Thursday Lecture – Genetically Engineered Plants

Quiz

Quiz

1. Plant family that gives us the plant that is the inspiration for the UT school colors; is also the largest plant family and a popular source of ornamentals

2. Plant family that is a source for many house plants, but must be used with caution because many members contain calcium oxalate crystals in their leaves and other parts. Dumb cane is an example, the common name providing an example of the effects.

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Polyploid?

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Is a grafted plant? Apple grafted onto Crabapple?

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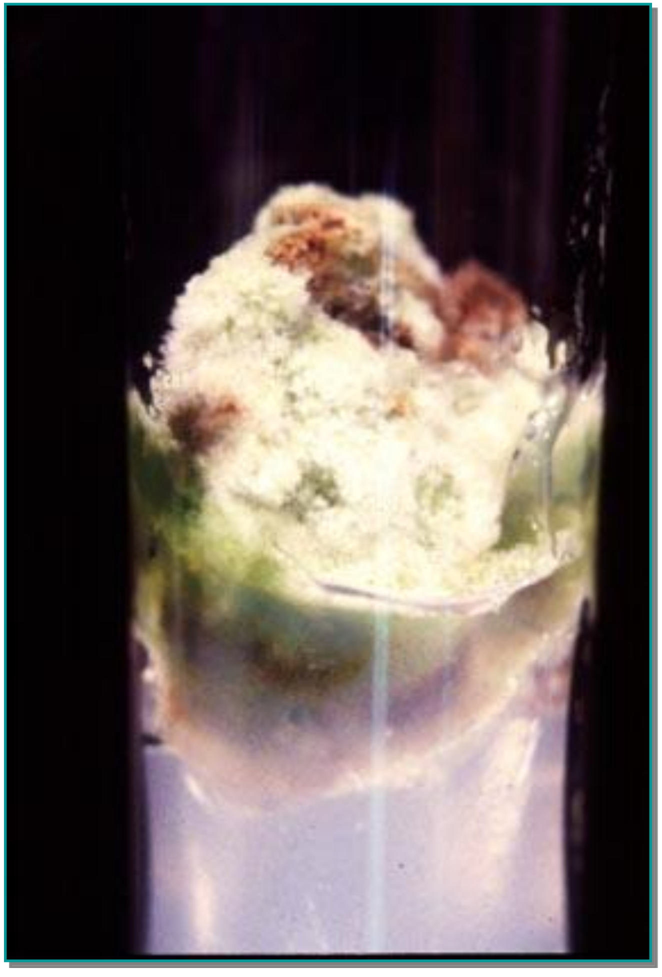
Generally accepted – GMO (genetically modified organism) or "transgenic organism" – has been altered using recombinant DNA technologies

Creation of Genetically Engineered Plants - Prerequisites

1. Growth of individual plant cells (protoplast or tissue culture)

Callus Tissues

Carrot



Tobacco

Creation of Genetically Engineered Plants - Prerequisites

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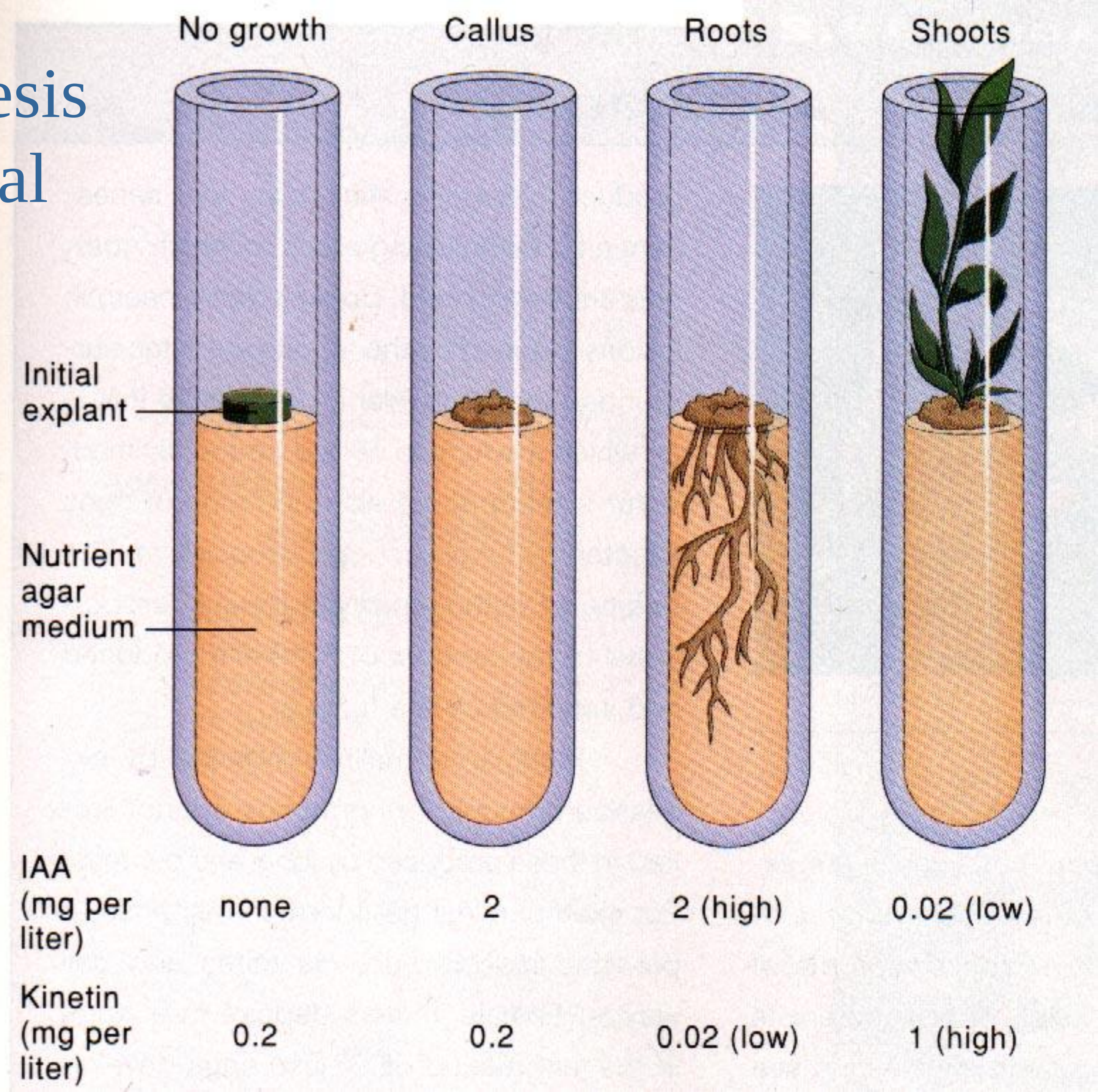
2. Regeneration of entire plants from protoplasts or tissue cultures

Organogenesis – Hormonal control



Auxin:

Cytokinin:

Creation of Genetically Engineered Plants - Prerequisites

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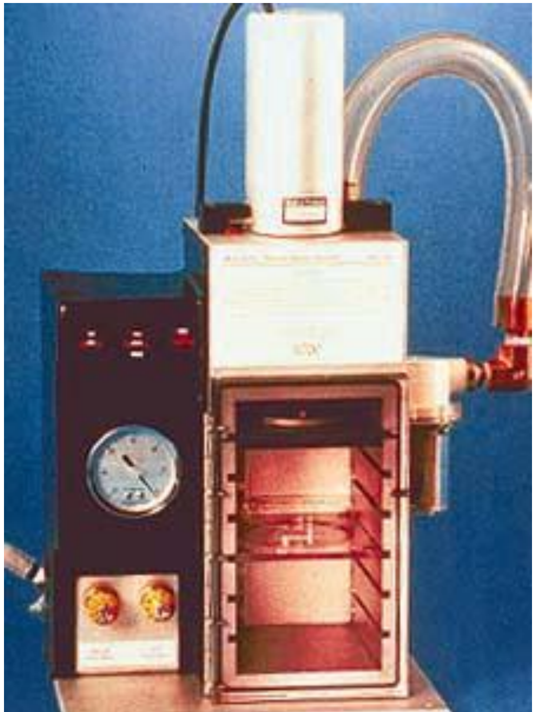
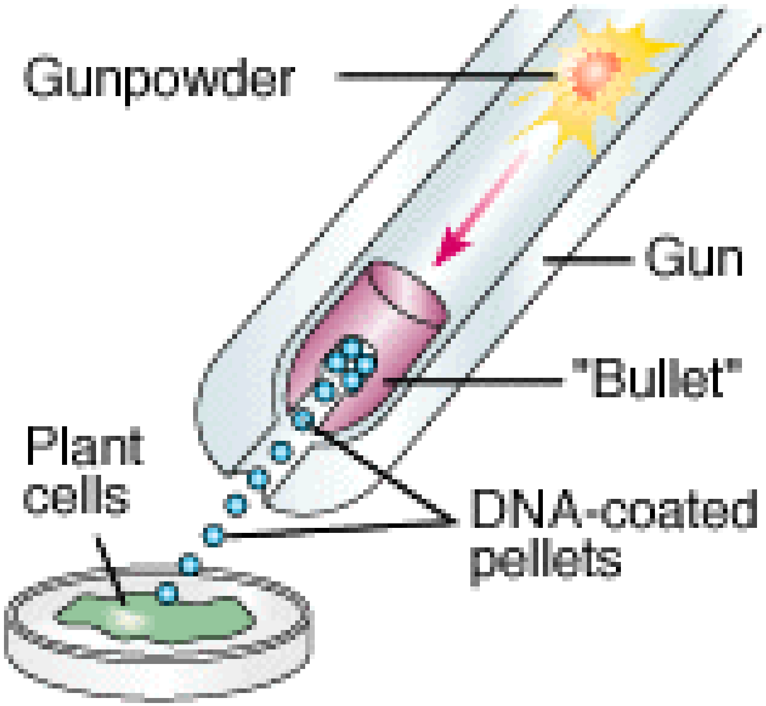
2. Regeneration of entire plants from protoplasts or tissue cultures
3. Alteration of nucleus of plant cell by inserting new genetic material:
   - Plasmid, carried by Agrobacterium
   - mechanically, by using “gene gun”



Gene Gun

Alternative Method of Gene Insertion – Gene Gun



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   Treat growing cell cultures with antibiotic → survivors transformed

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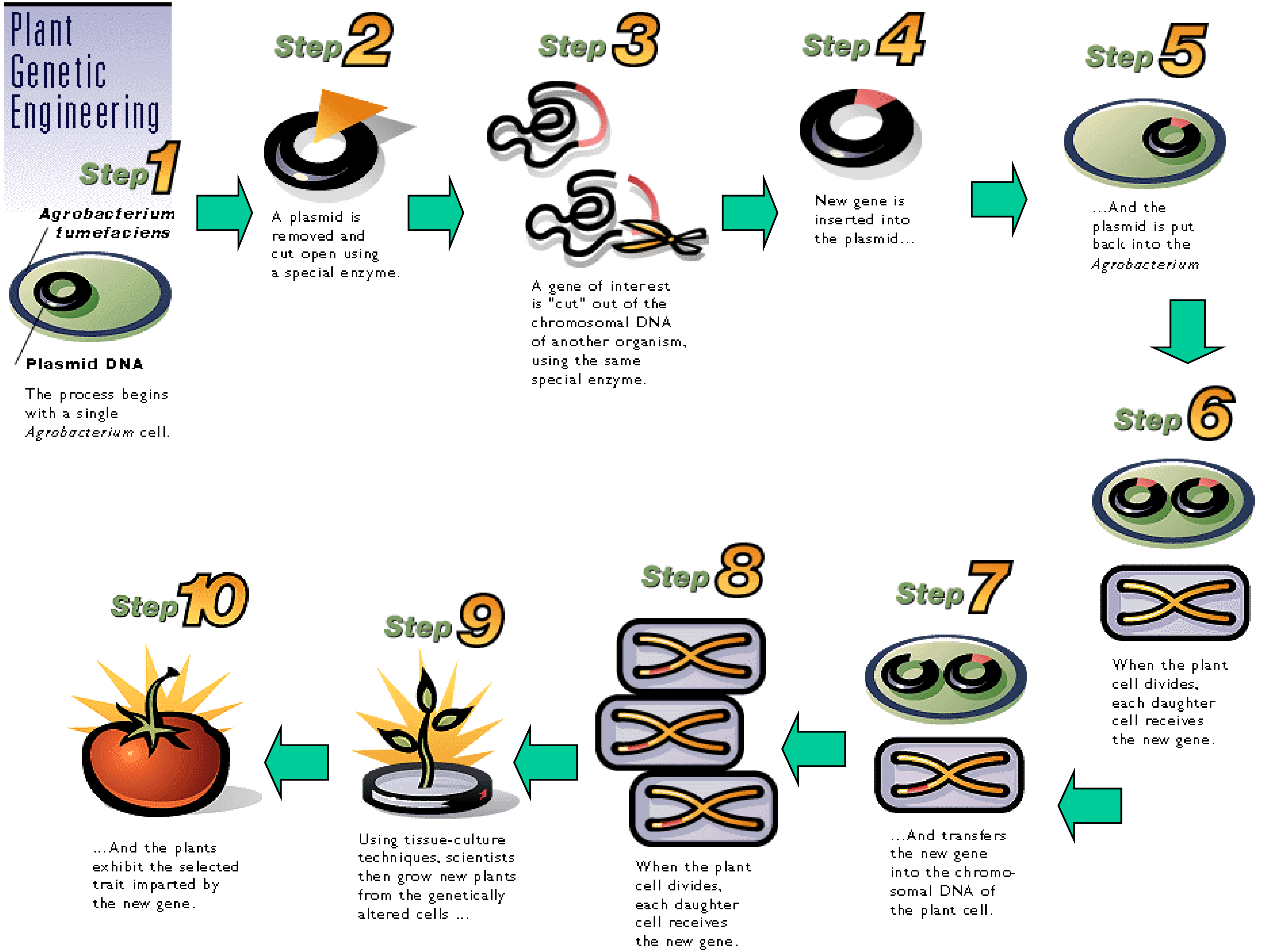
   New problem: does this spread antibiotic resistance?



Plant Genetic Engineering
Step 1
Agrobacterium tumefaciens
Plasmid DNA
The process begins with a single Agrobacterium cell.
Step 2
A plasmid is removed and cut open using a special enzyme.
Step 3
A gene of interest is "cut" out of the chromosomal DNA of another organism, using the same special enzyme.
Step 4
New gene is inserted into the plasmid...
Step 5
...And the plasmid is put back into the Agrobacterium
Step 6
When the plant cell divides, each daughter cell receives the new gene.
Step 7
...And transfers the new gene into the chromosomal DNA of the plant cell.
Step 8
When the plant cell divides, each daughter cell receives the new gene.
Step 9
Using tissue-culture techniques, scientists then grow new plants from the genetically altered cells ...
Step 10
...And the plants exhibit the selected trait imparted by the new gene.

Bt

Bt – *Bacillus thuringiensis* – common soilborne bacterium

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Produces proteins (“crystal proteins”, Cry) that selectively kill certain groups of insects

- stomach toxins, must be ingested to kill

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- stomach toxins, must be ingested to kill
- protein binds to receptors in intestines → insect stops eating
- used in granular or liquid form > 30 years as a pesticide
- many (>60) different Cry proteins → effective against different insects

Central Dogma of Molecular Biology

DNA → RNA → polypeptides → enzymes → chemical reactions → life

↓

DNA

↓

DNA

↓

Central Dogma of Molecular Biology

DNA → RNA → polypeptides → enzymes → chemical reactions → life

↓

DNA

↓

DNA

↓

Analyze amino acid sequence

Central Dogma of Molecular Biology

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DNA

DNA

Analyze amino acid sequence

Deduce RNA, DNA sequence

Find gene

Advances in Molecular Genetics

Gene – coding sequence (for polypeptide)

Advances in Molecular Genetics

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Promoter – controls where and how much protein is made

Advances in Molecular Genetics

Gene – coding sequence (for polypeptide)

Promoter – controls where and how much protein is made

(often): genes for detection – antibiotic resistance, herbicide resistance

Combination of gene + promoter + detecter = cassette


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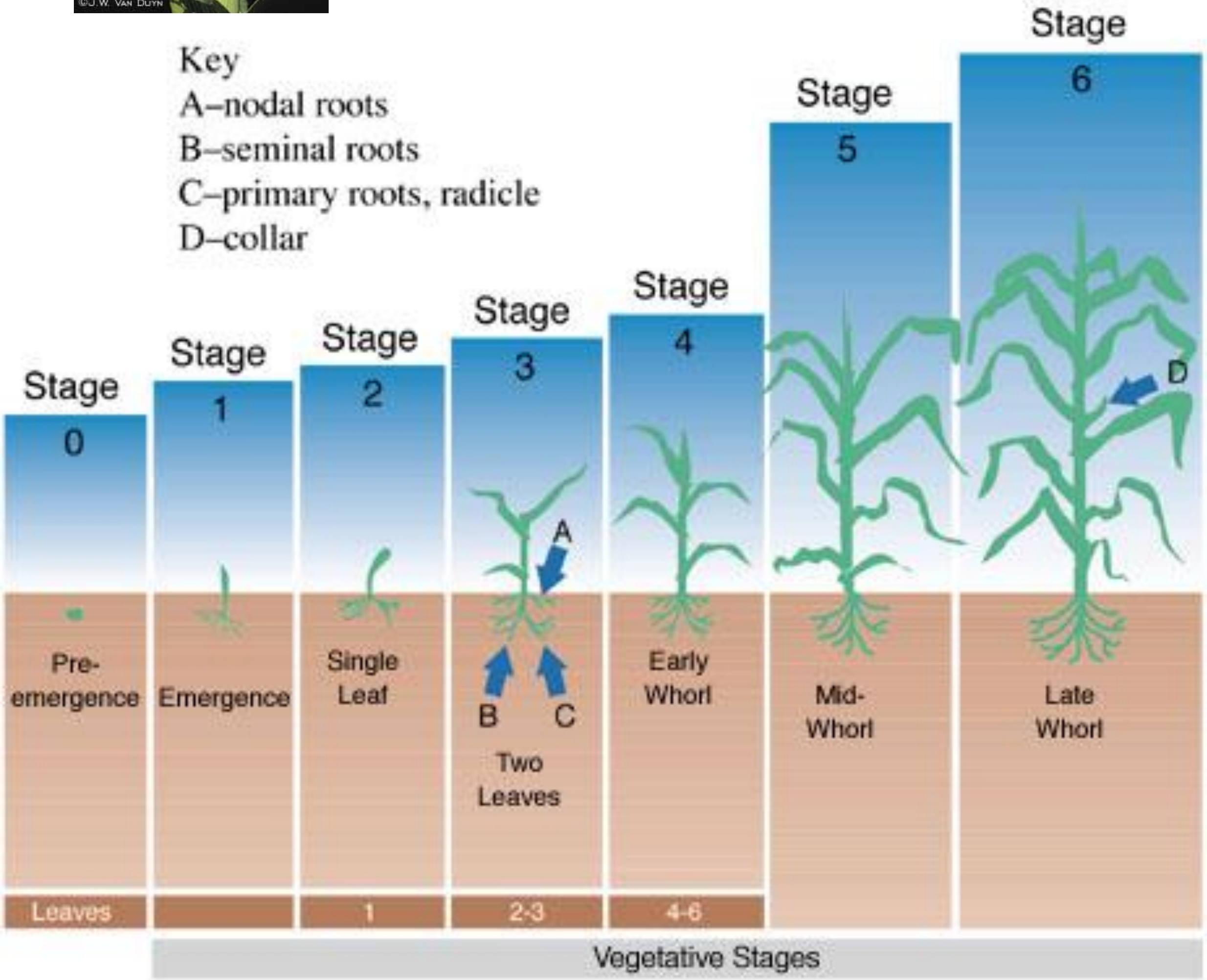
Key
A–nodal roots
B–seminal roots
C–primary roots, radicle
D–collar
Stage 0
Pre-emergence
Stage 1
Emergence
Stage 2
Single Leaf
Stage 3
A
B
C
Two Leaves
Stage 4
Early Whorl
Stage 5
Mid-Whorl
Stage 6
D
Late Whorl
Leaves
1
2-3
4-6
Vegetative Stages


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Corn Borer Impact

U.S. + Canada: > $1 billion per year, damage + control costs

Ca 25 bushels of corn/acre (studies in Iowa)

Bt Corn

Bt genes – inserted into corn genome (gene gun technology)

→ Corn plant that can produce Bt in every cell

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Advantages over topically applied powder:

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→ Corn plant that can produce Bt in every cell

Advantages over topically applied powder:

- Bt toxin not destroyed by UV, heat, dessication
- wider coverage of insect feeding sies
- no guessing as when to apply

Types of Bt Corn

Each transformation of corn = event

Each “event” potentially different:

- protein-coding region inserted
- location where insertion occurs

Event	Trade Name	Protein

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Event	Trade Name	Protein
“176”	KnockOut (Novartis)	Cry1Ab
BT111,Mon810	YieldGard (Monsanto)	Cry1Ab
DBT418	BT-Xtra (DeKalb)	Cry1Ac
CBH351	StarLink (Aventis)	Cry9c

Dangers of Bt Corn

1. Potential allergic response of people to Cry protein

   - toxicity of protein is basically nil for people

   - Cry1 – passed allergy test (digested rapidly)

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   * Cry9c in StarLink – Produced by Aventis:

   Tests to identify potential allergens:

   - stable at 90 C; not readily digestible under simulated gastric conditions

Starlink Corn & Fritos

2000: Starlink corn grown for animal food: <1% of farm crop

- some Starlink corn was found to occur in food products:

“Green” (environmental) groups – tested tacos, using DNA probe

→Recall of affected products; testing

Note: No actual health problems have been reported

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2001 – "volunteer" corn (and cross-pollinated "volunteer" corn) could still contain the Cry9c Bt protein

Dangers of Bt Corn

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2. May kill other insects (selective for lepidopterans):

   - Monarch butterfly larvae

Monarch Butterfly and Bt

Monarch butterflies – food plant = milkweeds

-milkweed commonly found weed at edge of cornfield

Monarch butterflies – lepidoptera, killed by Bt toxin

Paper in Nature magazine:

Dust food source for monarch larvae with Bt pollen, feed to larvae, observe response (→ significant number die in treatments)

→Warning about effect of Bt on Monarchs

[problems: Monarchs don't lay eggs on cornfield milkweeds; feeding doses >> than naturally occurring]

Swallowtail butterfly – no effect seen

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4. Produce resistance in target pest

U.S. Government Regulation of GMOs

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Examples:

Bt corn or RoundUp Ready soybeans – checked by EPA

Bt corn (ear) – checked by USDA

Bt corn (in cornflakes) – checked by FDA

Economics of Bt Corn

Factors to consider: increased cost of Bt vs regular corn ($7-10/acre); costs of regular pesticides that don't have to be used; yield; yield reduction by corn borers

1. "Benign Neglect" (no treatment) – costs $19.50/acre

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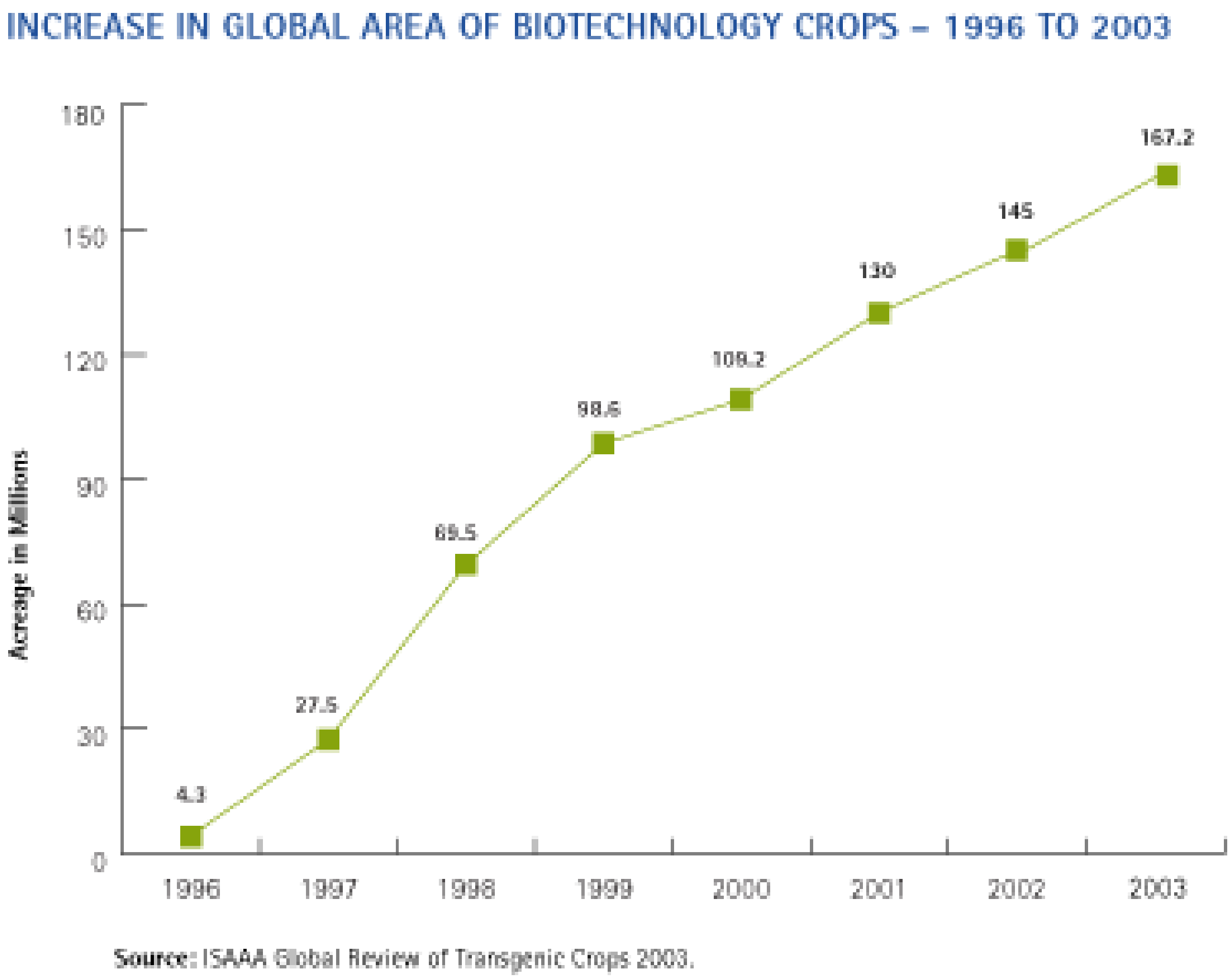
FURTHER NOTE: exact benefits will vary from year to year

Applications of GM Crops

- insect resistance (eg Bt)
- herbicide resistance (Round-up Ready)
- increase nutrient value (Golden Rice)
- produce drugs (eg vaccine for respiratory disease)
- alter properties of crop (eg polyester cotton)

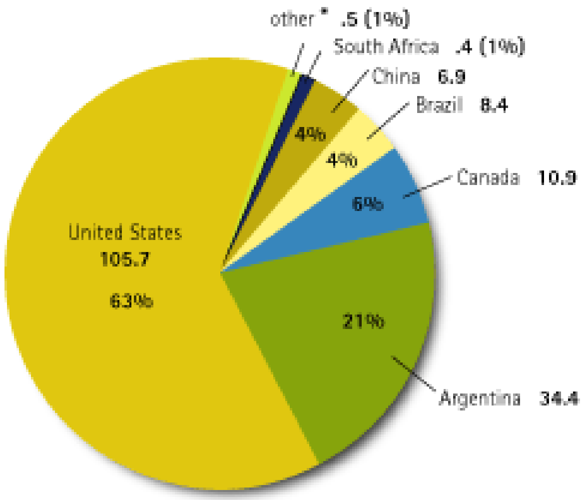
GM Crops – From Fringe Mainstream Agriculture



U.S. – Leading the Way in GM Crops

PERCENT OF GLOBAL LAND AREA PLANTED IN BIOTECHNOLOGY VARIETIES BY COUNTRY

(2003 total global land area: 167.2 million acres)



Acreage in Millions

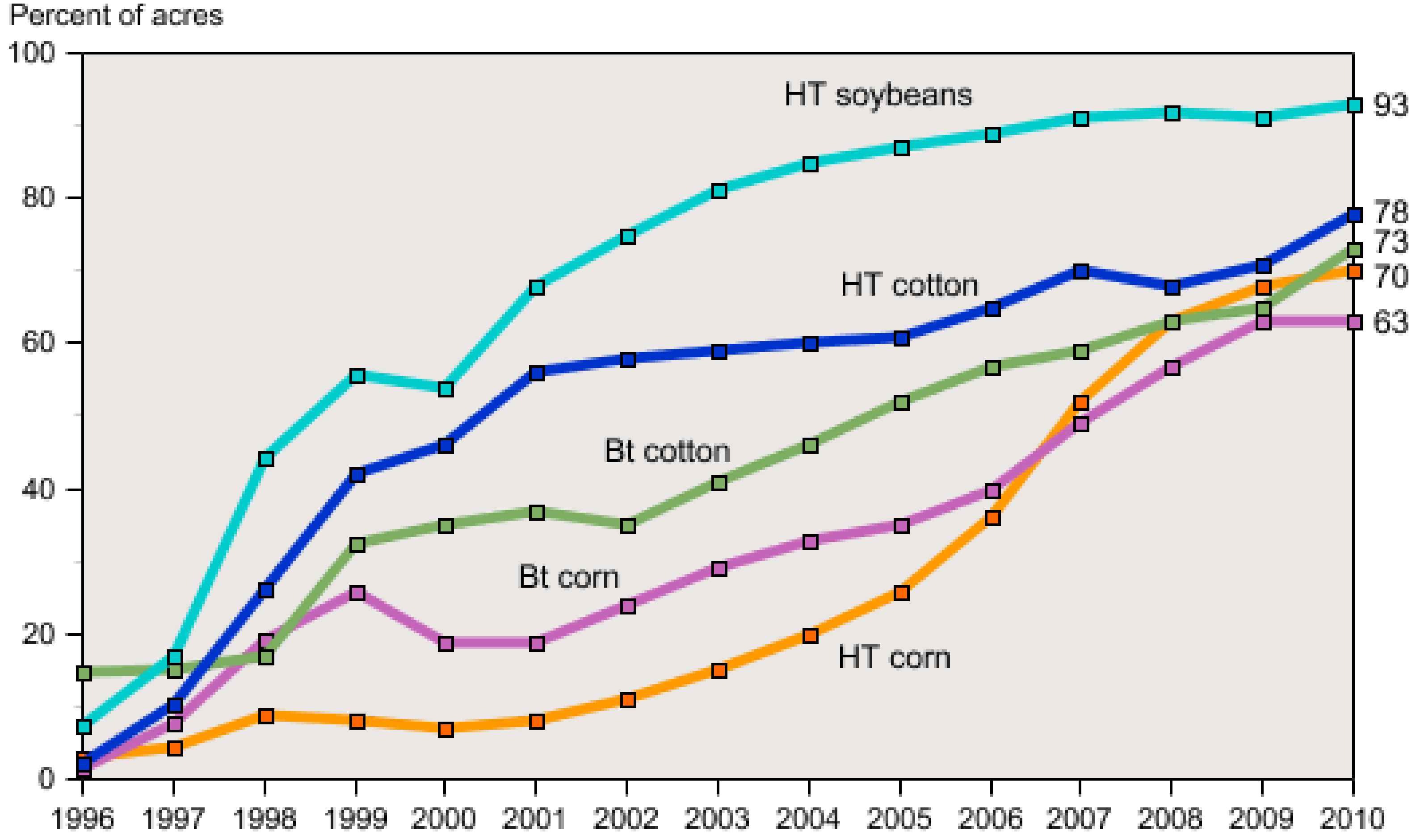
* The following countries planted genetically modified crops totaling one percent of global GM crop production: Australia, Mexico, Romania, Bulgaria, Spain, Germany, Uruguay, Indonesia, India, Columbia, Honduras, and the Philippines. Differences between values shown and those calculated (from percent and total global acreage) are a likely consequence of rounding.

Source: International Service for the Acquisition of Agri-biotech Applications (ISAAA) Global Review of Transgenic Crops 2003.

MAJOR U.S. GM CROPS

Crop	2001 Total Acreage	2002 Total Acreage	2003 Total Acreage	2004 Total Acreage
Corn	75,800 (26%)	79,000 (34%)	79,066(40%)	81,100 (45%)
Soybean	74,105 (68%)	72,993 (75%)	73,653 (81%)	74,724 (85%)
Cotton	15,499 (69%)	14,151 (71%)	13,924 (73%)	13,947 (76%)

Rapid growth in adoption of genetically engineered crops continues in the U.S.



Data for each crop category include varieties with both HT and Bt (stacked) traits.
Sources: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-10 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Roundup – Glyphosate – Mode of Action/Resistance

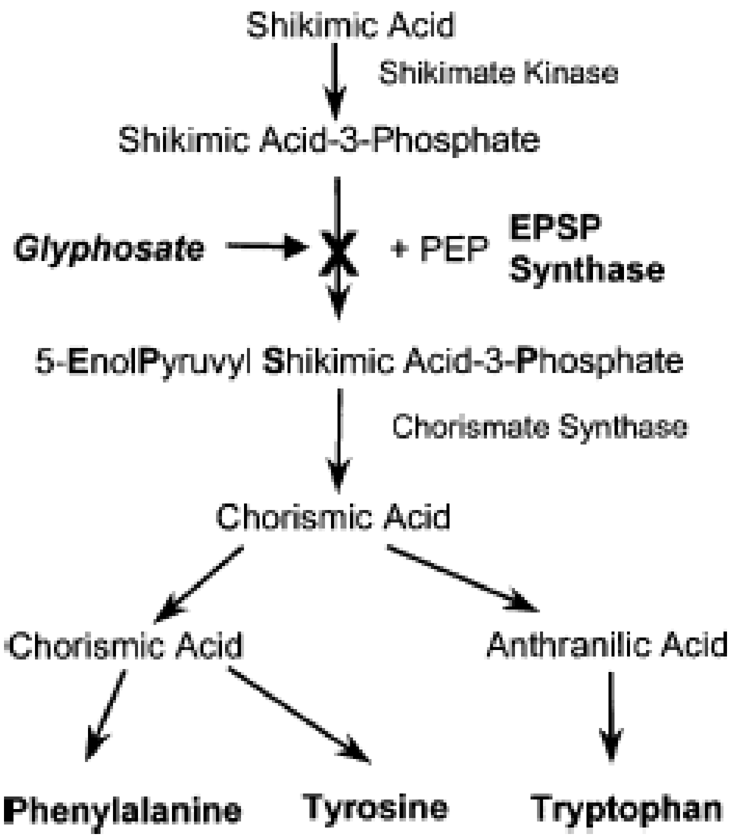


Figure 1. Glyphosate mode of action.

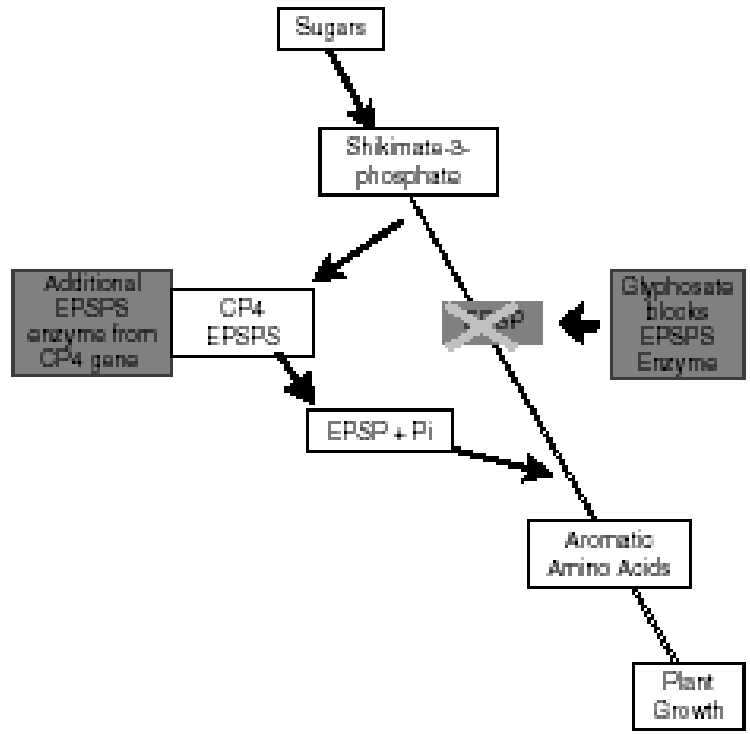


Figure 2. Strategy for the development of glyphosate-resistant crops.

Roundup-Resistant Crops – Cost/Benefit Analyses

Table 2. Hectares of herbicide-resistant crops planted globally in 2003 and the percentage of total crop plantings represented

Crop	Million hectares	% of transgenic crop plantings
Herbicide-resistant soybeans	41.4	61
Herbicide-resistant maize	6.4	10
Herbicide-resistant cotton	4.1	6
Herbicide-resistant canola	3.6	5

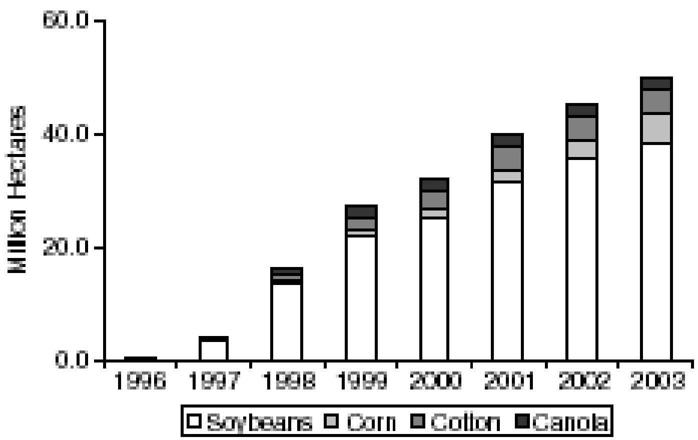


Figure 3. Global adoption rates of glyphosate-resistant crops since introduction.

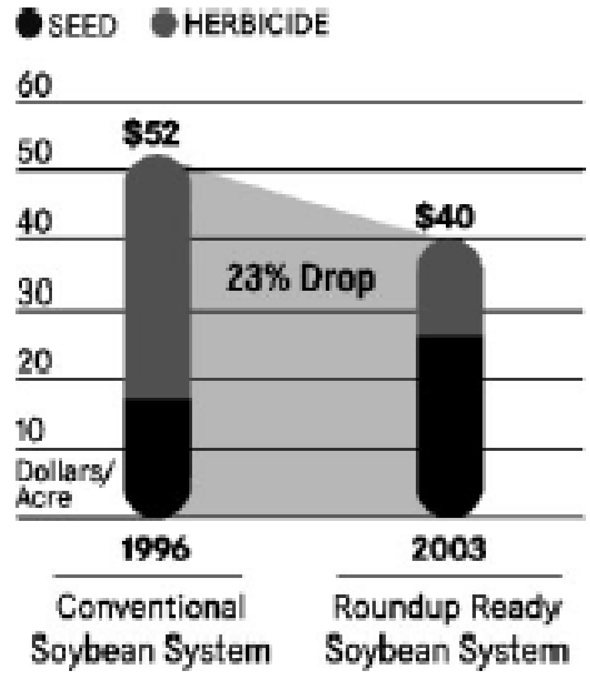


Figure 4. Since Roundup Ready Soybeans were introduced in 1996, total weed control input costs have dropped over $10 per acre. Source: Doane Market Research, 452 farmers in 19 states.

Roundup Ready Crops – Environmental Boon or Bane?

Upside: higher yields

Downsides:

- Potential for development of resistance
- Gene flow contaminates other crops
- Higher use of glyphosate → unexpected environmental effects:

soil accumulation could affect other organisms
(microorganisms such as mycorrhizae)

run off affects other organisms

Roundup Ready Crops – Fly (Frog?) in the Ointment?

THE IMPACT OF INSECTICIDES AND HERBICIDES ON THE BIODIVERSITY AND PRODUCTIVITY OF AQUATIC COMMUNITIES

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Abstract. Pesticides constitute a major anthropogenic addition to natural communities. In aquatic communities, a great majority of pesticide impacts are determined from single-species experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents an assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals.

Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions.

Key words: *amphibian decline;* Anax junius; Bufo americanus; Daphnia; Dytiscus; *frogs;* Hyla versicolor; Lestes; Pseudacris crucifer; Rana pipiens; Rana sylvatica; Tramea.

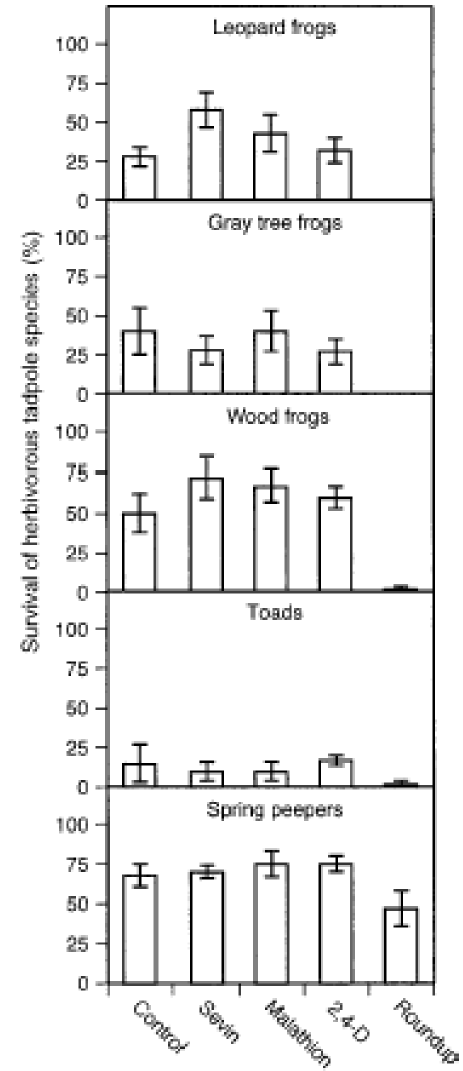

FIG. 5. The impact of four different pesticides on the survival of individual species of herbivorous tadpoles. Data are means ± 1 SE.

AgBioTech

Aventis (Hoechst + Rhone Poulenc)

Monsanto (Monsanto + Pharmacia+ Upjohn)

Dupont (Dupont + Pioneer Hybrid)

Syngenta (Novartis + AstraZeneca)

Dow Chemical (Dow + Elanco)

5 Firms – each Multinational:

68% of agrochemical market worldwide

20% of commercial seed worldwide

GM Crops – Other Issues

Threat to small farmers

Patenting Life Forms

Biopiracy

Terminator Technology

Sociology/Politics: Use of perjorative terms clouds discussion

e.g. Frankenfoods