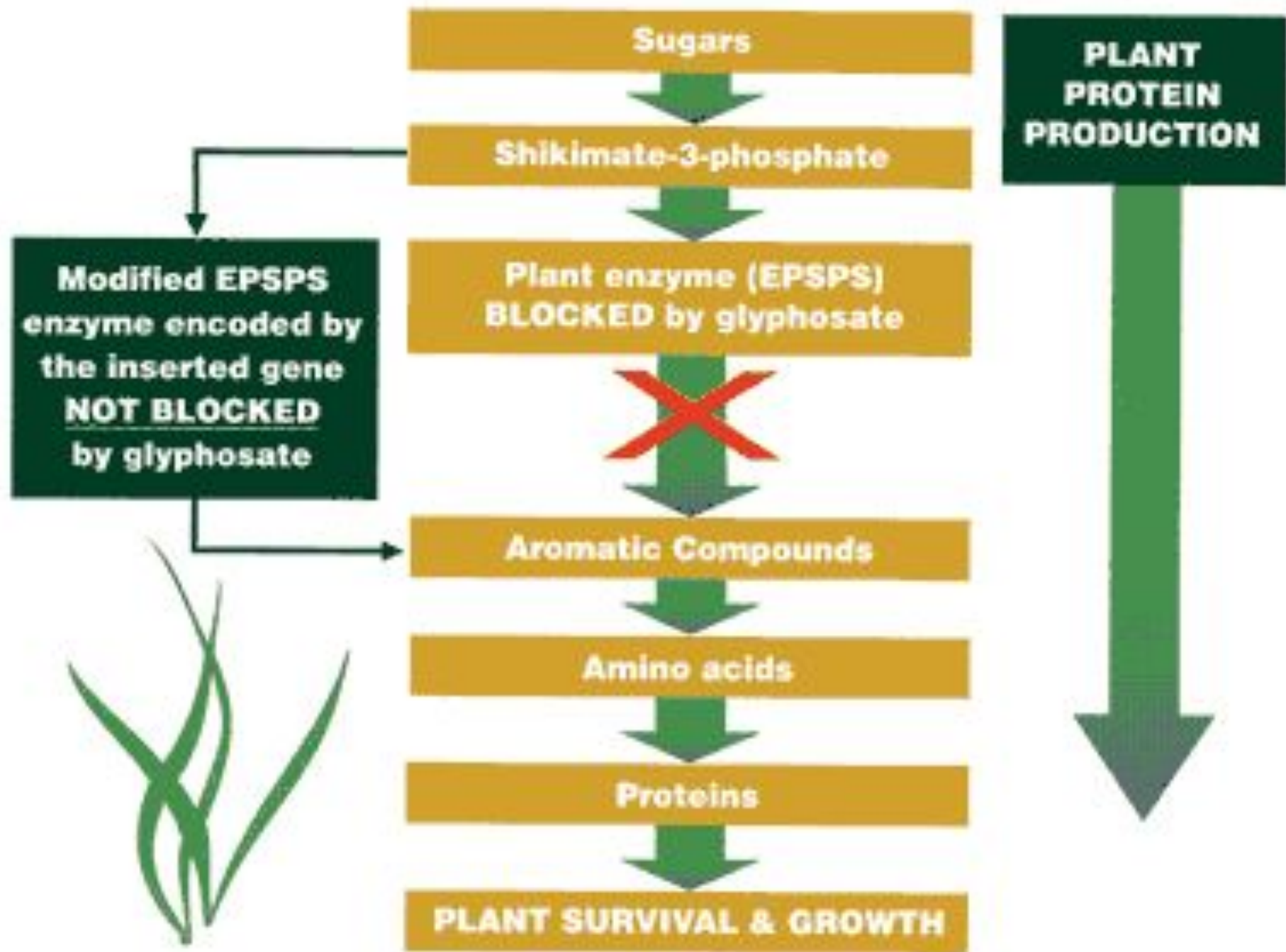
**Breaks down quickly in the soil,
eliminating
residual carry-over problems**

and

reducing environmental impact.

Roundup Ready®

**transgenic varieties
of common crops
completely resistant
to those herbicides**



EPSPS Transgene Introduced into Plants

Transit peptide from plant gene added to allow chloroplast import

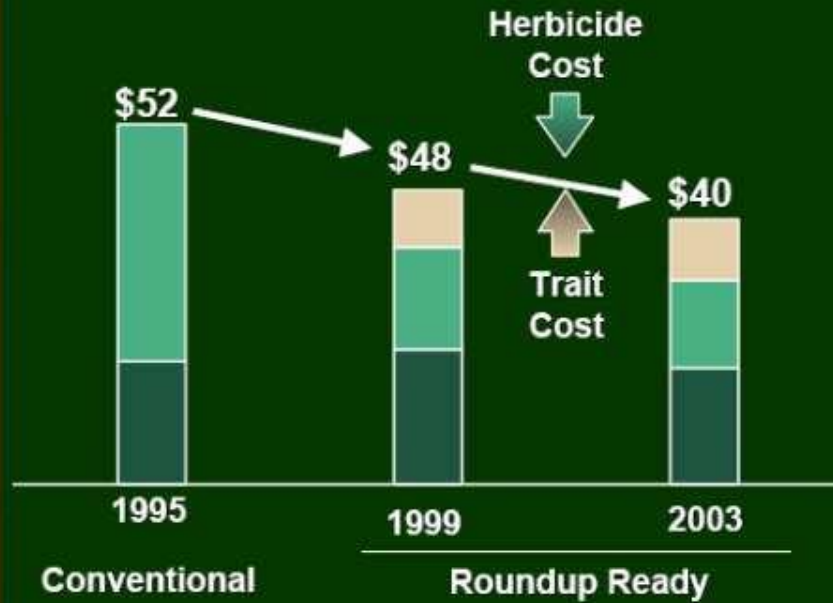
Codon usage modified for efficient expression in plants



Regulatory sequences recognised by plant (either from plant gene or plant virus gene). In this case 35S CaMV promoter

U.S. Grower System Cost Roundup Ready Soybeans

\$ per acre



Unmatched weed control in Roundup Ready Soybeans

Source: Monsanto and Doane Market Research

7



Benefits of Glyphosate Tolerance in Crops

Can use at any time

- can wait until there is a problem

Move to greener herbicide

Very effective

- Weeds very sensitive
- GM crop very resistant

Reduced herbicide use

GM canola surrounded by weeds



- glyphosate

+ glyphosate

Roundup® Ready Crops

Corn

Alfalfa

Soybeans

Canola

Sorghum

Cotton

Tomato

Potato

Wheat



1996 Roundup® Ready Gene Agreement

Terms:

- The farmer must pay a \$5 per bag "technology fee"
- The farmer must give Monsanto the right to inspect, monitor and test his/her fields for up to 3 years
- The farmer must use only Monsanto's brand of the glyphosate herbicide it calls Roundup®

- The farmer must give up his/her right to save and replant the patented seed (replanting seed is a practice as old as agriculture)
- The farmer must agree not to sell or otherwise supply the seed to "any other person or entity."
- The farmer must also agree, in writing, to pay Monsanto "...100 times the then applicable fee for the Roundup[®] Ready gene, times the number of units of transferred seed, plus reasonable attorney's fees and expenses..." should he violate any portion of the agreement.

Concerns of Roundup® Ready Crops

Spread of resistance genes to weeds

Problems with quality of crops

e.g.. Cotton bolls falling off prior to harvest

Farmers required to purchase seed annually

The herbicide is still toxic at high doses

Benefits of Roundup[®] Ready Crops

Fields no longer need tilling

Reduction in weed management costs of up to 37%

Decrease in herbicide use by >1lb/acre

Overall 74% increase in farmer profits¹

Roundup[®] patent recently expired

Researchers have designed new method of resistance

Sorted thru 100s of microbes to find a detoxifying enzyme

Found 3 genes in *Bacillus licheniformis* which encode glyphosate N-acetyltransferase (GAT)

Using directed evolution generated an enzyme 10000x more efficient

~5yrs to market

There are also varieties of various crops resistant to:

Glufosinate

Bromoxyl

Sulfonylurea

Virus Resistance

Yellow Squash resistant to three different viruses were developed by Asgrow Seed

Resistance was then transferred to zucchini

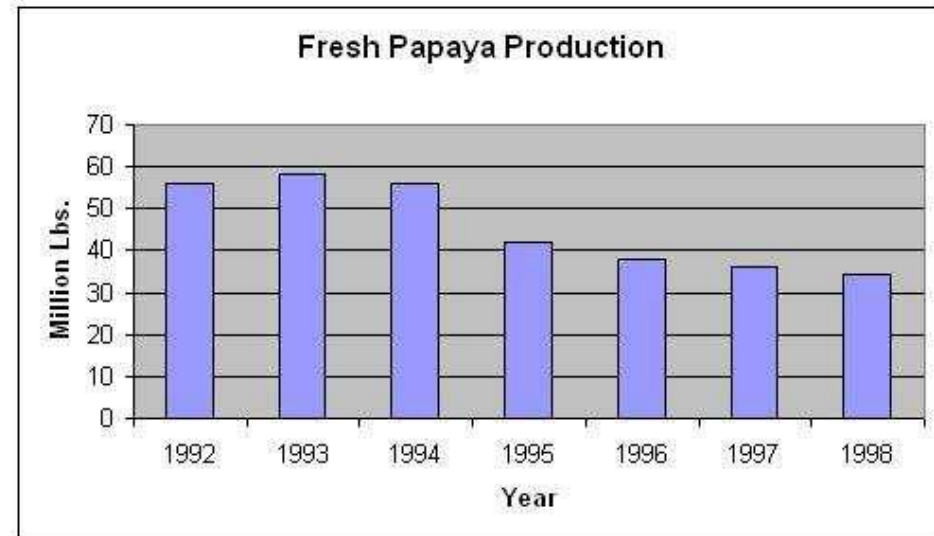
Virus resistant papaya were developed in the mid '90s

This was after a outbreak of papaya ring spot virus destroyed 40% of the Hawaiian crop

The varieties called Rainbow[®] & SunUp[®] are provided free to farmers

Increased virus resistance: Papaya ringspot virus (PRV)

Virus has had huge impact on papaya industry in Hawaii - reduction of fresh fruit production directly related to spread of PRV



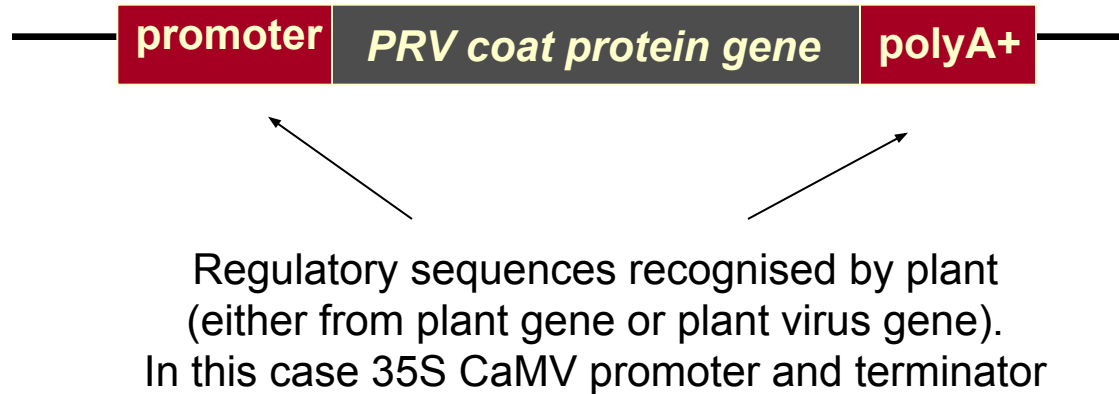
No naturally occurring resistance genes - without GM, papaya industry in Hawaii would be destroyed

Transgenic PRV-resistant papaya has been grown commercially in Hawaii since 1996

Papaya Resistant to PRV

PRV coat protein gene expressed from 35S CaMV promoter.

Papaya transformed by particle bombardment



Confers partial resistance to PRV in one variety ('Rainbow') and complete resistance in another ('SunUp')

Growers have to sign up to careful crop management - minimize virus pressure on transgenics to maintain resistance

Insect Resistance

Various Cry genes (CryIA(b), CryIA(c), & Cry9C) have been inserted into crops such as corn, cotton, potatoes, & rice

Pest must ingest a portion of the plant for the toxin to be effective

Within hours the gut breaks down and the pest dies

Wt Corn

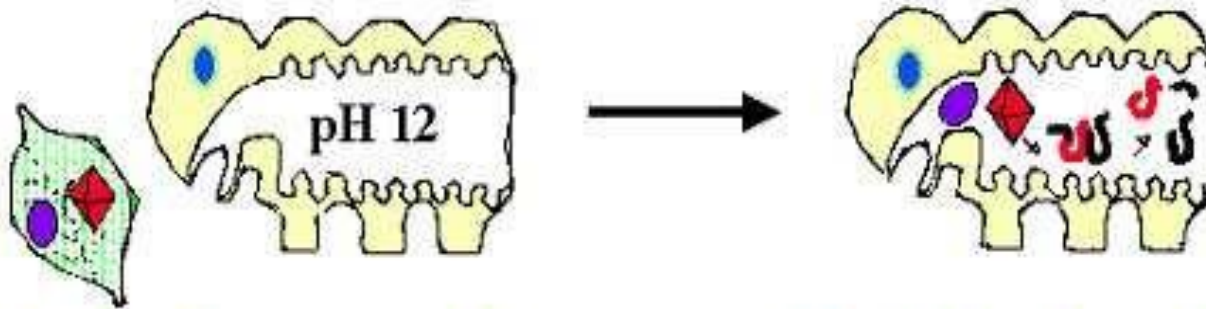


Bt Corn

What is BT

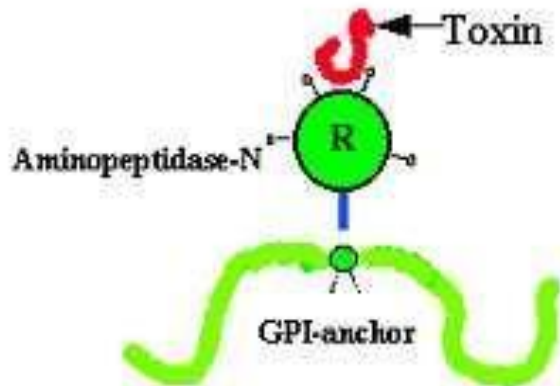
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Molecular basis of the Bt action

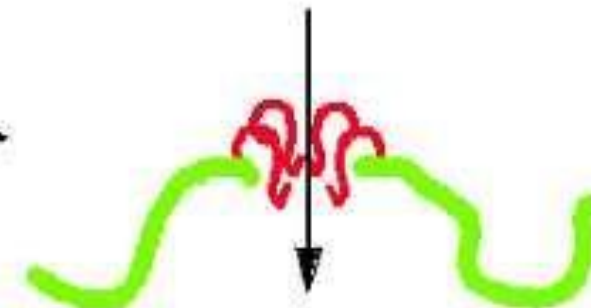


1 Crystal and spore eaten by insect

2 Crystal dissolves and protoxin is processed to smaller 'active' form by gut enzymes



3 Activated toxin binds to receptor (R) in the midgut epithelium



4 Toxin inserts into the membrane making it permeable to ions and small molecules so that the cell bursts

Bt Corn & Monarch Butterflies



Cry toxin is expressed in all of the plant as well as pollen

Corn pollen can blow onto milkweed growing near corn fields

Monarch caterpillars feed exclusively on milkweed

An early study showed a possible toxic effect of Bt pollen on monarch caterpillars



Biodiversity / NTO Studies



- Monarch Butterfly, symbol of nature and “wildness” in North America.

- The reports of Bt effects on Monarch butterflies have fueled much emotional debate on the use of biotech crops.



Potential Concerns

- Effect on target organisms
 - Development of resistance
 - Effect on non-target organisms
 - Lack of resistance
 - Escape of transgenes from transgenic cultivars to other cultivars or to wild relatives
 - Reduction in genetic diversity
 - Development of resistance
-

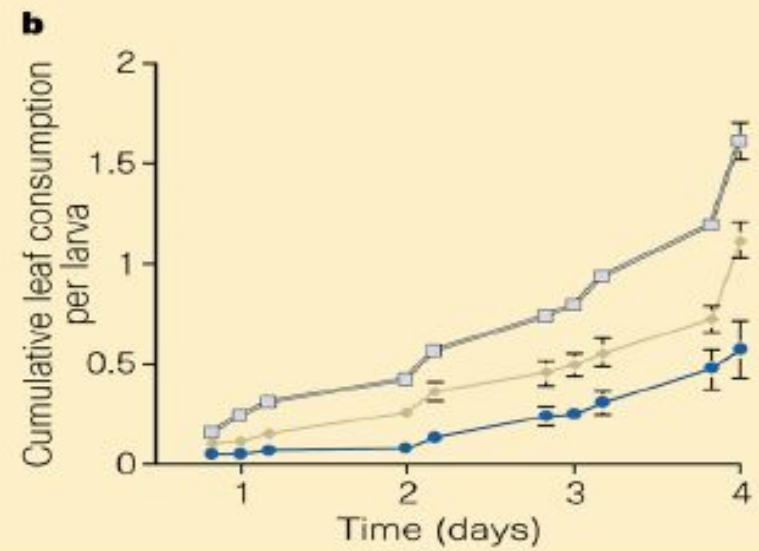
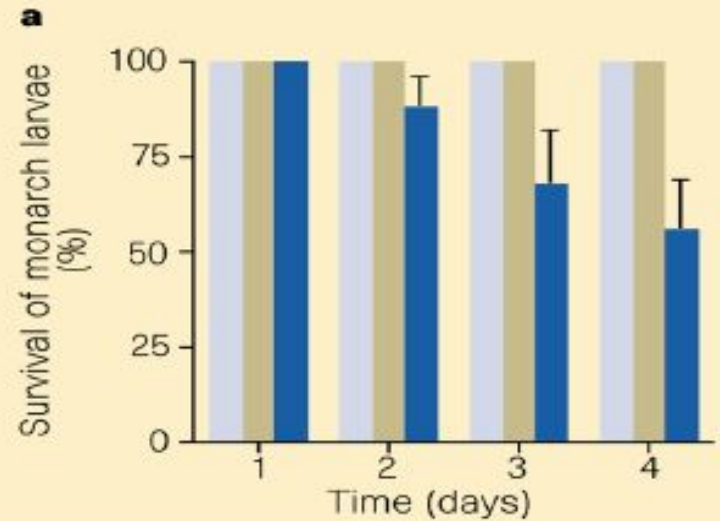
Effect on Bystanders?

- Monarch butterfly studies



- Losey et al. 1999

- Pollen sprinkled on milkweed leaves
- Criticisms:
 - pollen dosage?
 - lab study



Effects of Bt pollen: Lethal doses vs. Actual doses (Hellmich et al. 2001; Pleasants et al. 2001; Stanley-Horn et al. 2001)

- Lethal doses: linked to expression levels/events
 - Bt11, MON810 and TC1507
 - > 1,000 grains/cm²
 - 176
 - ≈ 10 grains/cm²
- Actual doses:

Average pollen density levels on milkweed leaves (/cm ²)				
Within field	0 m from edge	1 m from edge	2 m from edge	4-5 m from edge
161	63	35	14	8

Unanswered concerns about Bt crops

(Scriber 2001)

- Long-term resistance management (refugia)
 - Fate of Bt in the environment
 - Aerial sprays against gypsy moth: 4-6 weeks effect on non-target
 - Escape of transgenes into wild relatives
 - Effects of sublethal levels of Bt toxin
 - Multitrophic interactions; ecosystem level interaction
-

Effect of pesticides

(Pimentel and Raven 2000)

- 115×10^6 kg/yr of pesticides on corn:
 - corn rootworm: 45% yield reduction; other control: rotation
 - European corn borer: 20% yield reduction; difficult to control when inside the plant --> Bt
 - Effect of pesticides:
 - pesticide poisonings, cancer
 - 35% of food samples have pesticide residues
 - 70×10^6 birds/yr killed
 - 10^9 s of insects, including beneficials: pollination, biological control
 - Reasons for decline of monarch: insecticide use, loss of habitat
-

Bt Corn & Allergies

Bt corn is approved for human consumption

However Starlink[®] brand corn is approved *only* for animal feed

Contains Cry9C which may be a potential allergen

In 2000 Starlink[®] was found in Taco Bell-brand taco shells

EPA determined that no one who ate the food was allergic, but they found that it had a moderate potential allergenicity

All the products were recalled and Starlink[®] corn is not approved for human consumption

Benefits of Bt Corn

Crop yield increases by up to 33%

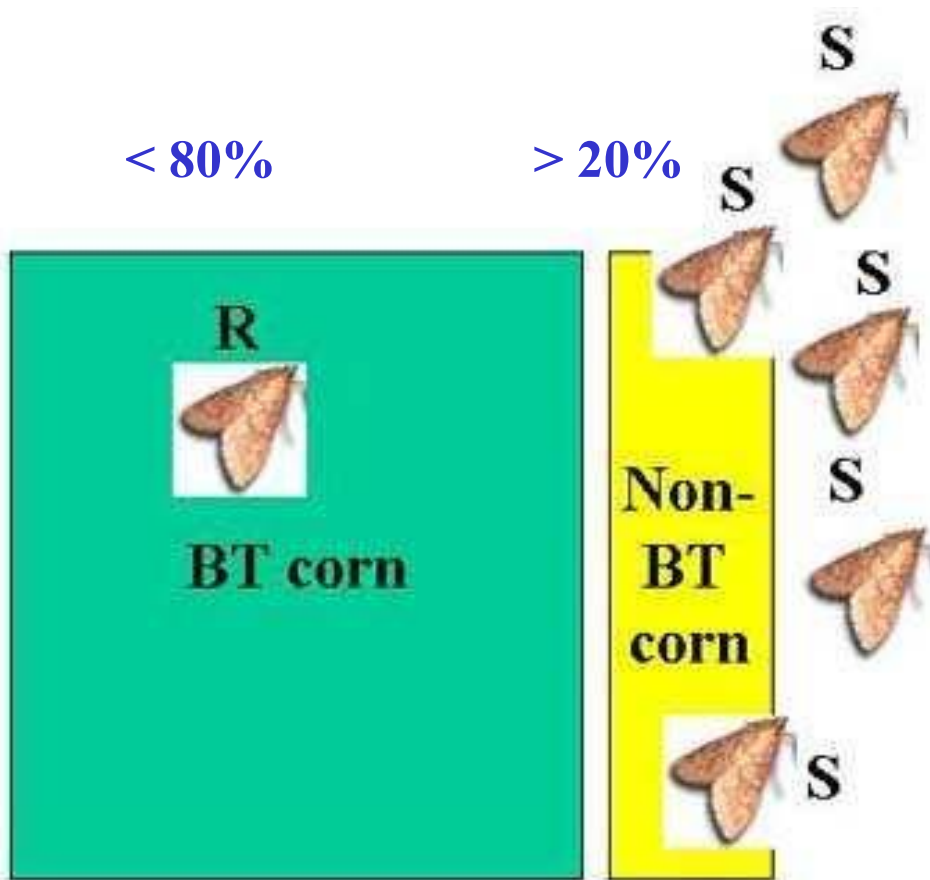
39% less insecticide used

Increase in monetary gains by 18%¹

Concerns associated with GM crops

1. Possible production of **allergenic or toxic proteins** not native to the crop
2. Adverse effects on **non-target organisms**, especially **pollinators** and biological control organisms
3. Loss of **biodiversity**
4. Genetic pollution (**unwanted transfer of genes** to other species)
5. Development of **pest resistance**
6. Global concentration of **economic power and food production**
7. **Lack of "right-to-know"**
(i.e., a desire for labeling transgenic foods)

How to prevent development of Bt resistance in insects?



at least 20%
of a farm's corn acreage
must be planted
to non-BT corn.

R = resistant European borer;
S = susceptible borer.

few Bt-resistant insects
surviving in the Bt field
would likely mate
with susceptible individuals
that have matured
in the non-Bt refuge.
Thus, the resistance alleles
would be swamped
by the susceptible alleles.

Strategy will not work if resistance is dominant !!!

Proposed Ban on Genetically Modified Corn in Europe

JAMES KANTER
Published: November 23, 2007

PARIS, Nov. 22 — [European Union](#) environmental officials have determined that two kinds of genetically modified corn could harm butterflies, affect food chains and disturb life in rivers and streams, and they have proposed a ban on the sale of the seeds, which are made by [DuPont Pioneer](#), [Dow Agrosciences](#) and [Syngenta](#).

 [Enlarge This Image](#)



Thad Alender for The New York Times
A study says exposure to the corn can affect the larvae of the monarch butterfly.

 [Enlarge This Image](#)



stránce se vyskytla chyba.

The preliminary decisions are circulating within the [European Commission](#), which has the final say. Some officials there are skeptical of a ban that would upset the powerful biotechnology industry and could exacerbate tensions with important trading partners like the United States. The seeds are not available on the European market for cultivation.

In the decisions, the environment commissioner, Stavros Dimas, contends that the genetically modified corn, or maize could affect certain butterfly species, specifically the monarch, and other beneficial insects. For instance, research this year indicates that larvae of the monarch butterfly exposed to the genetically modified corn “behave differently than other larvae.”

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Crop Improvements

Current research into crop improvements include:

- Increased growth rate
- Increased salt tolerance
- Increased drought resistance
- Modification of seed oil content

Drought / Salinity Resistance

Trehalose is a protectant against many environmental stresses; freezing, osmotic pressure (salinity), heat and desiccation.

Trehalose (1- α -D-glucopyranosyl-glucopyranoside) is synthesised in a two-step process in yeast.

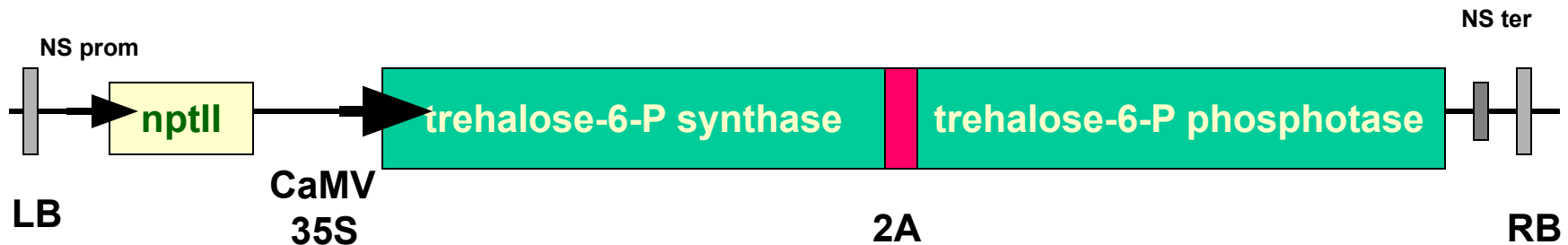
Zygosaccharomyces rouxii is one of the most highly osmo-tolerant yeasts – especially to salt

Kwon, S.J., Hwang, E.W. & Kwon, H.B. (2004). Genetic engineering of drought resistant potato plants by co-introduction of genes encoding trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase of *Zygosaccharomyces rouxii*. *Korean J. Genet.* 26, 199-206.



Figure 4. Drought stress tolerance of ZrTPS2-2A-ZrTPS1 transgenic plants. One month old plants rooted in the soil mixture were not watered for 3 weeks. (A) is the empty vector transgenic control plant, and (B–D) are ZrTPS2-2A-ZrTPS1 transgenic lines.

Transgenic potatoes morphologically identical to parents.



Modification of Seed Oil Content

Plant Seed Oils

Oils either for cooking or industrial uses are usually extracted from seeds such as,

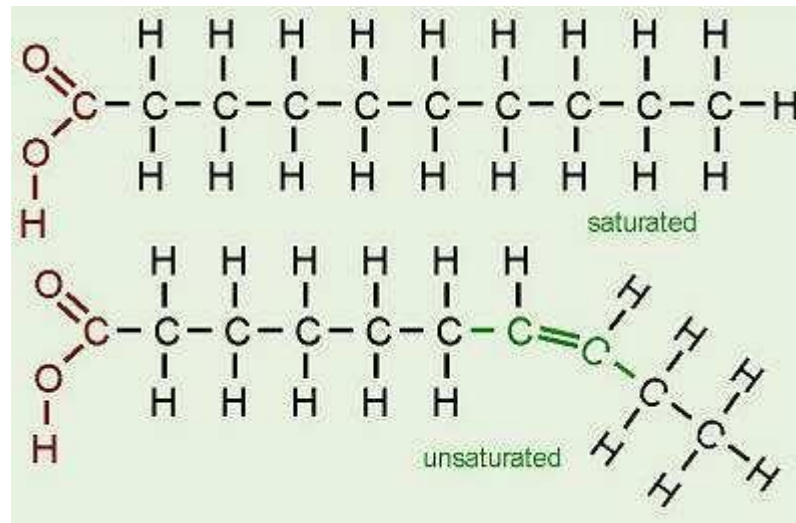
Corn, safflower, sunflower, canola, coconut, flax

Seeds rely on their stored oil as an energy & carbon source for germination

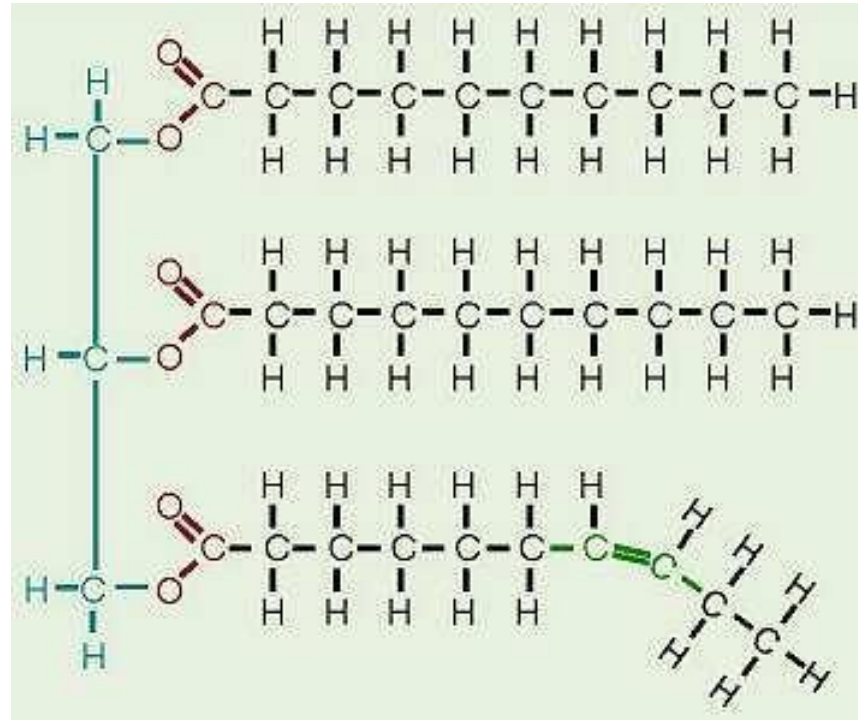
Most seed oils are made up of unsaturated fatty acids

Some tropical oils such as palm & coconut have significant levels of saturated fatty acids

Fatty Acids



Triglyceride

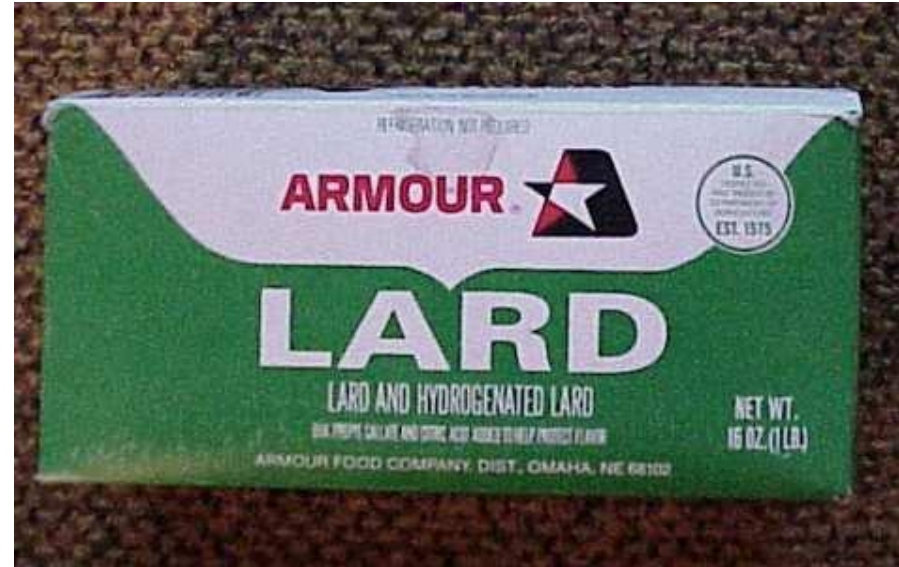




Canola Oil -10°C
5% Saturated



Coconut Oil $20-25^{\circ}\text{C}$
85% Saturated



Lard 40.5°C
100% Saturated

<http://food.oregonstate.edu/images/fat/lard1.jpg>

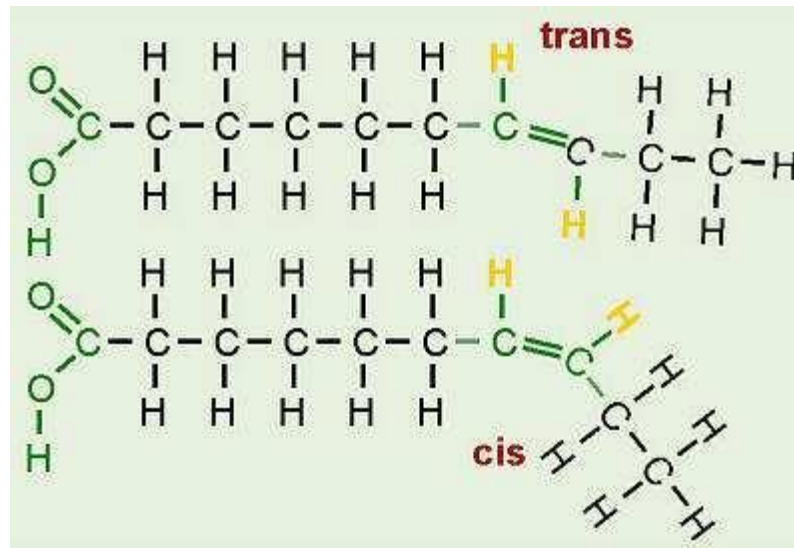
<http://www.plattsalat.de/Gawang.html>

<http://www.aces.edu/dept/extcomm/newspaper/feb23b01.html>

Cis-fatty acids, naturally occurring unsaturated acids

Trans-fatty acids, artificially generated to keep unsaturated fatty acids from going rancid

Trans-fatty acids increase LDL (bad cholesterol) increasing the risk of heart disease

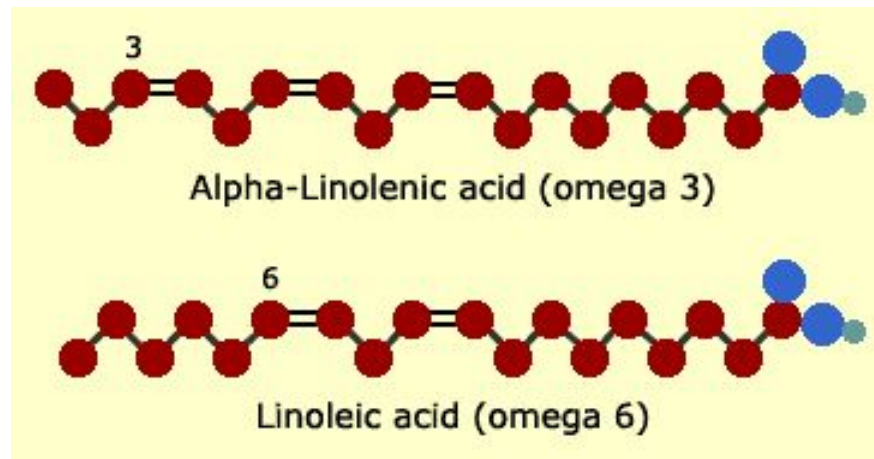


Soybeans with Modified Oil Content

Soybeans normally have high amounts of the unsaturated fatty acid linolenic acid

Vistive by Monsanto is Roundup Ready soybeans bred to have low amounts of linolenic acid

This eliminates the need to hydrogenate the oil



Biotechnology...what's down the road

- 🌱 Healthier soybean oil
- 🌱 Improved flavor
- 🌱 Improved digestibility



The cloudy hydrogenated soybean oil on the left contains unhealthy trans-fatty acids while the clear 1% linolenic soybean oil on the right contains no trans-fatty acids.

“Food manufacturers are scrambling to eliminate trans fat from their products” Basic
Harvard Health Watch

Market Need

- Trans fat labeling on foods mandated by FDA in 2006

Features

- Linolenic acid < 3%
- A more stable soy oil requiring less hydrogenation: reduce/eliminate trans fats
- Better flavor profile
- Full agronomic and yield performance

Product



Technical Status

- 2005 prototype testing
- 2006 commercial launch

Likely Uses

- Substitute for hydrogenated oil and blends for low trans solutions

MONSANTO



Over 60 million tons of seed oil are used for edible purposes

About 15 million tons is employed in industrial usage

One of the first plants modified was **Rape** (*Brassica napus oleifera*)

A member of the **mustard family**, Rape has been grown for centuries as animal feed and natural lubricant

In the 1970s selective breeding led to strains of Rape lacking erucic acid

The removal of erucic acid made the oil fit for human consumption, LEAR (low-erucic acid rapeseed)

Rape field full of brilliantly yellow flowers



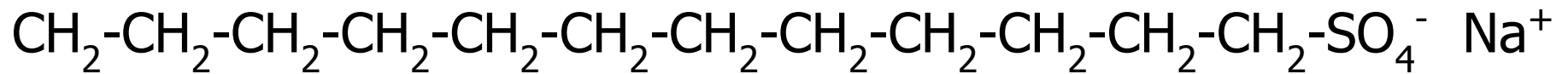
The first transgenic crop with modified oil content was a high lauric oil rapeseed.

Rapeseed oil normally contains about 0.1% lauric acid

Modified rapeseed contains ~40% lauric acid

Lauric acid from GM rapeseed would be used in place of oils from palm or coconut

The primary use of lauric acid is in detergents



Sodium Lauryl Sulfate a.k.a SDS

Other varieties of GM rapeseed could provide:

Steric acid as a substitute for hydrogenated oils

Jojoba waxes for use in cosmetics and lubricants

Various acids for use in biodiesel

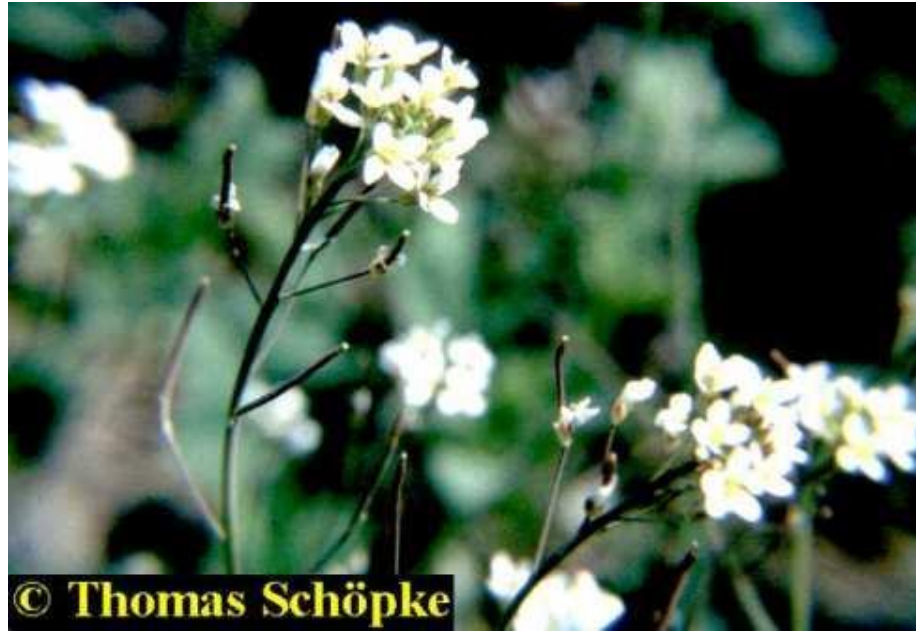
Phytases for animal feed

Novel peptides for pharmaceuticals

Why is rapeseed so versatile?

One reason is because it is related to...

Arabidopsis thaliana (Cress)



Arabidopsis is a model organism used by scientists to investigate plant development and genomics

The *Arabidopsis* genome was recently completed

Brassica (Mustard) Family



Nutritional Enhancement

Improving Protein Quality

Animals and humans are incapable of making 10 'essential' amino acids
- must obtain in diet

Nutritional value of seed storage proteins is often limited

- may lack one or more amino acid essential to human health

e.g. legume seeds lack cysteine and methionine; other seeds can lack lysine

Amino acid balance in seeds has been manipulated in laboratory experiments using a number of strategies:

- introduce seed storage protein from another species

- alter sequence of seed storage protein gene *in vitro*

- manipulate amino acid biosynthetic pathway to increase abundance of particular amino acids

Similar strategies have been used to improve protein content and composition in non-seed food crops...

'Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from Amaranthus hypochondriacus'
Chakraborty et al., PNAS 97, 3724-3729 (2000)

Potato is the fourth most abundant global crop and used for food, animal feed and production of starch and alcohol

Limited in lysine, tyrosine, methionine and cysteine

Transformed potato with seed albumin from *Amaranthus hypochondriacus* which has good amino acid balance

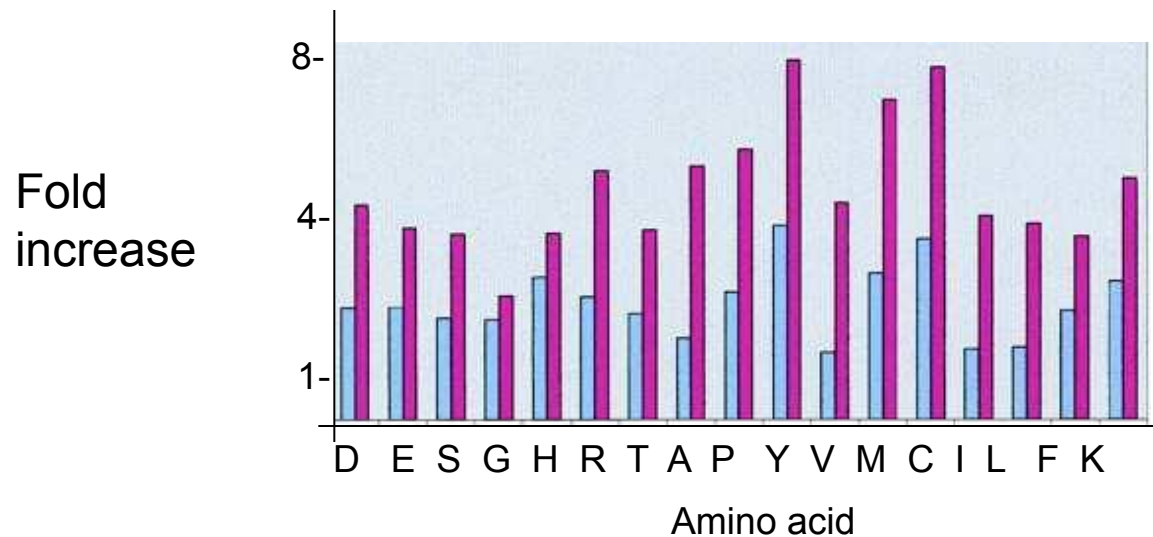


Expression in tuber 5-10 fold higher with GBSS promoter than with 35S promoter

Changes in protein quality in *Amaranthus* albumin potatoes

5-8 fold higher essential amino acids in pSB8G transgenics

Total protein content also increased (35-45%)



Golden Rice

Inserted genes from other plants & bacteria to produce β -carotene

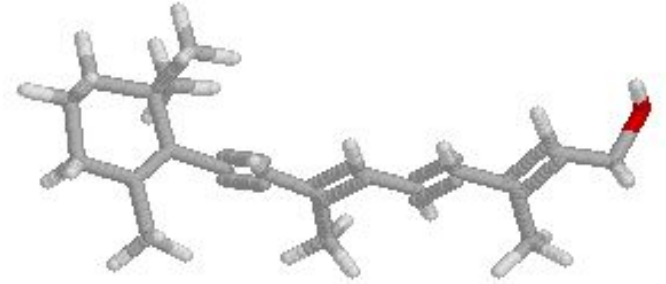
Vitamin A deficiencies affect >124 million children worldwide



Vitamin A

Vitamin A (retinol) is essential to human growth

Our bodies cannot make vitamin A,



All carotenoids that contain a β -ring can be converted into retinol, and one of the most important carotenoid pro-vitamins is β -carotene

β -carotene is a pigment required for photosynthesis

- produced in all plant green tissues

Vitamin A deficiency

400 million people are at risk of vitamin A deficiency (VAD), particularly in Asia and Africa

implicated in up to 2.5 million deaths annually in children under 5



VAD makes children especially vulnerable to infections

0.5 million children go blind each year because of VAD

Supplementation programmes have reduced child mortality by up to 50% in target areas

supplementation not universal; expensive; misses remote areas

VAD is most serious in regions where rice is the staple food ; up to 70% children under 5 affected



Wild type



Golden Rice 1



Golden Rice 2

Vaccine Foods

In the early 1990's tomatoes, bananas, & potatoes were proposed as delivery vehicles for vaccines

Touted as a simple method of delivering vaccines especially to developing countries

Studies have shown plant-produced oral vaccines to increase immunity in mice

Potatoes containing Hepatitis B vaccine have been shown to boost immunity in humans

There are concerns about dosing when these crops are directly consumed.

Would a dose be? 2 bananas and a tomato

What if a person eats too many vaccine potatoes?

Also there is concern if the vaccine foods enter the food supply of people who are vaccinated the traditional way

Plants as Bioreactors

Plants (crops or cell culture) can be used to produce proteins currently produced by microbes or animal cells

The advantage over microbes:

The proteins are more like human proteins

The advantage over animal cells:

Plants cannot become contaminated with mammalian pathogens

Large Scale Biology Corp. (LSBC) uses tobacco plants for drug manufacturing

LSBC uses an engineered tobacco mosaic virus (TMV)

Recombinant gene is inserted into TMV which infects & replicates in the plants

During replication large amounts of the drug are generated

The drug accumulates in the leaves which are harvested

α -galactosidase A purified from tobacco is as effective in treating Fabry's disease as the animal cell derived drug

More and More Plants Are Being Used to Produce Proteins

- USDA just approved the use of rice to produce lactoferrin and lysozyme
- Sigma-Aldrich now sells aprotinin and trypsin made in tobacco
- Duckweed is being used to produce interferon- α

Plant BioFuels

Cellulosic Ethanol

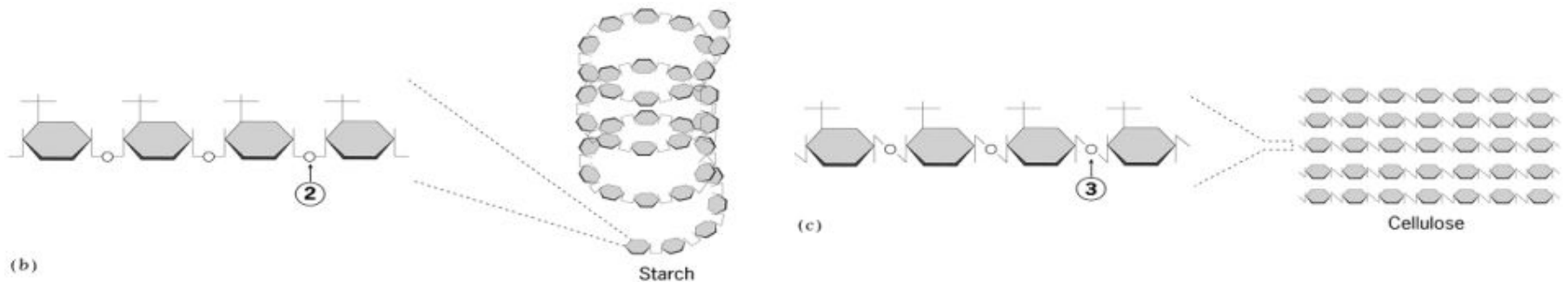
Has higher yield due to the fermentation of sugar released from cellulose

Requires the addition of cellulase or acid

Agricultural plant wastes (corn stover, cereal straws)

Plant wastes from industrial processes (sawdust, paper pulp)

Crops grown specifically for fuel production (switchgrass)

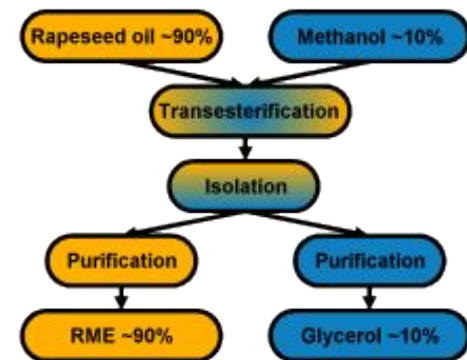
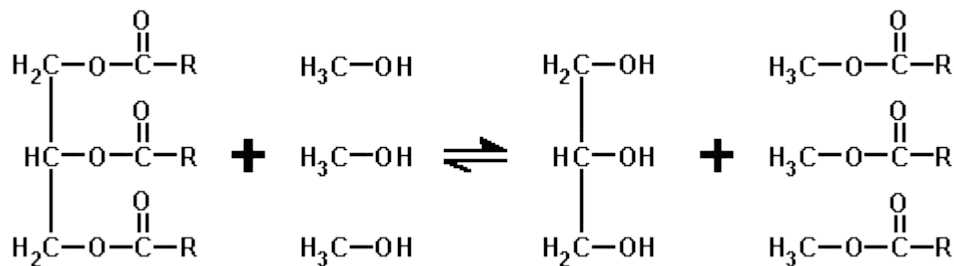


BioDiesel

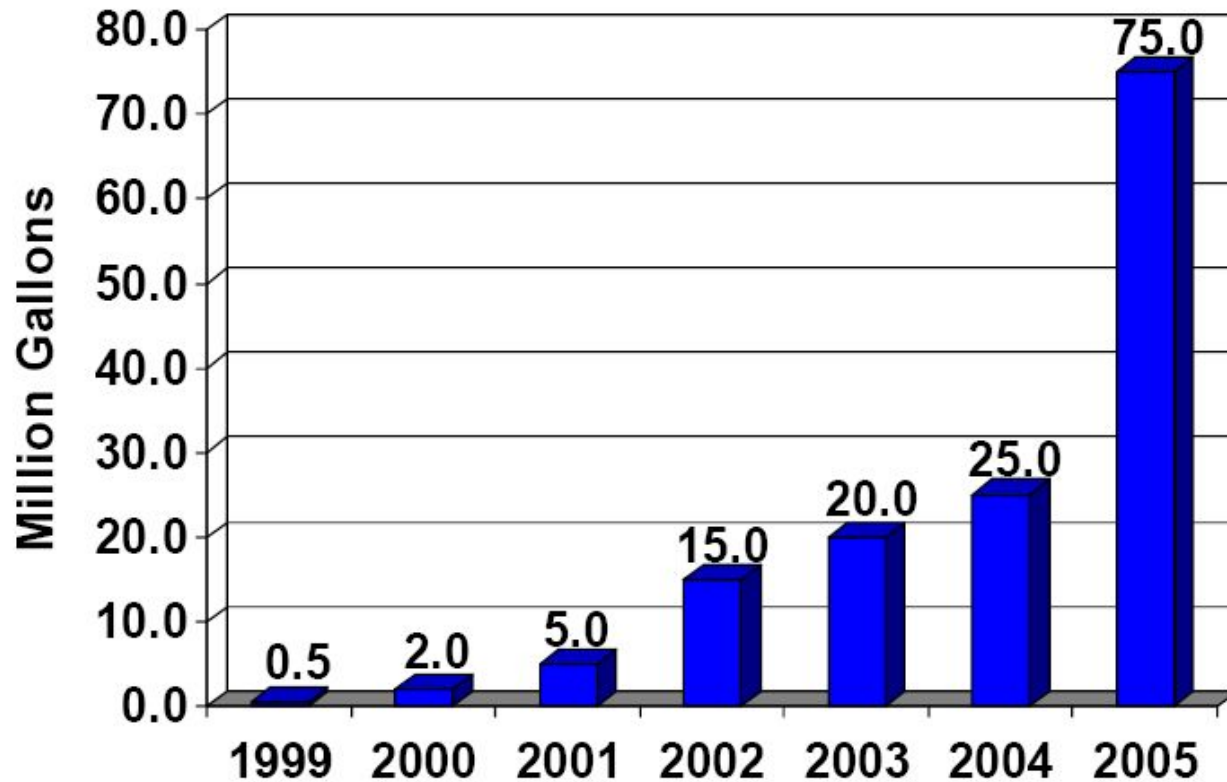
1900 Rudolph Diesel runs his engine on peanut oil

Biodiesel is defined as "a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats"

Transesterification converts triglycerides into methyl esters of fatty acids



Estimated US Biodiesel Production



U.S. consumes 40 billion gallons of diesel/yr

Timber Biotechnology



Reduction in generation time

Trees can take years to flower

Those overexpressing the *LEAFY* (*LFY*) gene can flower in as little as 7 months.

This is of particular value in fruit bearing trees

Also allows for rapid analysis mature traits

Alteration in tree size or form

Altered expression of a gene involved in hormone synthesis can give wildly differing results

Overexpression of GA 20-oxidase gives faster growing trees both in height and diameter and longer wood fibers

Inhibition of GA 20-oxidase gives dwarf trees

Six Weeks Old Hybrid Aspen



From left to right:

antisense-GA 20-oxidase, wild type, & GA 20-oxidase overexpressing

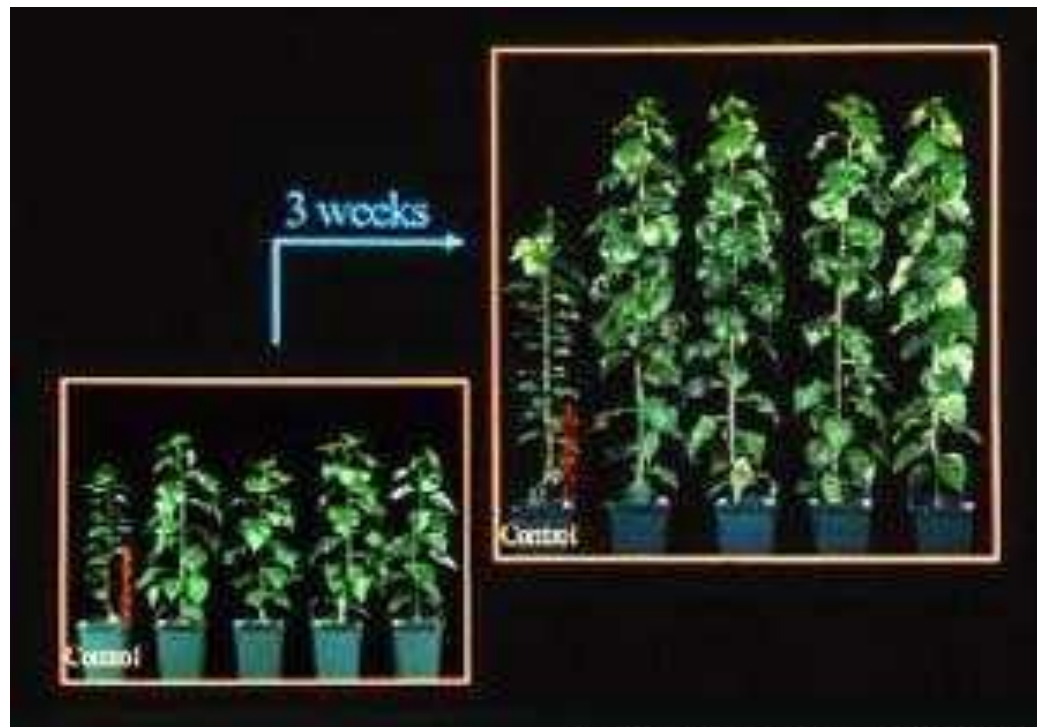


Leaves from GM poplar

Trees engineered to produce lower amounts of **lignin**

These trees grow faster and have greater cellulose content

Lignin is a glue-like compound that must be chemically removed from pulp prior to papermaking



Courtesy Michigan Technical University

Bioremediation

Bioremediation using bacteria has limitations

The engineered or specialized bacteria used are often unable to compete with indigenous soil bacteria

An alternative is **phytoremediation**, the use of plants to mop up toxic waste

A standard technique for treating soils contaminated with heavy metals (lead or cadmium), or organic pollutants (pesticides) in a cost-effective way





Flex Babin is on vacation

Anderson / Courier Journal