Eco-Friendly Plants At Work

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Outline

- What is genetic engineering?
- Why genetically engineer plants?
- Advantages, Problems
- Methods

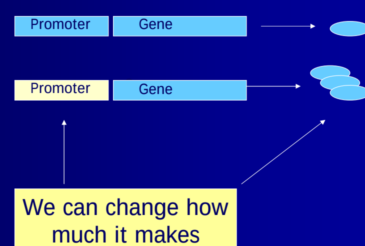
- Examples of g.e. of plants
- How I use it

Genetic Engineering

- Transferring specific genes to an organism
  - A gene is a segment of DNA that encodes a protein.

Genetic Engineering: Example 1

- 1. Gene can be from the same species



Genetic Engineering Example 2

- 2. It can be a gene transferred from a wild relative

Genetic Engineering: Example 3

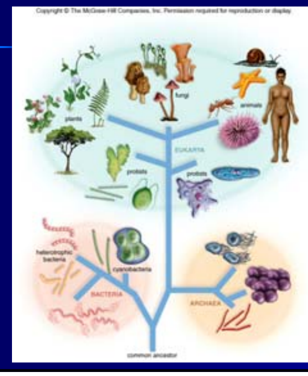
- Can be cross-kingdom transfer (mammalian to plant, bacteria to plant)

- The genetic code is universal

Genetic code is in 3-letter codons → amino acids → protein

FIRST LETTER	SECOND LETTER: U	C	A	G	THIRD LETTER
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	Stop	Stop	A
	Leucine	Serine	Stop	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	(Start) Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartate	Glycine	U
	Valine	Alanine	Aspartate	Glycine	C
	Valine	Alanine	Glutamate	Glycine	A
	Valine	Alanine	Glutamate	Glycine	G

Cross-kingdom transfer



Genes from any living source are “readable” by other living cells

Why engineer plants

- Traditional breeding is too generalized; highly variable; random mixing of 1000s of genes
  - Crossing to get large fruit resulted in the loss of many other valuable traits

Why engineer plants

- Often, only a specific trait is the focus
  - Example: pathogen resistance from a wild relative

Why engineer trees in particular

- Traditional breeding is often not an option
- Traditional breeding would take decades
- Phytoremediation- discuss next time
- Bioenergy- discuss later
- Lumber production- more efficient, less land needed

Benefits of Bioengineered Trees

- Intensify production in a limited area
  - Plant and manage for high yields so that 50% of world forests can remain undeveloped
- Increased stress tolerance, use marginal lands

Teosinte ear *(Zea mays* ssp *mexicana*) on the left, maize ear on the right, and ear of their F1 hybrid in the center

- Only a few genes are different between wild and cultivated maize.

(photo by John Doebley)

Disadvantages

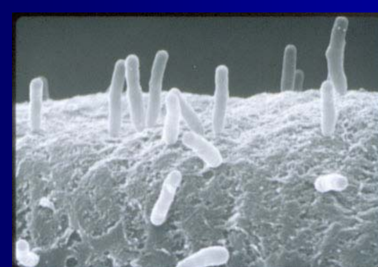
- Public perception; fear and violence
- Tightly regulated
- Outcrossing issues; containment. But this issue can be addressed:
  - Harvest before sexually mature
  - Buffer zones
  - Note: agricultural crops have not eliminated the wild varieties

Methods

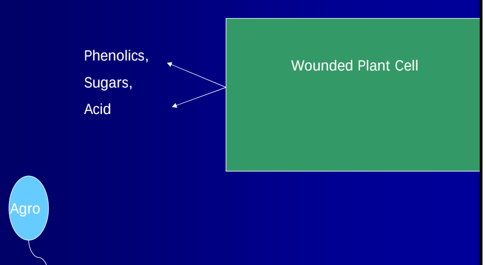
- *Agrobacterium tumefaciens*
- *Agrobacterium rhizogenes*

- Biolistics

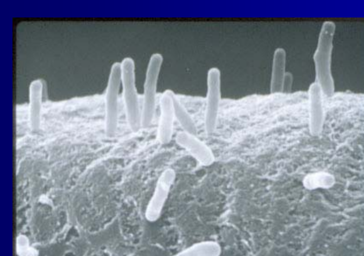
Agrobacterium is commonly used to introduce specific genes into plants



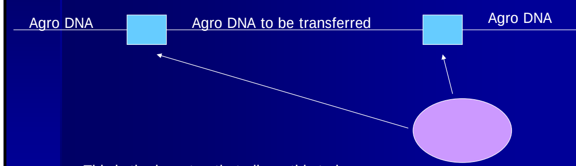
1. Step One: *Agrobacterium* detects a wounded plant cell



Step 2. Agrobacterium attaches to the plant cell



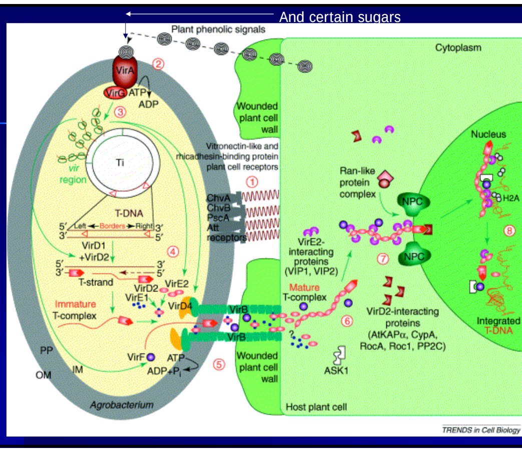
Step 3. Agrobacterium prepares a piece of DNA for transfer



This is the key step that allows this to be a tool for plant genetic engineering

Agrobacterium-mediated Transformation

- 4. T-strand is exported out of the bacteria and into the plant cell
- 5. Nuclear targeting signals guide it to nucleus
- 6. T-strand is integrated into plant genome



Why does Agrobacterium do this?

1. Food
2. Safe home away from competitors

Agro-Induced Tumor

How Agrobacterium is used to transform plants

- 1. Remove the genes that cause the tumor
- 2. Replace them with “genes of interest”
- 3. Agrobacterium transfers anything that is between the borders

Transformation of Poplar using *Agrobacterium tumefaciens*

Agro is killed; hormones provided for plant regeneration →

A. rhizogenes Transformation of Poplar Leaves

- *Agrobacterium rhizogenes* carries root induction genes on T-DNA

Biolistic Transformation

- Coat particles with DNA of interest
- Particle bombardment of callus cells

Examples of genetically-engineered plants

- B.t. Cotton
- Salt-tolerant crops
- Decaf coffee trees
- Papaya resistant to viral pathogen
- American Chestnut project

Enhancing Phytoremediation

Removal of pollutants from our environment using plants

The Pollution Problem

- Solvents, PAHs, PCB's, BTEX
- Occurs from spills or deliberate dumping
- $6-8 billion spent annually in U.S.
- $25-50 billion worldwide
- Enormous health costs too

Super Fund Sites

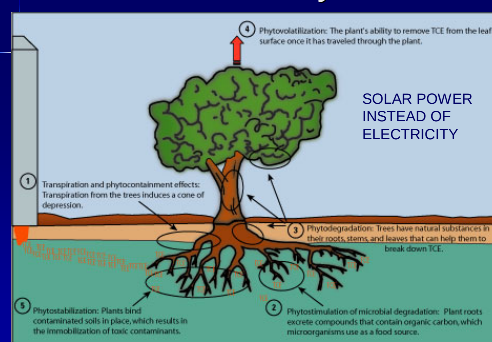
- EPA's list of the nation's most contaminated hazardous waste sites
- 12,000 (US alone)
- Washington State too
- 400,000 in Western Europe

Brownfields

Traditional treatments-Engineering

- Excavation to another site
- Indefinite storage
- Capping
- Soil washing
- Incineration

Phytoremediation=Solar-Powered Pollution Removal System



Advantages to Phytoremediation

- Uses the plant's natural ability to extract chemicals from water, soil, and air
- Less intrusive and more aesthetically-pleasing
- Cheaper
- Easier to monitor (plant and chemical)

More Advantages!

- Soil stabilization
- Buffer of dust, noise, garbage from site
- Wildlife habitat
- Carbon sequestration
- Usable product- biofuel, wood

Why Enhance Phytoremediation?

- 1. Some pollutants are phytotoxic

Photo from Neil Bruce

Why Enhance Phytoremediation?

- 2. Plant species that can degrade the pollutant sometimes cannot grow in the required environment

Why Enhance Phytoremediation?

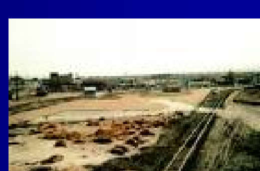
- 3. Phytoremediation is too slow and transient

Why Enhance Phytoremediation?

- 4. Plant species with the desired activities are of low biomass

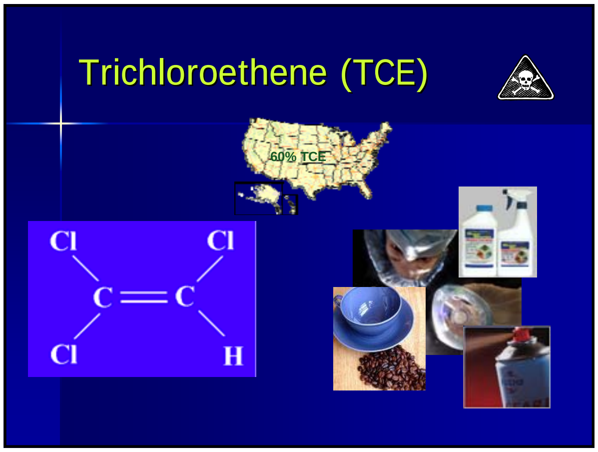
Why Enhance Phytoremediation?

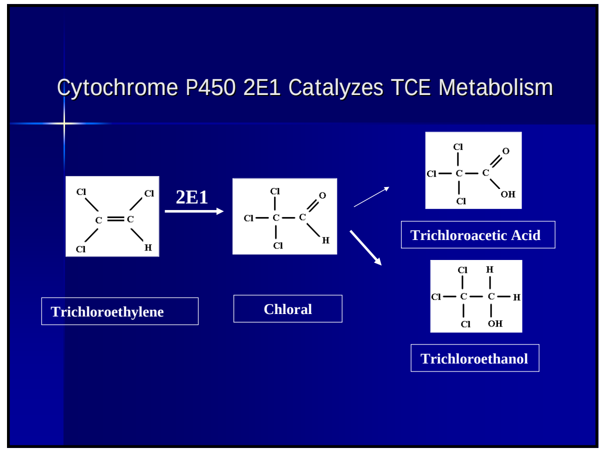
- 5. Plants might be tolerant to one type but killed by another type of pollutant
- 64% of polluted sites have mixed pollutants

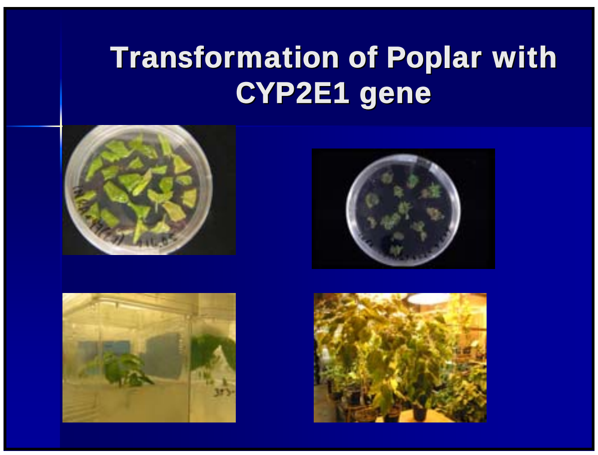


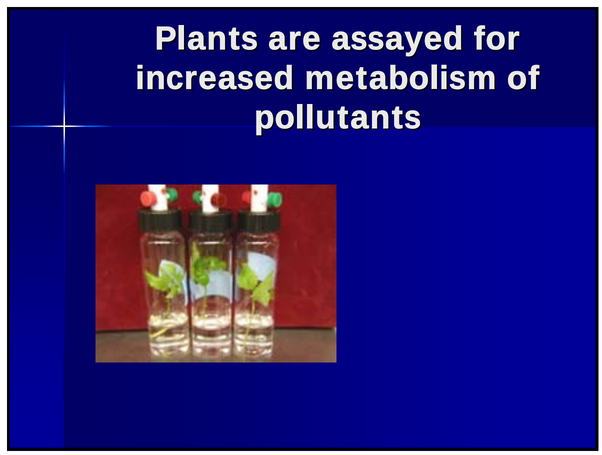
Enhancing phytoremediation of small, volatile chemicals

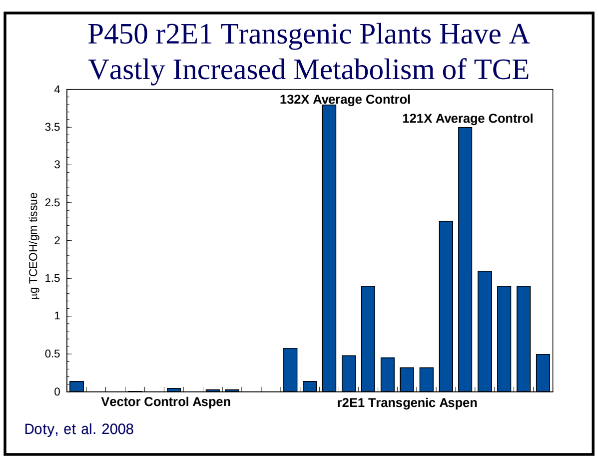
Principal Investigator: Prof. Stuart Strand (Dept. of Civil and Environmental Engineering; CFR)
Co-P.I.: Prof. Sharon Doty (CFR)
Funding: NIEHS and DOE

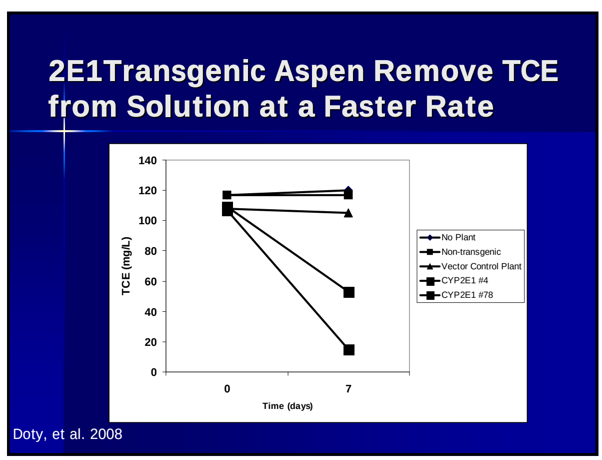

Trichloroethene (TCE)
60% TCE
Cl
Cl
C
C
Cl
H


Cytochrome P450 2E1 Catalyzes TCE Metabolism
2E1
Trichloroethylene
Chloral
Trichloroacetic Acid
Trichloroethanol


Transformation of Poplar with CYP2E1 gene


Plants are assayed for increased metabolism of pollutants

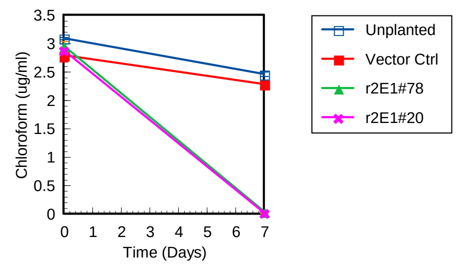

P450 r2E1 Transgenic Plants Have A Vastly Increased Metabolism of TCE
132X Average Control
121X Average Control
µg TCEOH/gm tissue
0
0.5
1
1.5
2
2.5
3
3.5
4
Vector Control Aspen
r2E1 Transgenic Aspen
Doty, et al. 2008


2E1Transgenic Aspen Remove TCE from Solution at a Faster Rate
TCE (mg/L)
0
20
40
60
80
100
120
140
0
7
Time (days)
No Plant
Non-transgenic
Vector Control Plant
CYP2E1 #4
CYP2E1 #78
Doty, et al. 2008

P450 2E1 Has Multiple Substrates

Chloroform
Carbon Tetrachloride
Vinyl Chloride
Benzene

Increased Removal of Chloroform from Solution by r2E1 Transgenic Aspen



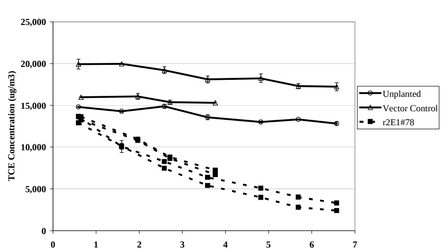
Doty, et al. 2008

Increased removal rates of several important pollutants

- 1. TCE 53X faster
- 2. Chloroform 9X faster
- 3. Vinyl chloride 3X faster

Removal of pollutants from air

P450 2E1 aspen plants remove more TCE from air



Doty, et al. 2008

Increased removal of benzene from air

- 10X faster removal using transgenic poplar than non-transgenic poplar

Acknowledgements

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Andy James

Allison Moore

Reference:

Doty, S. L., James, C., Moore, A., Vajzovic, A., Singleton, G. L., Ma, C., Khan, Z., Xin, G., Kang, J., Park, J., Meilan, R., Strauss, S., Wilkerson, J., Farin, F., and Strand, S. E. (2007). *Proceedings of the National Academy of Sciences* 104(43):16816-16821.