Phytoremediation of Organic Pollutants

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Poplar Trees

- Fast-growing trees (3-5 meters per year)
- Take up large volume of water (100 L/day)
- Long-lived
- Successfully used in phytoremediation projects

Phytoremediation

1. Overview of Phytoremediation
2. Organic (degradable) pollutants

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

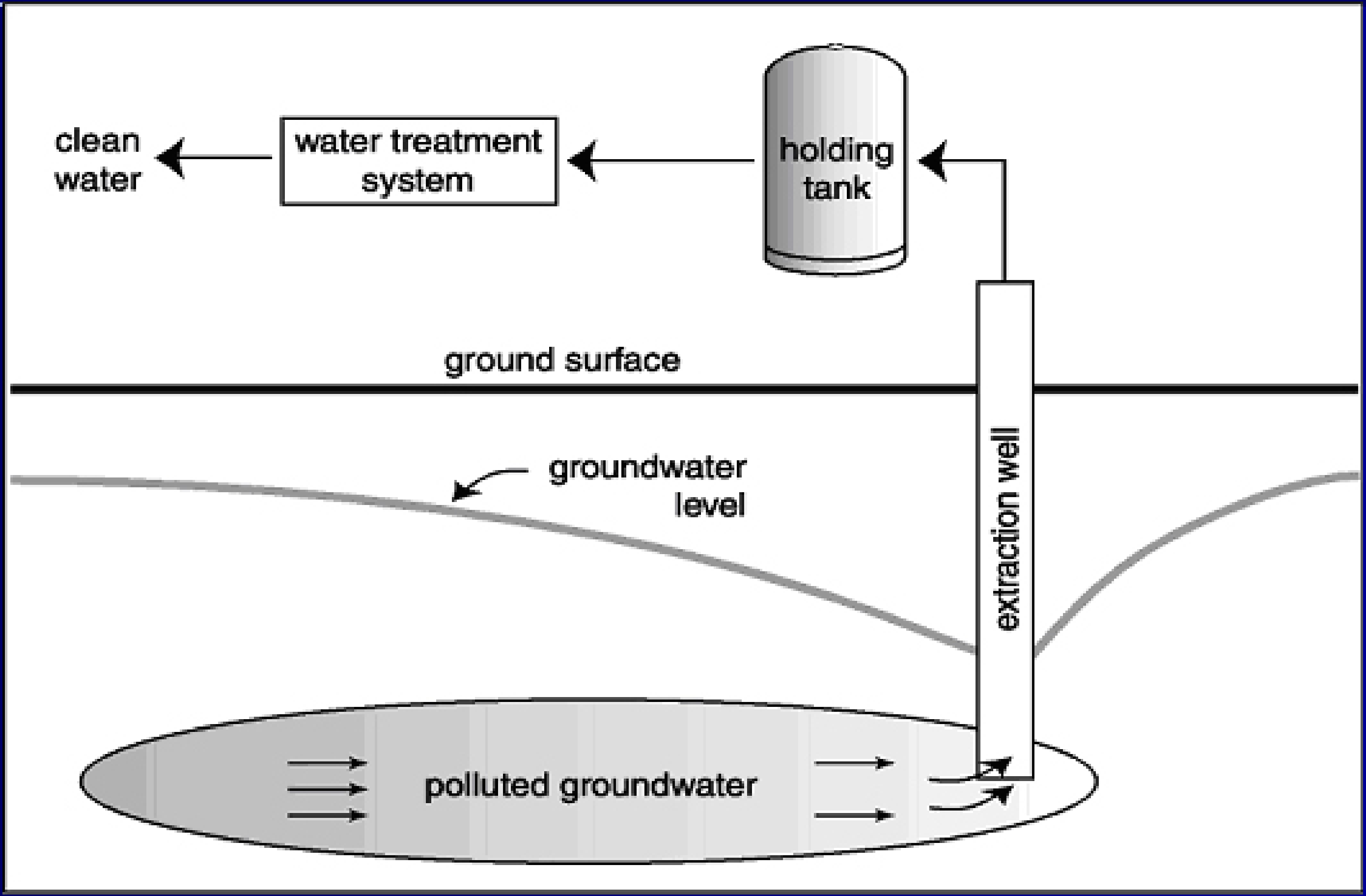
Brownfields

- Abandoned, polluted commercial properties
- More than 500,000 in U.S.
- Owners would rather abandon the site than pay for clean up

Traditional treatments-Engineering

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

Engineering: Pump&Treat



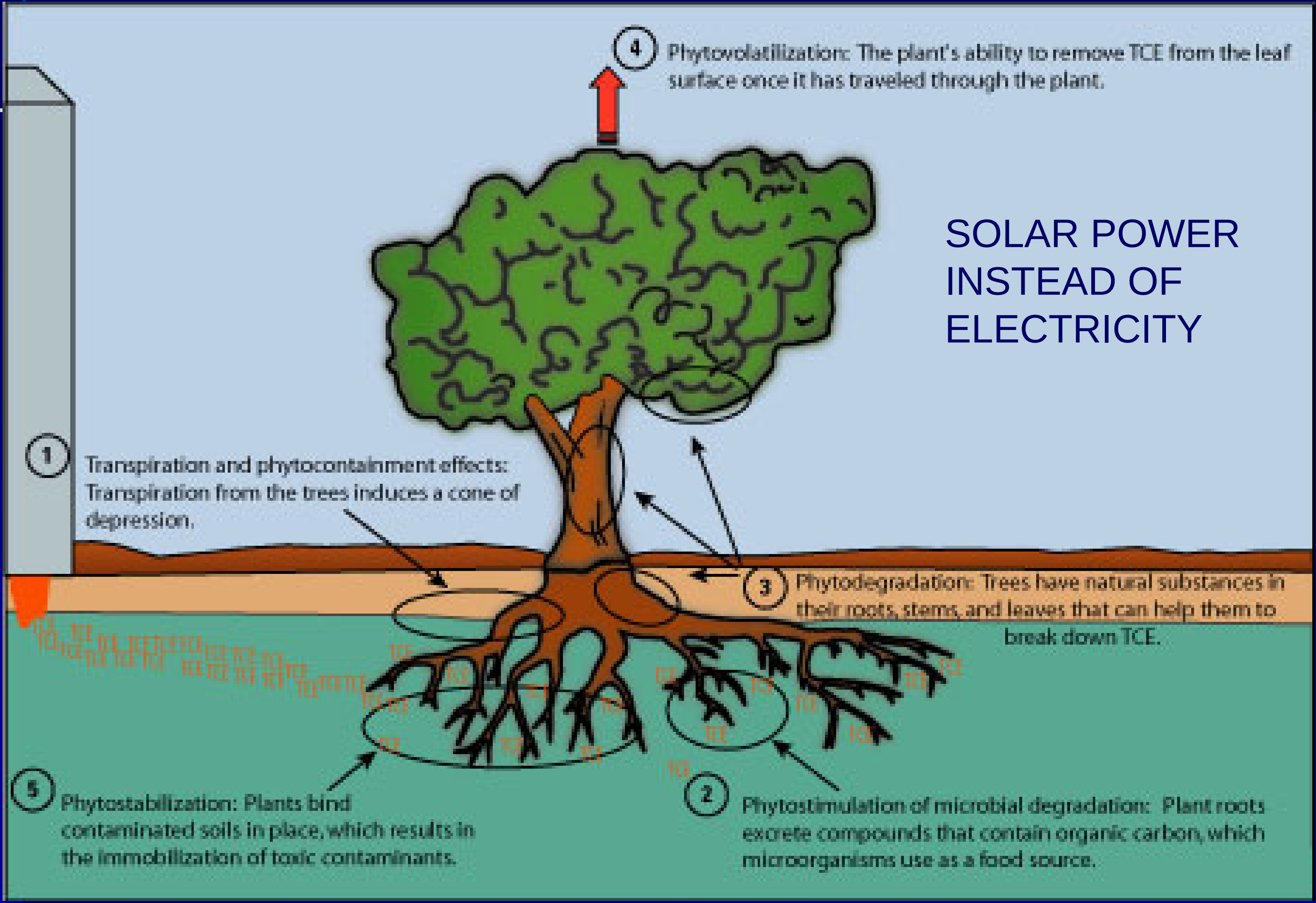
Chemical Treatment

- Add 1000s of gallons of oxidant (potassium permanganate or hydrogen peroxide)

Bioremediation

- “Bioaugmentation” with bacterial strains known to degrade the pollutant
- Issues:
  - – Monitoring
  - – competition with other microbes
  - – anaerobic metabolism
  - – Feeding the microbes

Phytoremediation = "Solar Pump&Tree"



SOLAR POWER INSTEAD OF ELECTRICITY

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)
- Soil stabilization
- Carbon sequestration
- Usable product- biofuel, wood

Disadvantages

- Too slow
- Seasonal
- Plant species must be adapted to the environment of the site

How phytoremediation works

How Phytoremediation Works

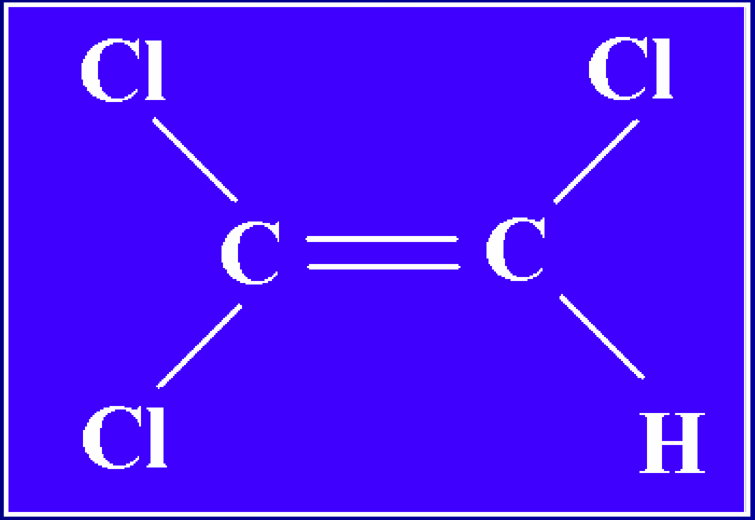
- A. Phytoextraction (metals)
- B. Rhizofiltration (metals)
- C. Phytostabilization and containment
- D. Phytovolatilization
- E. Phytostimulation
- F. Phytodegradation

Phytodegradation

- Take up and biochemically degrade the pollutants to harmless biproducts
- Chlorinated solvents, explosives
- Phytoremediation at its best!

Solvents: TCE

- Trichloroethylene (TCE)- 50% of SuperFund sites
- Metal degreaser
- Toxic
- Plants can take it up



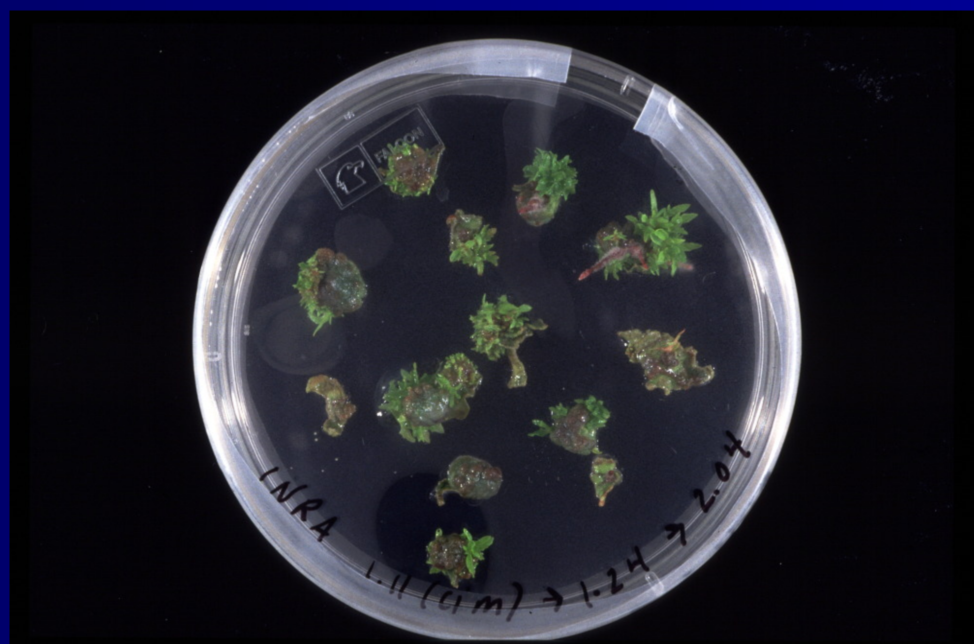
Metabolism of TCE by Poplar

- Pure poplar cells
- Oxidizes TCE to trichloroethanol
- Adds on a sugar
- Stores it while it slowly degrades

Why Enhance Phytoremediation?

- Pollutants are phytotoxic
- Plant species that can metabolize the pollutant sometimes cannot grow in the required environment
- Phytoremediation is too slow and transient
- Plant species with the desired activities are of low biomass
- 64% of polluted sites have mixed pollutants

Genetic Engineering of Plants



Genetic Engineering

- Transferring specific genes to an organism
  - A gene is a segment of DNA that encodes a protein. The genetic code is universal
- Can be the same species (overexpress or underexpress a native gene)
- Can be a transfer from wild relative
- Can be cross-kingdom transfer (mammalian to plant, bacteria to plant)

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

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

Why engineer plants

- Traditional breeding is too generalized; highly variable; random mixing of 1000s of genes
  - Crossing to get large fruit results in loss of many other valuable traits
- Often, only a specific trait is the focus
  - Example: pathogen resistance from a wild relative

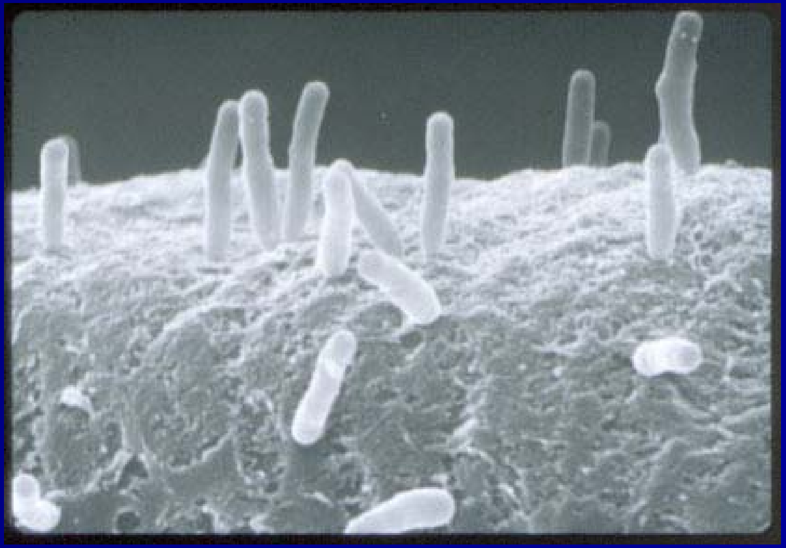
Methods

- *Agrobacterium tumefaciens*
- *Agrobacterium rhizogenes*
- Both are soil bacteria that naturally transform plant cells by introducing a piece of their DNA (T-DNA) into the plant nuclear genome

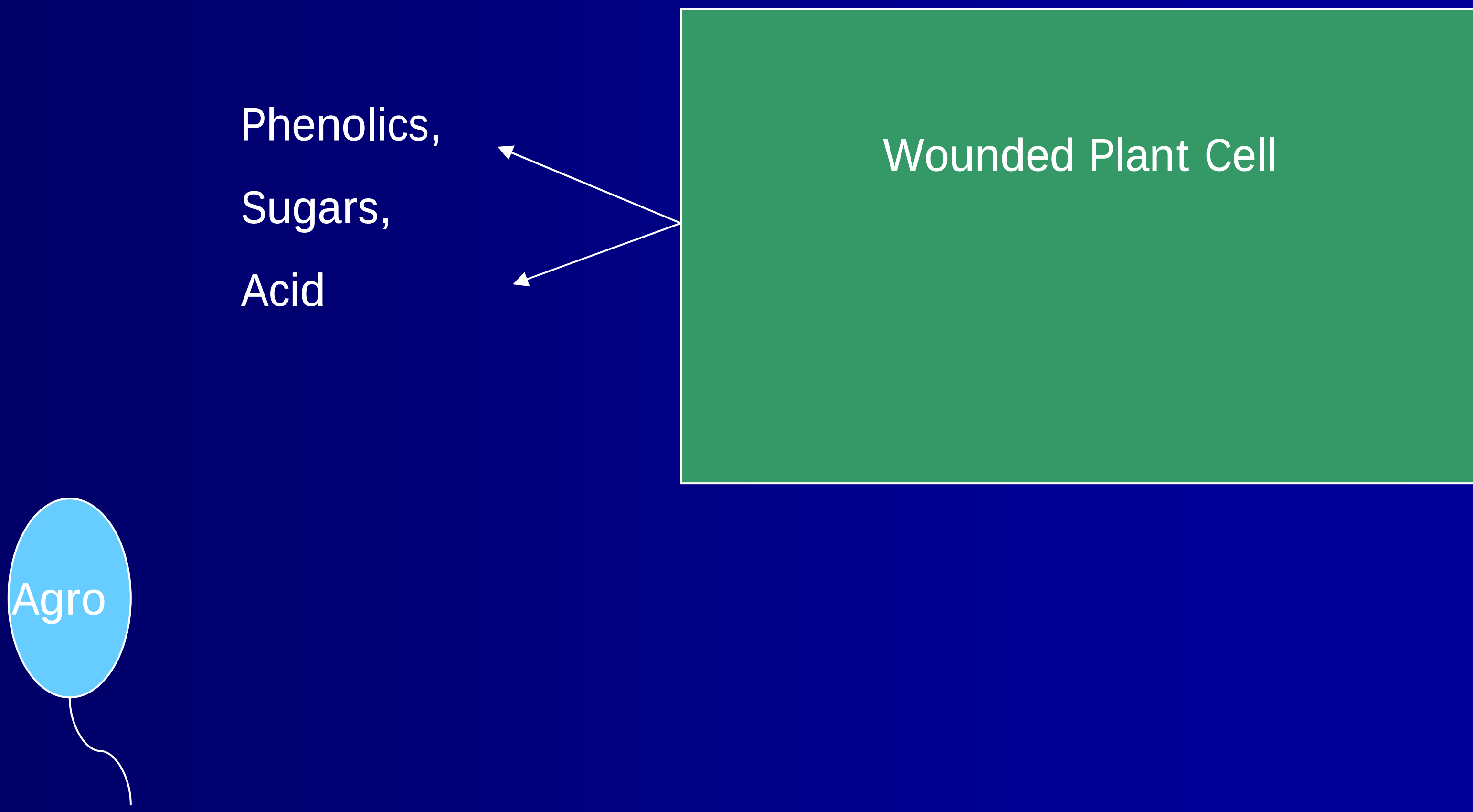
- Biolistics

Agrobacterium is commonly used to introduce specific genes into plants

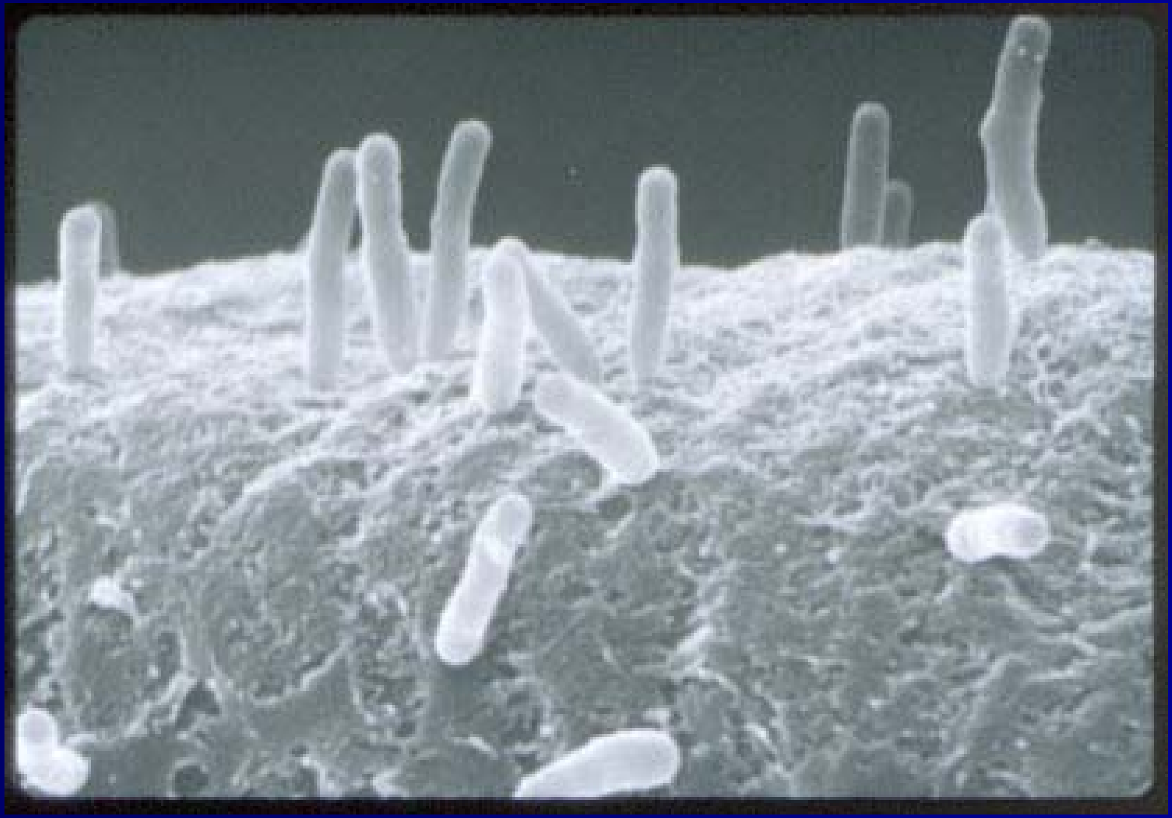
- *Agrobacterium* is a common soil bacterium
- It naturally transfers DNA into the plant cell genome
- The plant treats the new DNA as its own
- Mechanism discovered here at UW



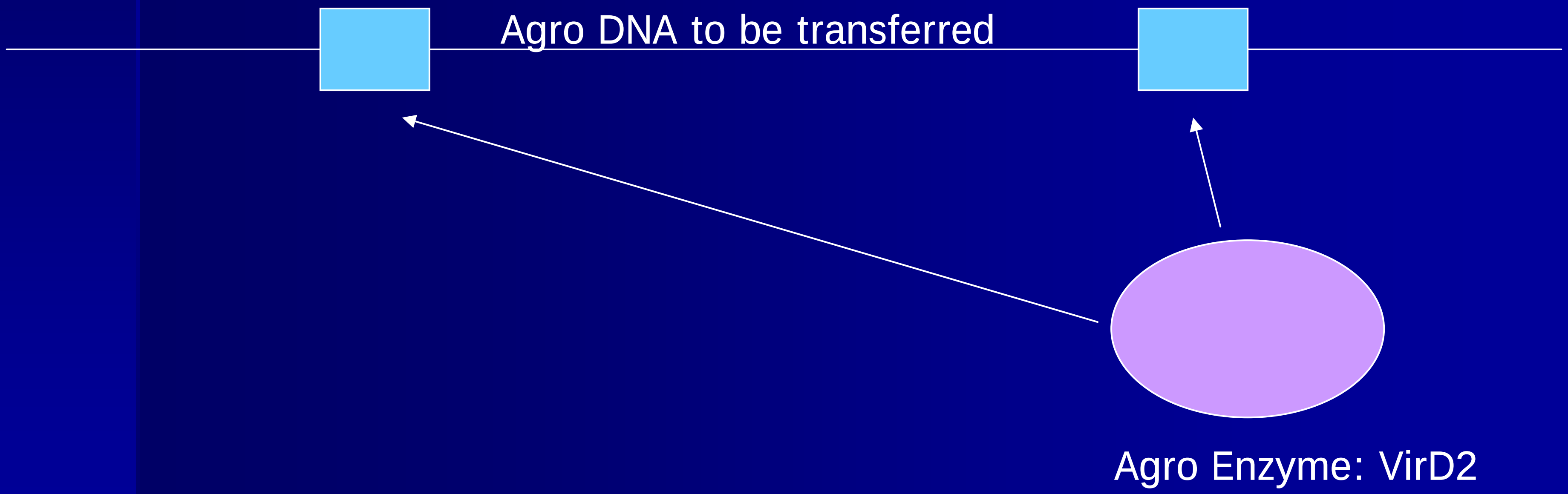
1. Step One: *Agrobacterium* detects a wounded plant cell



Step 2. Agrobacterium attaches to the plant cell



Step 3. Agrobacterium prepares a piece of DNA for transfer



Why does Agrobacterium do this?

1. Food
2. Safe home away from competitors

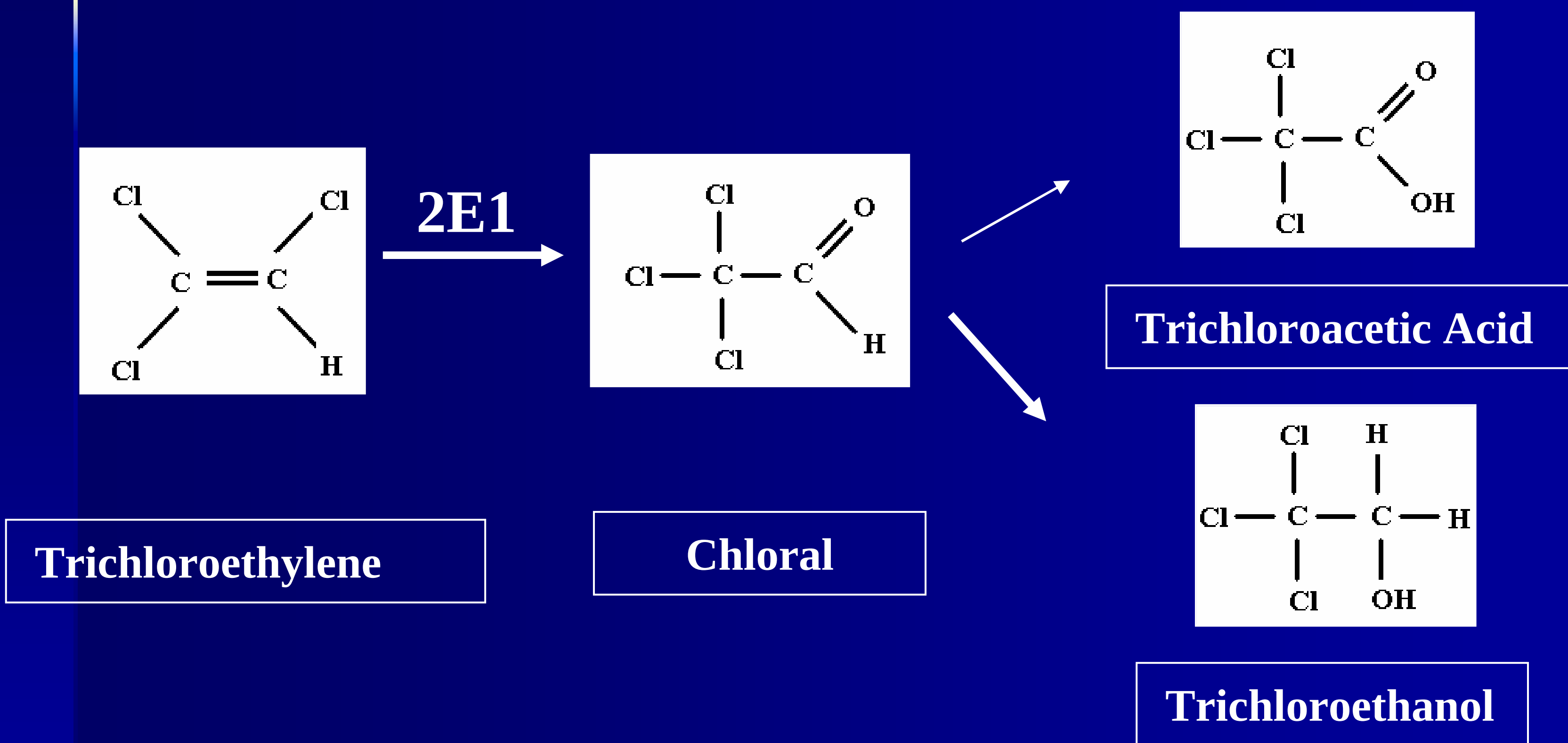
How Agrobacterium is used to transform plants

- 1. Remove the genes from Agrobacterium that cause the tumor and “Agro food” production
- 2. Replace them with “genes of interest”
- 3. Agrobacterium only “looks” at the border sequences, and transfers anything that is between them

Enhancing phytoremediation of small, volatile chemicals

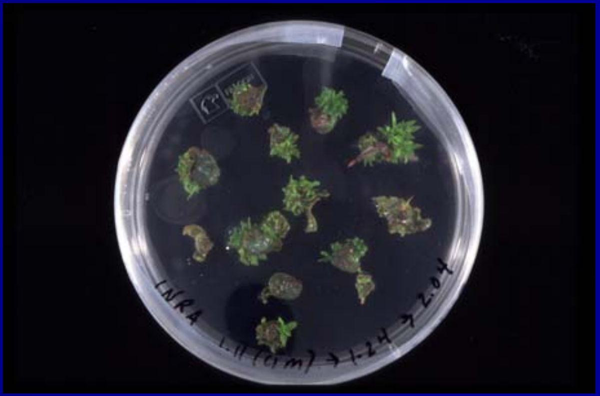
- Principal Investigator: Prof. Stuart Strand (Dept. of Civil and Environmental Engineering; CFR)
- Co-P.I.: Prof. Sharon Doty (CFR)
- Funding: NIEHS and DOE

Cytochrome P450 2E1 Catalyzes TCE Metabolism



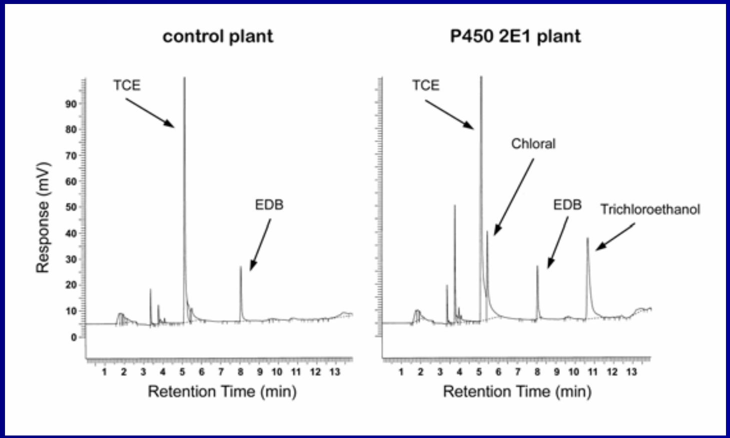
Transformation of Poplar using *Agrobacterium tumefaciens*

Agro is killed; hormones provided for plant regeneration →

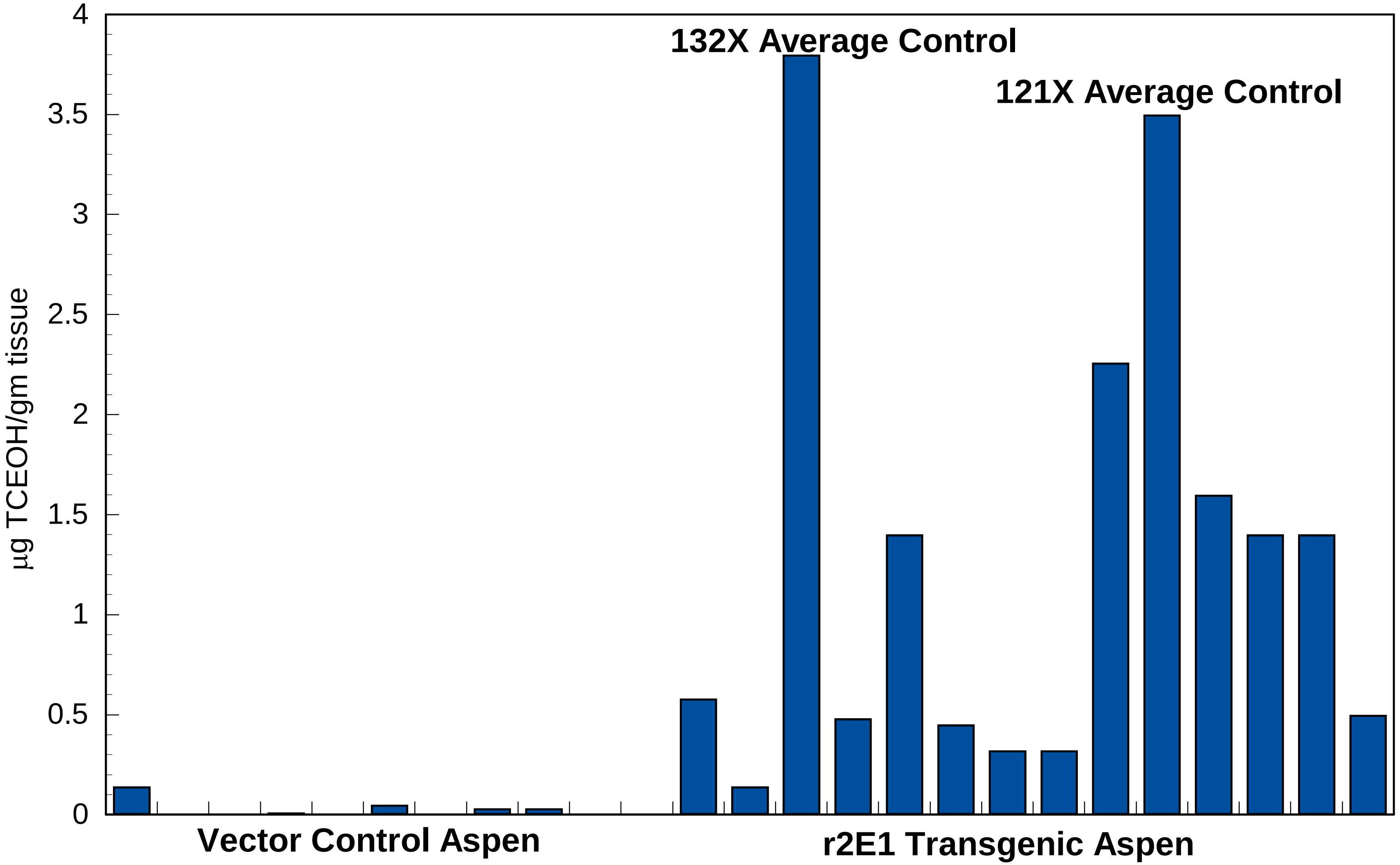


Plants are assayed for increased metabolism of pollutants

- The pollutant is added
- The concentration is monitored
- The metabolites are quantified by gas chromatography

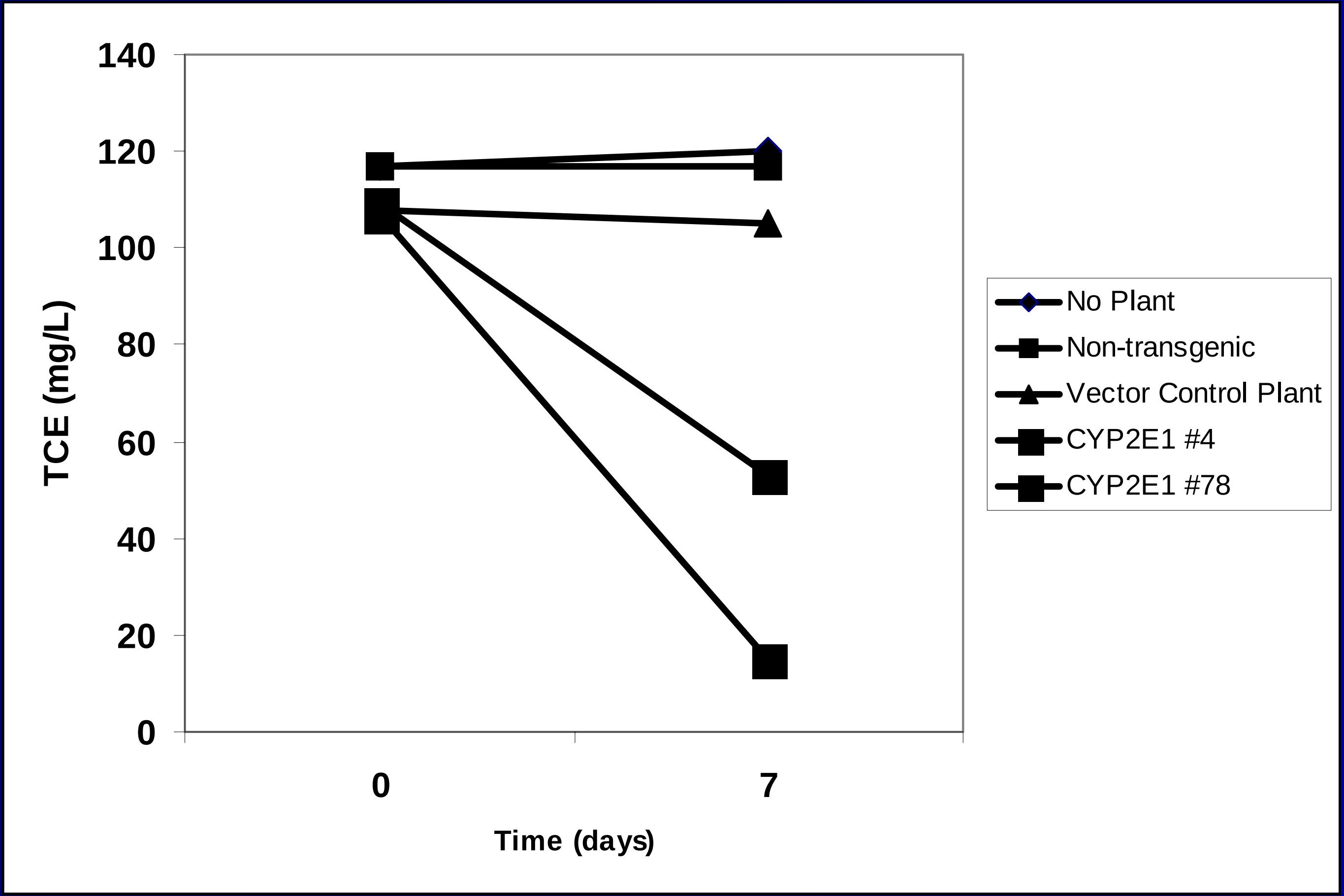


P450 2E1 Transgenic Plants Have Increased Metabolism of TCE



S. L. Doty, et al. (2007): Proc. Natl. Acad. Sci 104(43) 16816-16821

2E1 Transgenic Aspen Remove TCE from Solution at a Faster Rate



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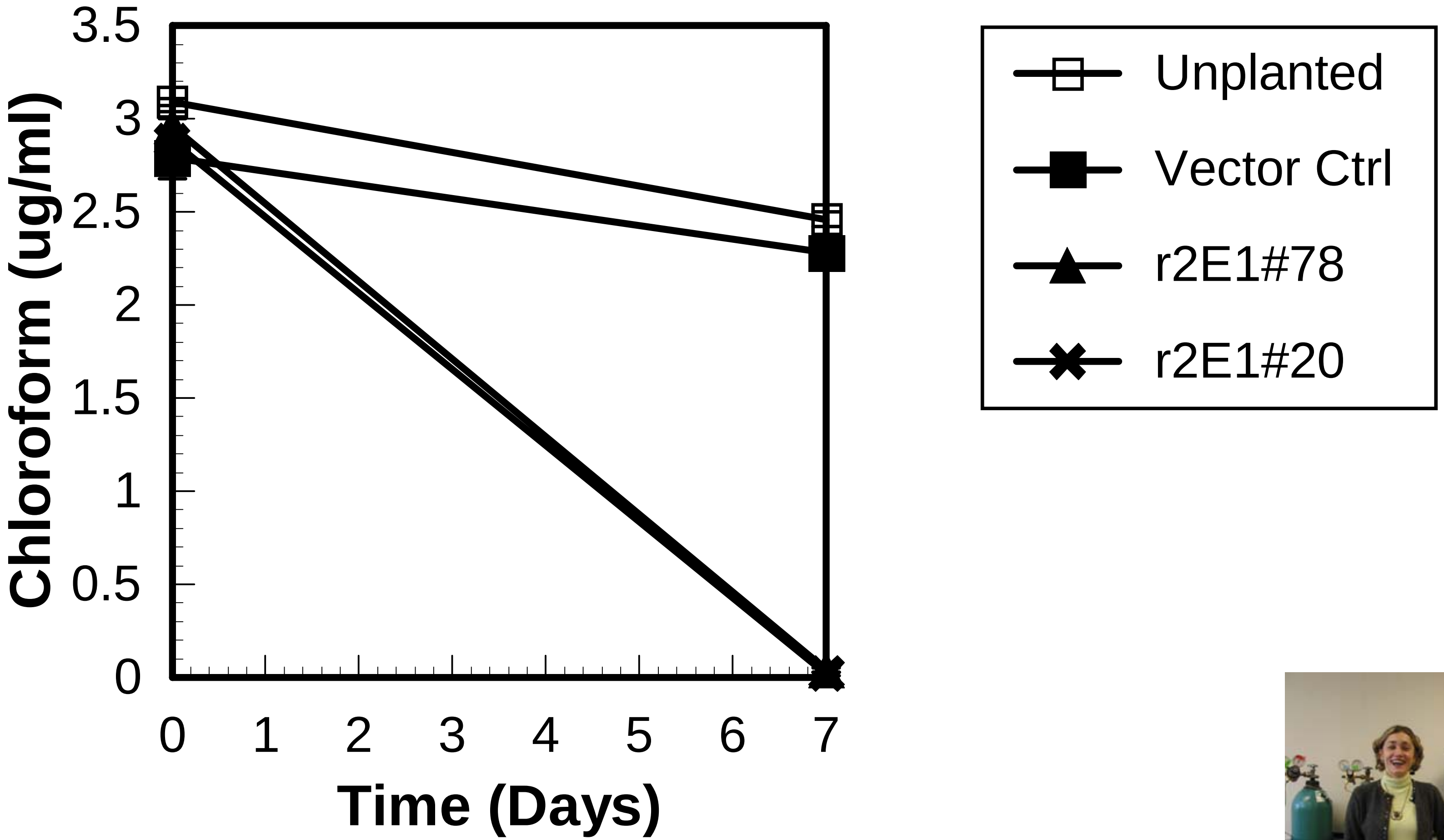
P450 2E1 Has Multiple Substrates

Chloroform
Carbon Tetrachloride
Vinyl Chloride
Benzene

Increased Removal of Chloroform from Solution by rCYP2E1 Transgenic Aspen



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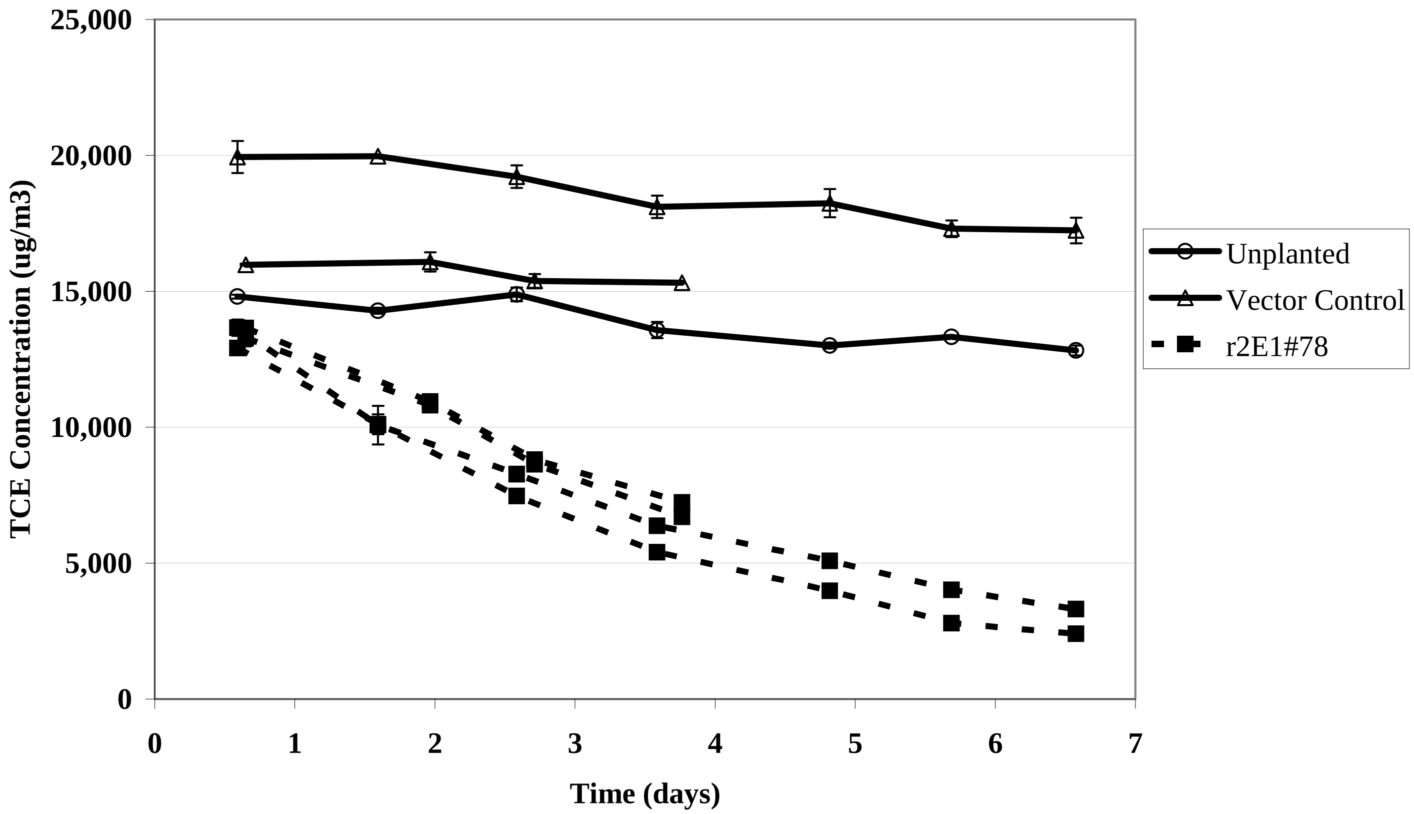
Increased removal rates of several important pollutants

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

Removal of pollutants from air

CYP2E1 aspen plants remove more TCE from air



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Increased removal of benzene from air

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

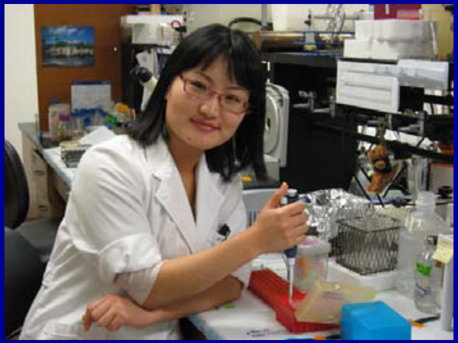
Identification of Plant Genes Involved in TCE Metabolism

Graduate students:

Jun Won Kang and

Keum Young Lee

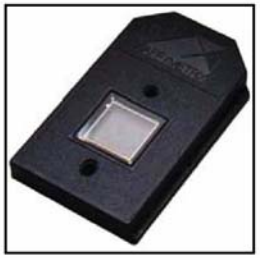
Funding: NIEHS

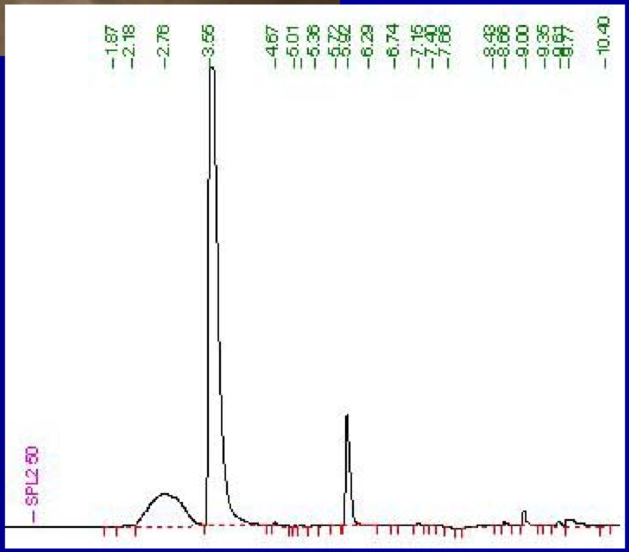


Approaches to identify plant genes involved in TCE metabolism

- Testing in yeast
  - 272 P450s in At
- Microarrays

[Affymetrix] Hybridization
Oligo "GeneChip" Array

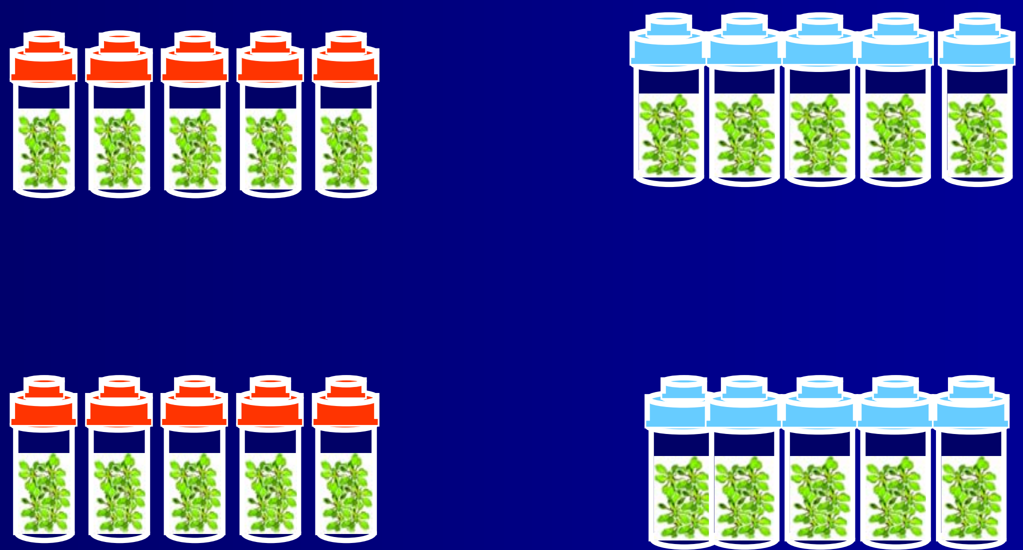




Which poplar genes are involved after the initial exposure to TCE?

Microarray -4 chips

50ug/ml TCE dosed **undosed**

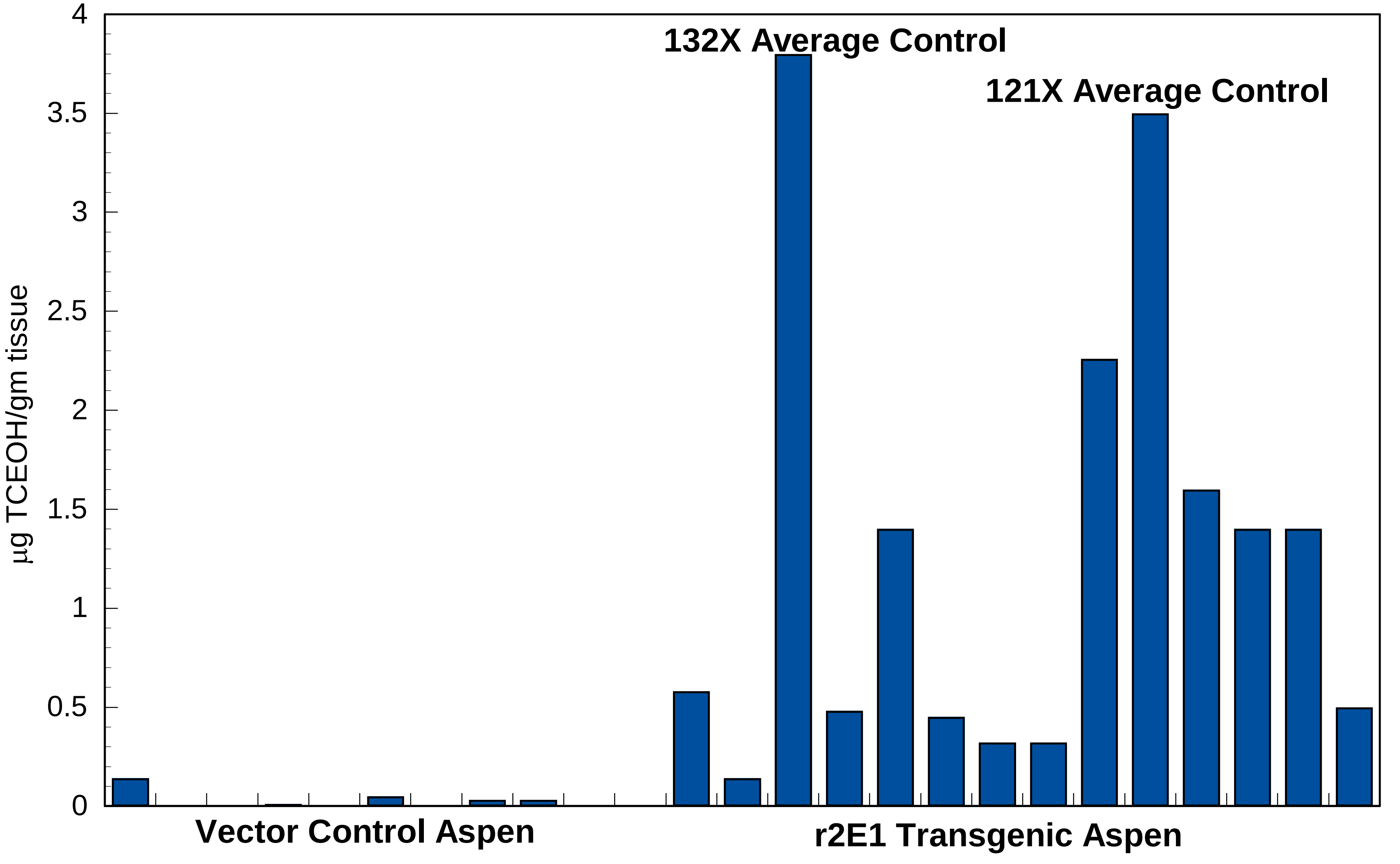


r2e1 #78

KH200 (vector control)

Slide by Jun Won Kang

P450 2E1 Transgenic Plants Have Increased Metabolism of TCE



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Phases of detoxification in plants

Phase I	**Transformation**	**Oxidation, Reduction or hydrolysis**	**Cytochrome P450 monooxygenases, Reductases, Dehalogenases**
Phase II	**Conjugation**	**With carbohydrates, Glutathione, Carboxylic acids**	**Glycosyltransferases (UGT) , Glutathione-S trasnferases (GST), Acyltransferases**
Phase III	**Compartmentation**	**into vacuole or apoplast**	**ATP binding cassette (ABC transporter)**

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	Function	Enzyme	KH200+TCE	#78+TCE
		Upregulated	68	472
Phase I	Transformation	Cytochrome P450, Reductases, Dehalogenases	2	5 5
Phase II	Conjugation	Glycosyltransferases (UGT) , Glutathione-S trasnferases (GST), Acyltransferases	1	8 11
Phase III	Compartmentation	ATP binding cassette (ABC transporter)	1	7
		Peroxidase Oxidase Oxidoreductase	3	4 6 7
		Unknown	…	…

Log FC >2

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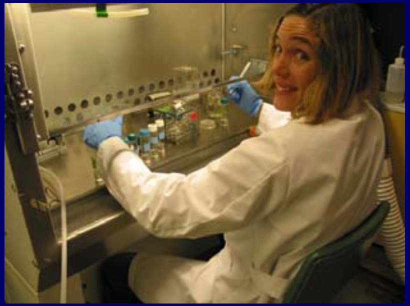
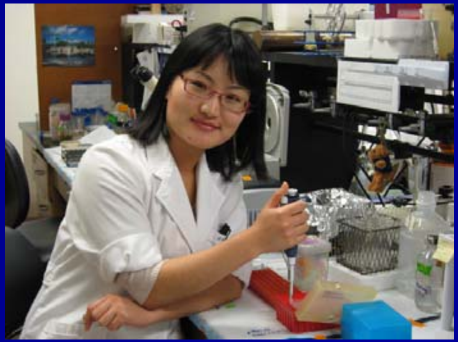
Summary

- Introduction of one gene can increase the removal rates of a whole class of serious environmental pollutants from both water and air.

- Research into the plant genes involved in pollutant metabolism is continuing

Acknowledgements





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