

Enhancing Phytoremediation and Plant Growth in Poplar and Willow

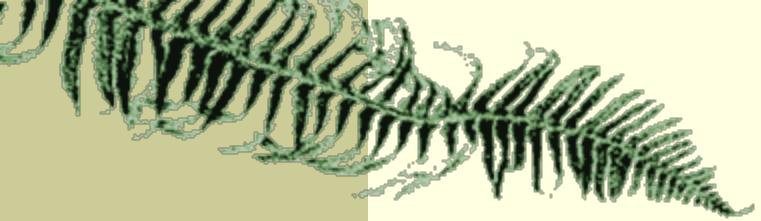


 UNIVERSITY OF
WASHINGTON

Sharon Doty
Assistant Professor
College of
Forest Resources

Why do we need to enhance phytoremediation?





Removal of Pollutants by CYP2E1 Transgenic Poplar



Funding: Superfund Basic Research
Program of NIEHS

P.I. Stuart Strand, UW

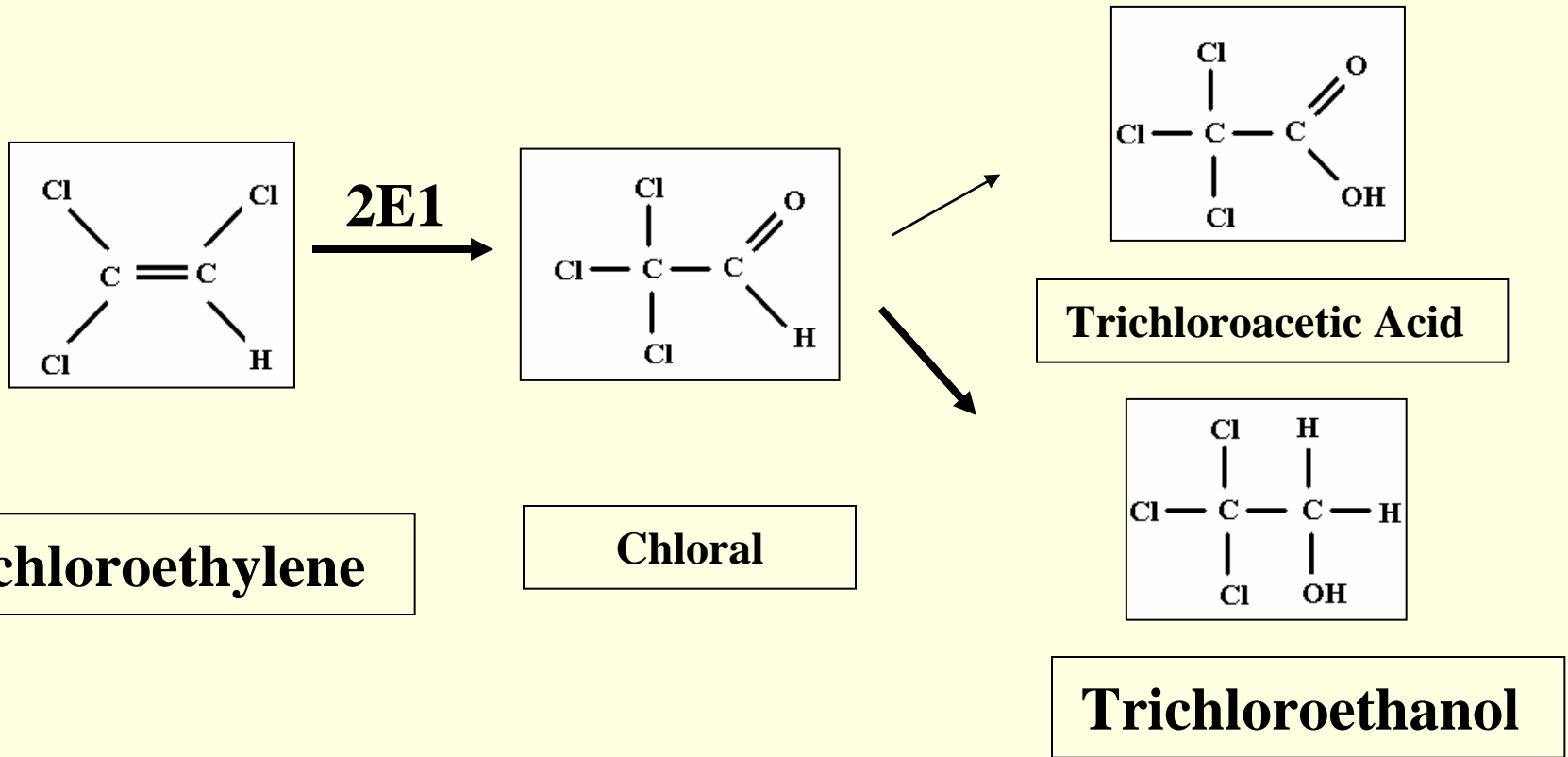
Co-P.I. Sharon Doty, UW

Trichloroethylene (TCE)

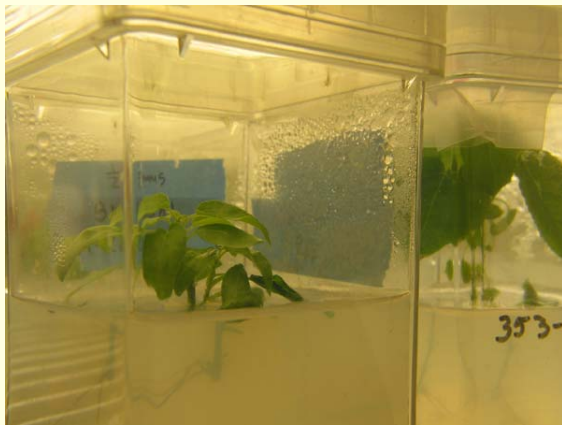


- Used for decades as metal degreaser, dry cleaning agent, solvent, and anesthetic
- Following use, it was dumped outside
- One of the most widespread contaminants in the environment (60% of SuperFund sites)
- Toxic to the liver, kidney, CNS, and likely carcinogenic
- Persistent in the environment

Mammalian Cytochrome P450 2E1 Catalyzes TCE Metabolism



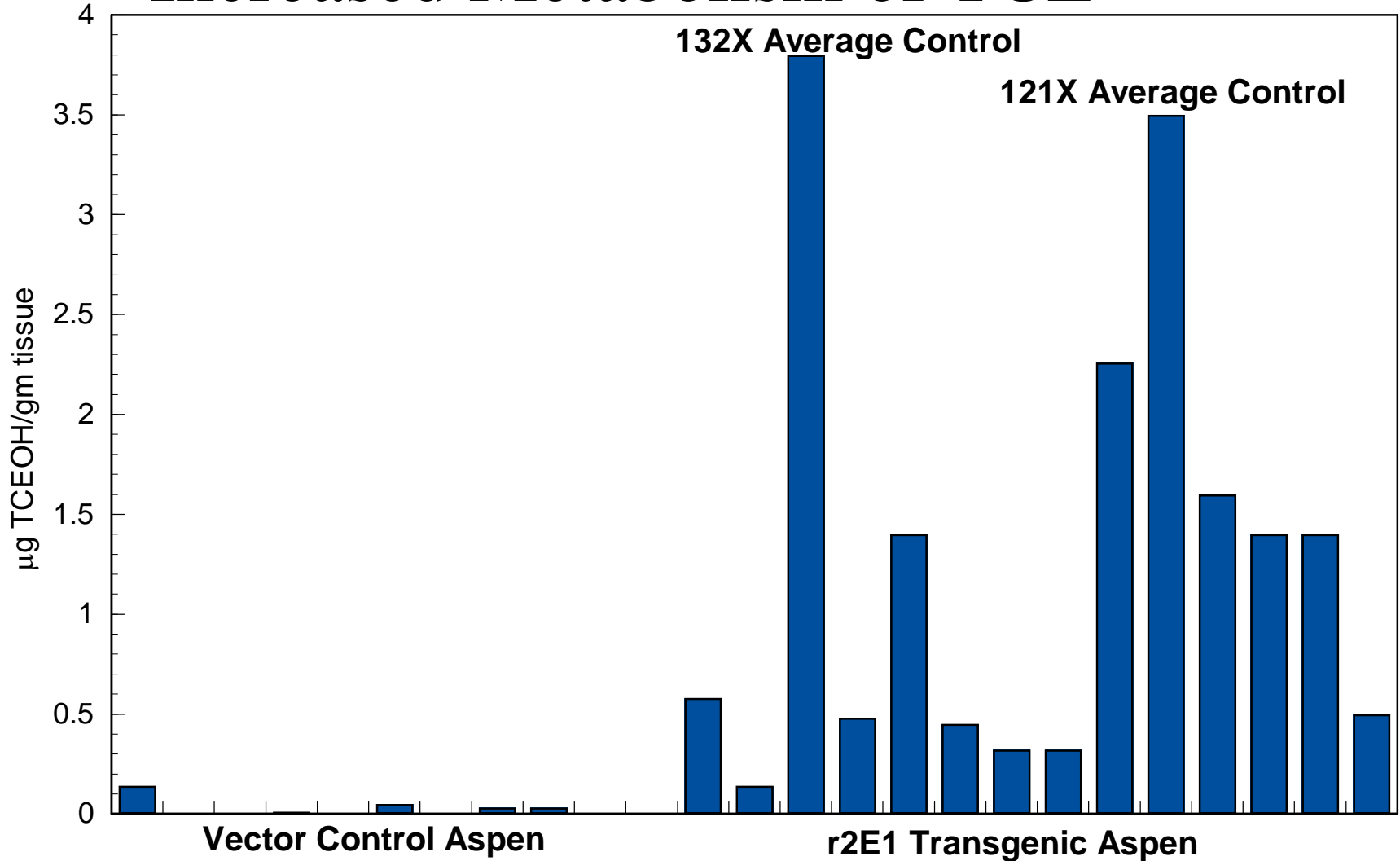
Transformation of Poplar (*P. tremula* x *alba* N717-1B4) using *A. tumefaciens*



Transgenic plants were assayed for increased metabolism of pollutants



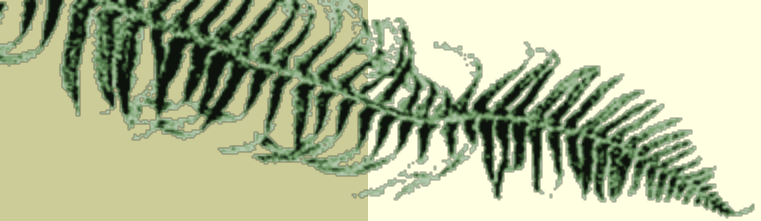
CYP2E1 Transgenic Poplar Had Increased Metabolism of TCE



CYP2E1 transgenic poplar removed TCE at a faster rate

<u>Transgenic Plant</u>	<u>% Removal</u>	<u>Rate *</u>
No plant control	0.8 +/- 1.1	0.1 +/- 0.1
Vector Control	2.6 +/- 0.3	0.4 +/- 2.8
CYP2E1 #78	86.9 +/- 11.4	20.3 +/- 4.6

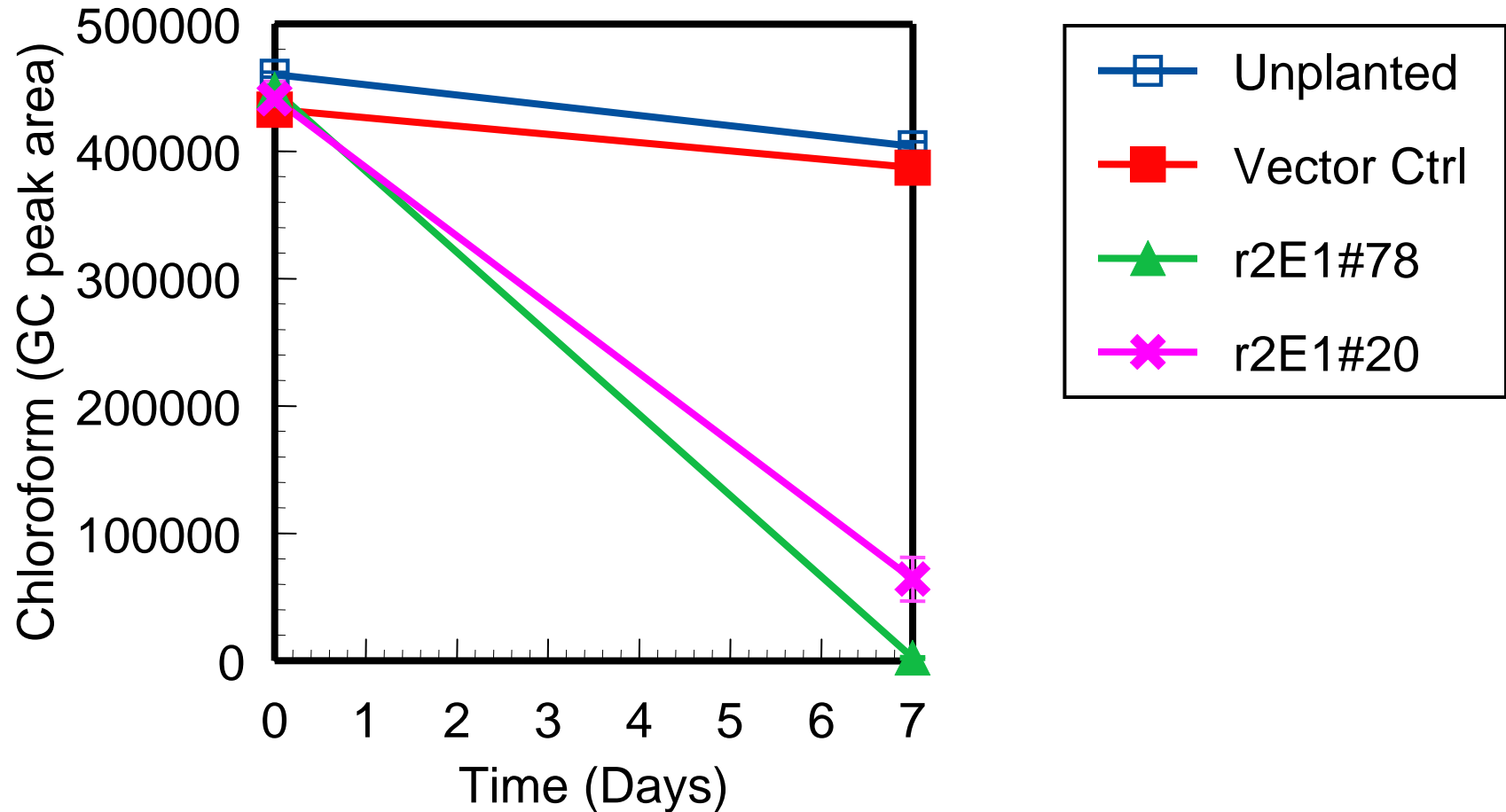
Rate: ug TCE/day*gm fresh weight



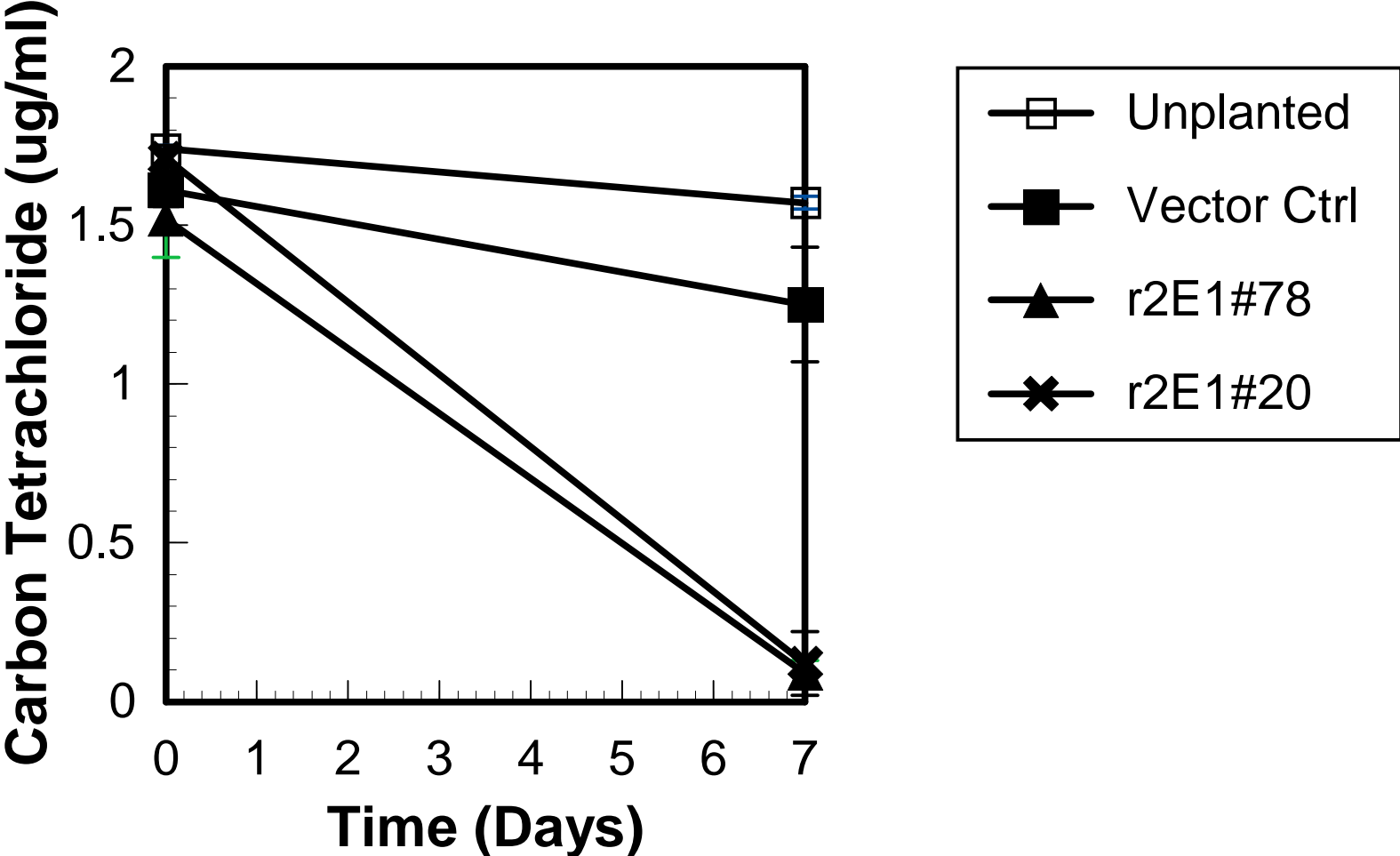
Cytochrome P450 2E1 Has Multiple Substrates

Chloroform
Carbon Tetrachloride
Vinyl Chloride
Benzene

Increased Removal of Chloroform from Solution by CYP2E1 Transgenic Poplar



Increased Removal of Carbon Tetrachloride from Solution By CYP2E1 Transgenic Poplar



CYP2E1 Transgenic Poplar Removed More Pollutants from Air

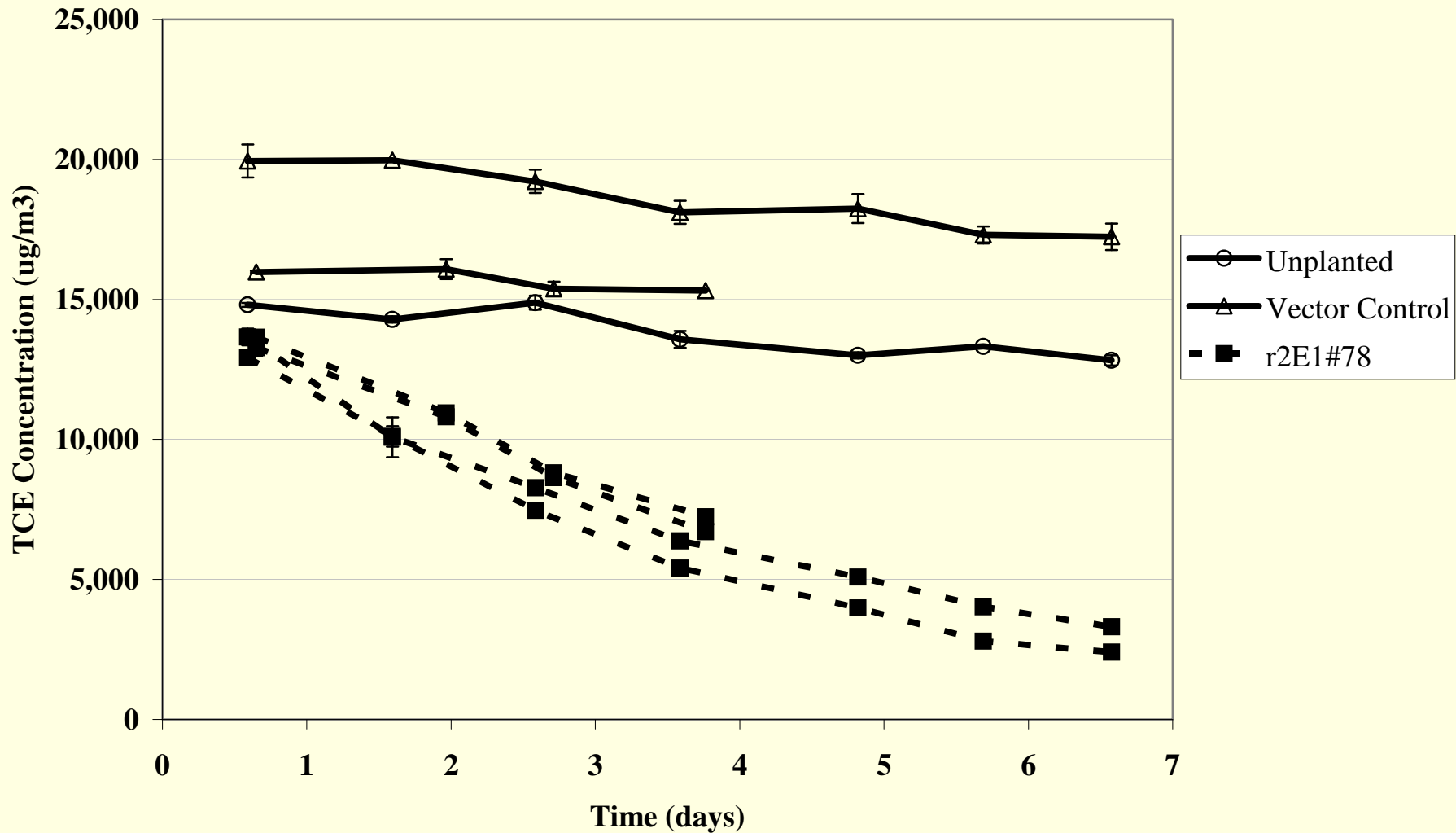


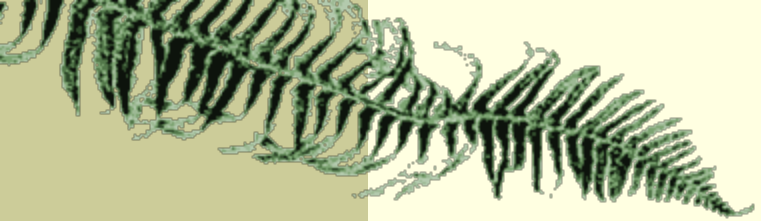
TCE

Benzene

Vinyl Chloride

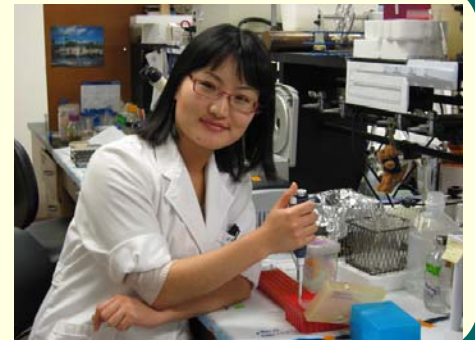
CYP2E1 Poplar Plants Removed TCE from Air At A Faster Rate



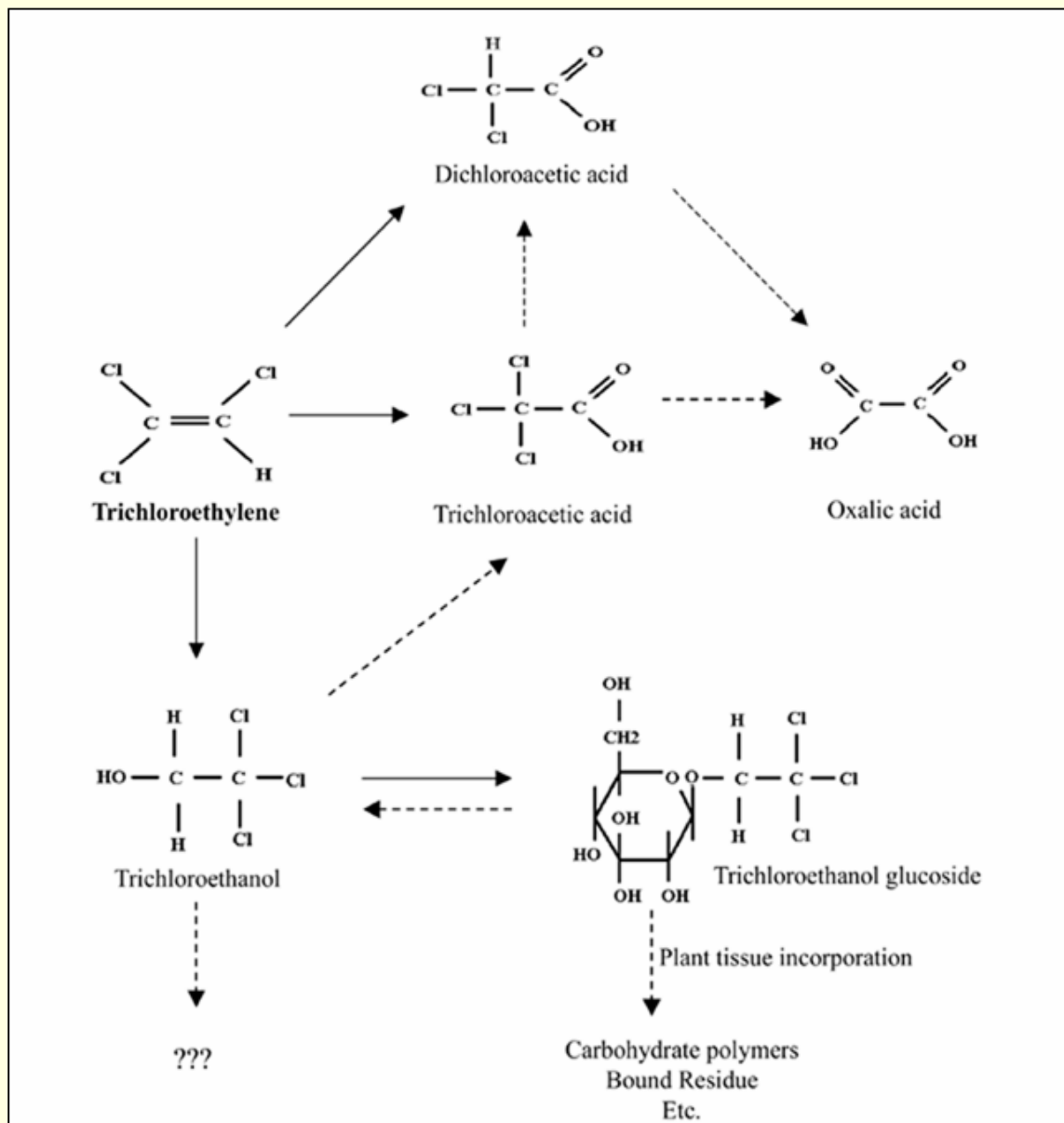


Identification of Plant Genes Involved in TCE Metabolism

[Affymetrix] Hybridization
Oligo "GeneChip" Array



Proposed Fate of TCE in Plants



Shang, T. Q., et al. (2001) *Phytochemistry* 58:1055-1065.

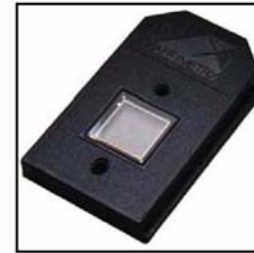
Approaches to identify plant genes involved in TCE metabolism

- Poplar microarrays (Dr. Fred Farin, UW)
- Arabidopsis microarrays (Dr. Mary Schuler, UI)

TCE dosed Un-dosed

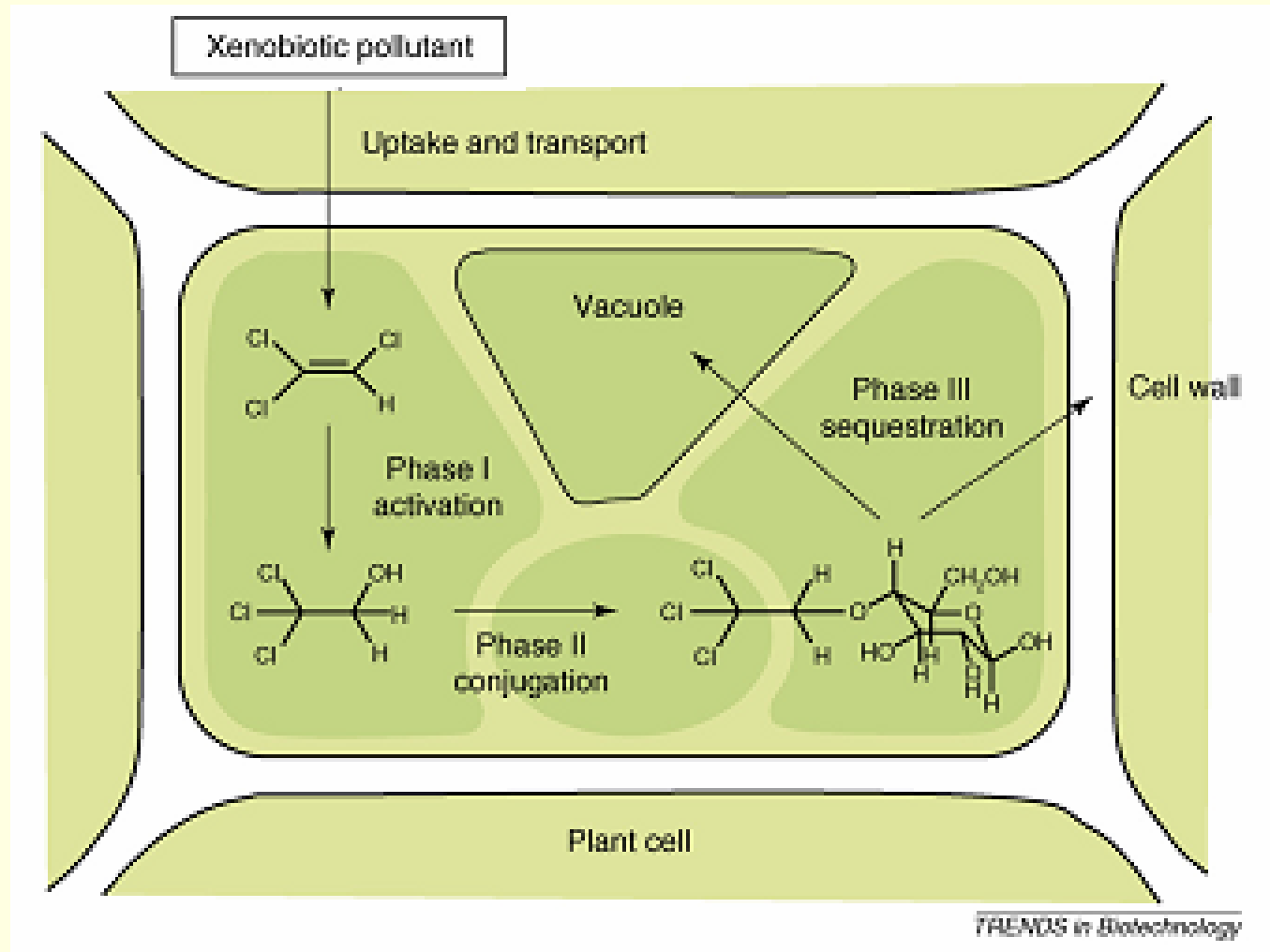


[Affymetrix] Hybridization Oligo "GeneChip" Array



Test candidate genes in yeast

Phases of detoxification in plants



(Benoit Van Aken, 2008 Trends in Biotechnology 26(5):225-227)

Microarray Results: Vector Control vs CYP2E1 Poplar Genes Induced by TCE Exposure

	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 transferases (GST), Acyltransferases	1	8 11
Phase III	Compartment- ation	ATP binding cassette (ABC transporter)	1	7
		Peroxidase Oxidase Oxidoreductase	3	4 6 7
		Unknown

Log FC >2

Current Research

- Field testing of transgenic poplar (NIEHS funded)
- Analysis of plant genes involved in TCE metabolism (NIEHS funded)
- Developing transformation protocols for willow (CPBR funded)



Summary of Transgenic Phytoremediation

- Expression of mammalian P450 2E1 gene in transgenic poplar led to a strong increase in TCE metabolism and removal of TCE (53X), and a strong increase in removal of chloroform (9X), carbon tetrachloride, vinyl chloride (3X) and benzene (10X).
- Analysis of upregulated genes in response to TCE in poplar revealed many genes known to be involved in detoxification of pollutants. Analysis of these genes may lead to improved phytoremediation.

*Enhancing Plant Growth
using Nitrogen-Fixing Endophytes of
Poplar and Willow*



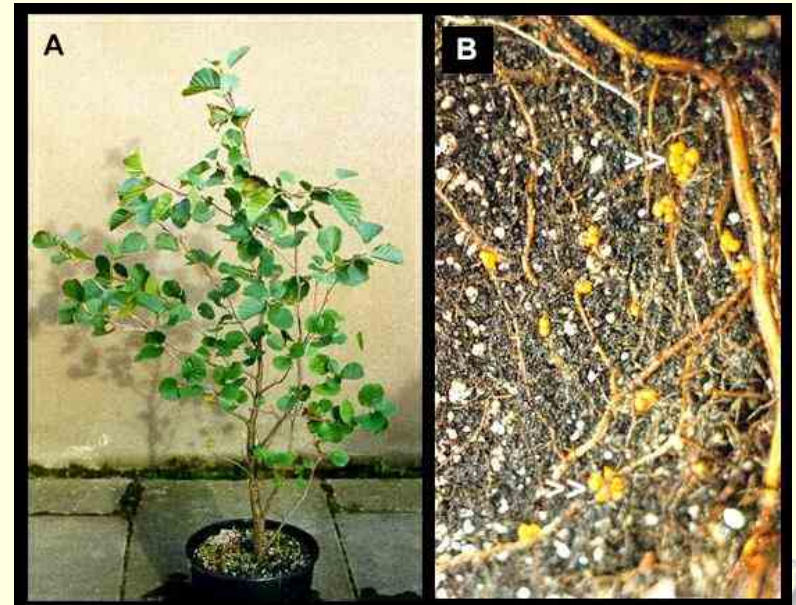
Contaminated areas often have poor soil

- Abandoned, polluted commercial properties
- Phytoremediation depends on good plant growth



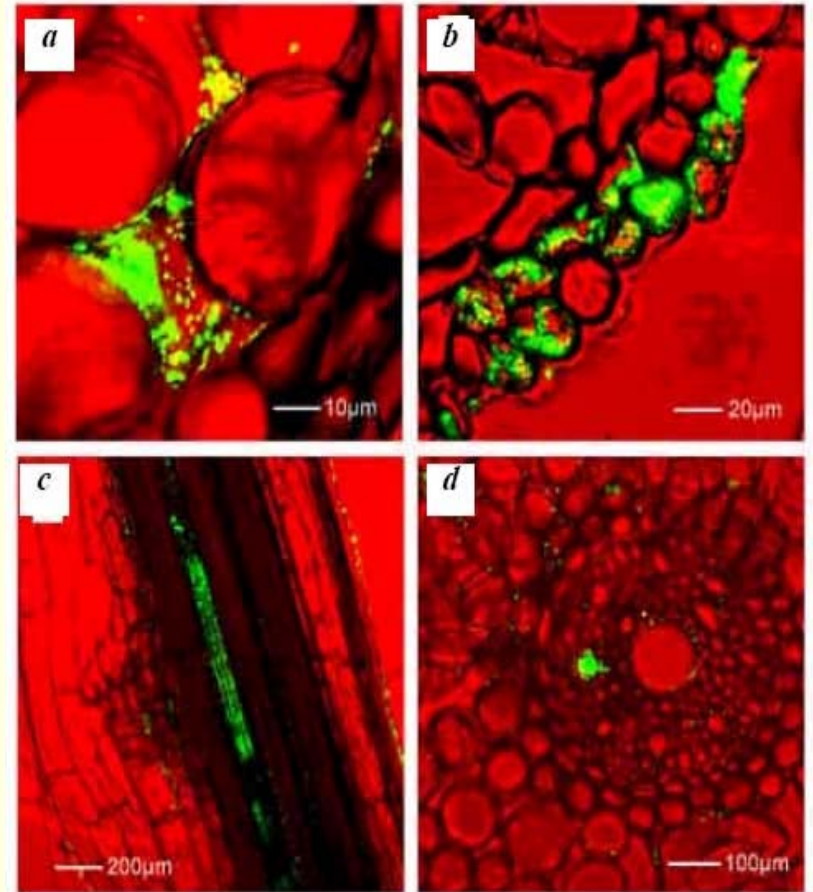
Nitrogen fixation in root nodules

- Chemical fertilizers are petroleum-based; getting costlier
- Some microbes convert N_2 gas to ammonia
- Nitrogen fixation occurs within root nodules of only some plant species



Endophytes

- Microorganisms that live within plants without causing disease
- Increased nutrient acquisition, pathogen resistance, stress tolerance



Nitrogen-fixing endophytes of poplar and willow



Doty, et al. (2005) *Symbiosis* 39: 27-35

Doty, et al. (2009) *Symbiosis* 47:23-33



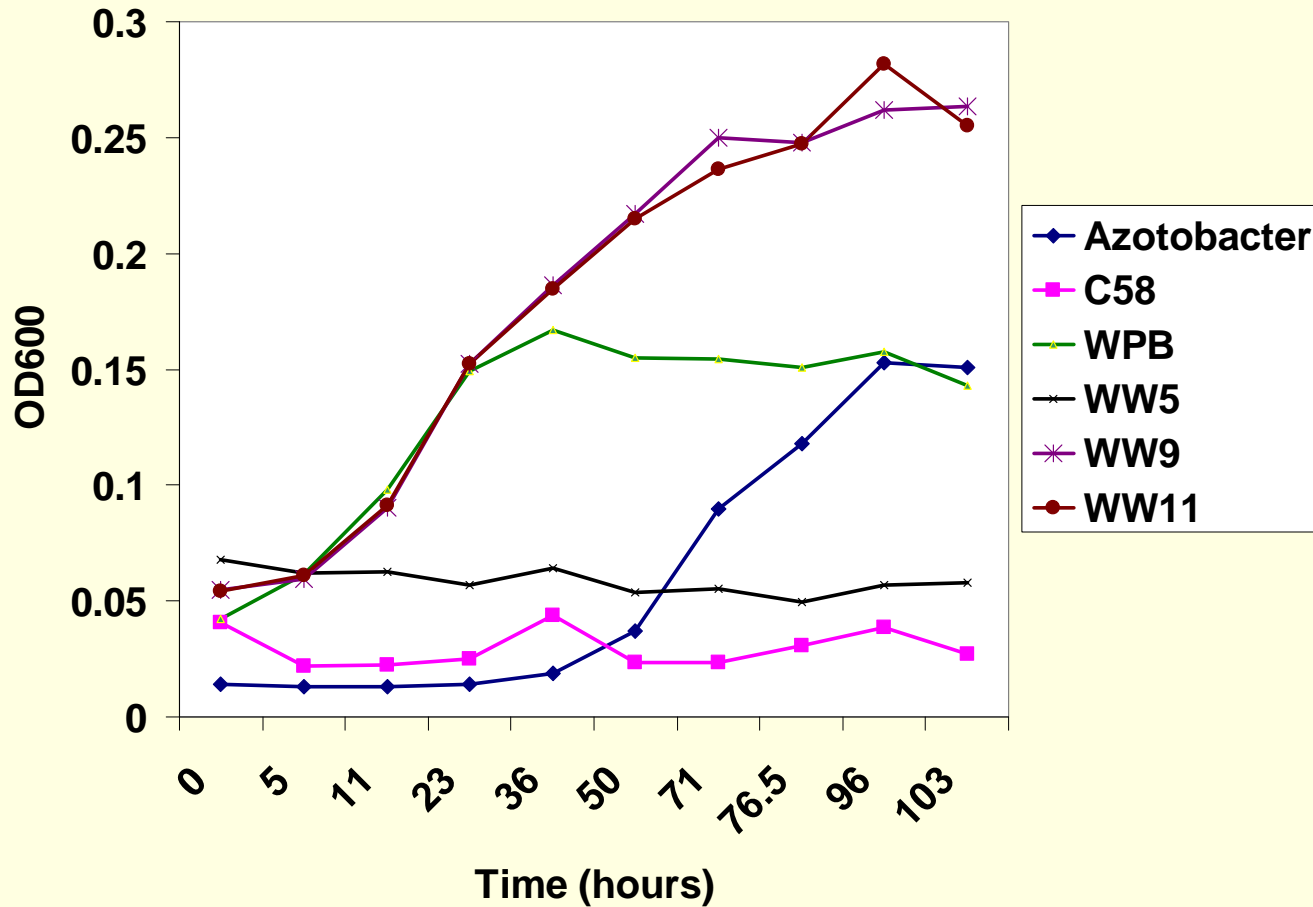
In wild settings, poplar and willow grow under low-nitrogen conditions



Isolated
endophytes;
identified using
16S rRNA gene
sequencing

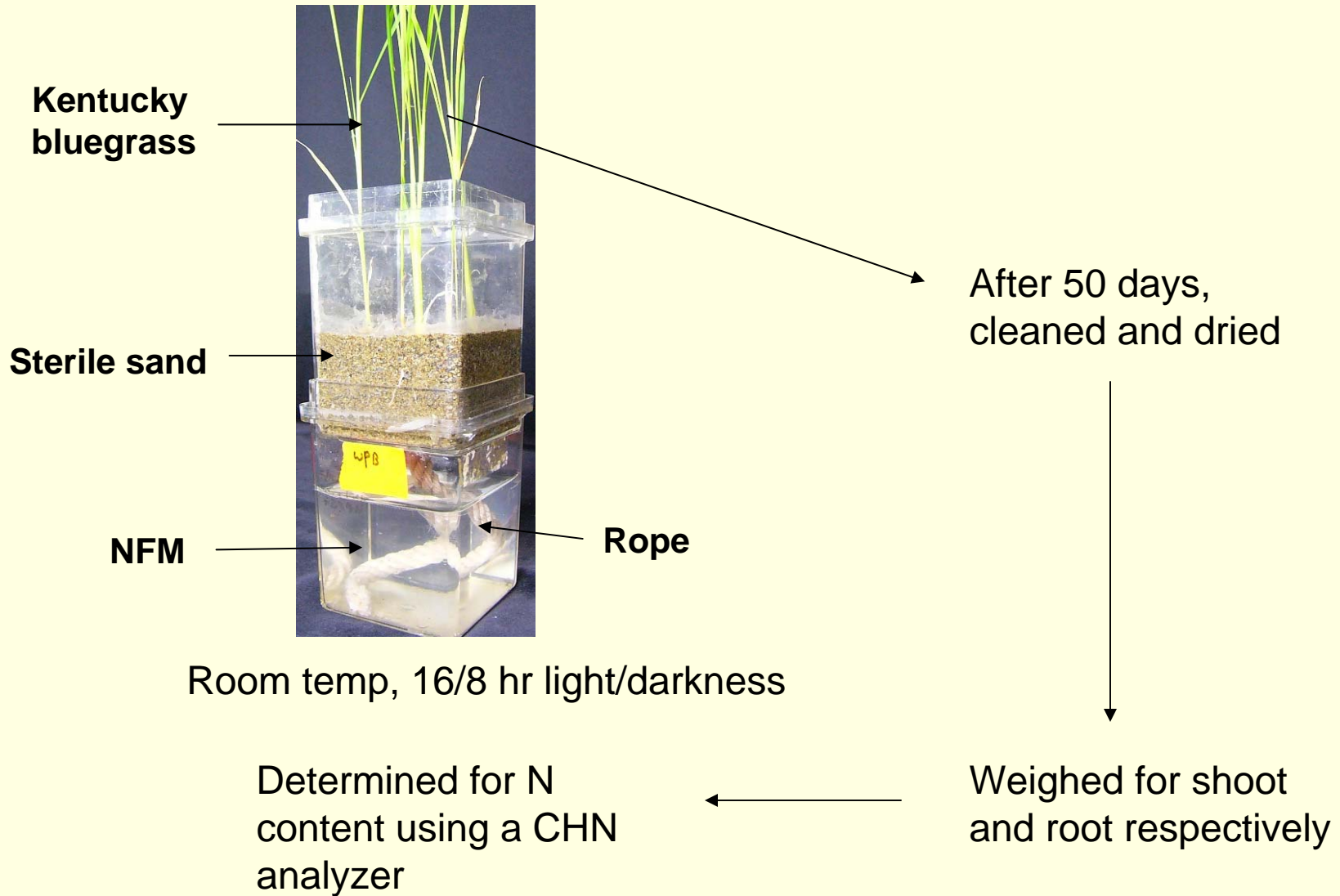


Some poplar and willow endophytes grow well in medium without ammonium or nitrate



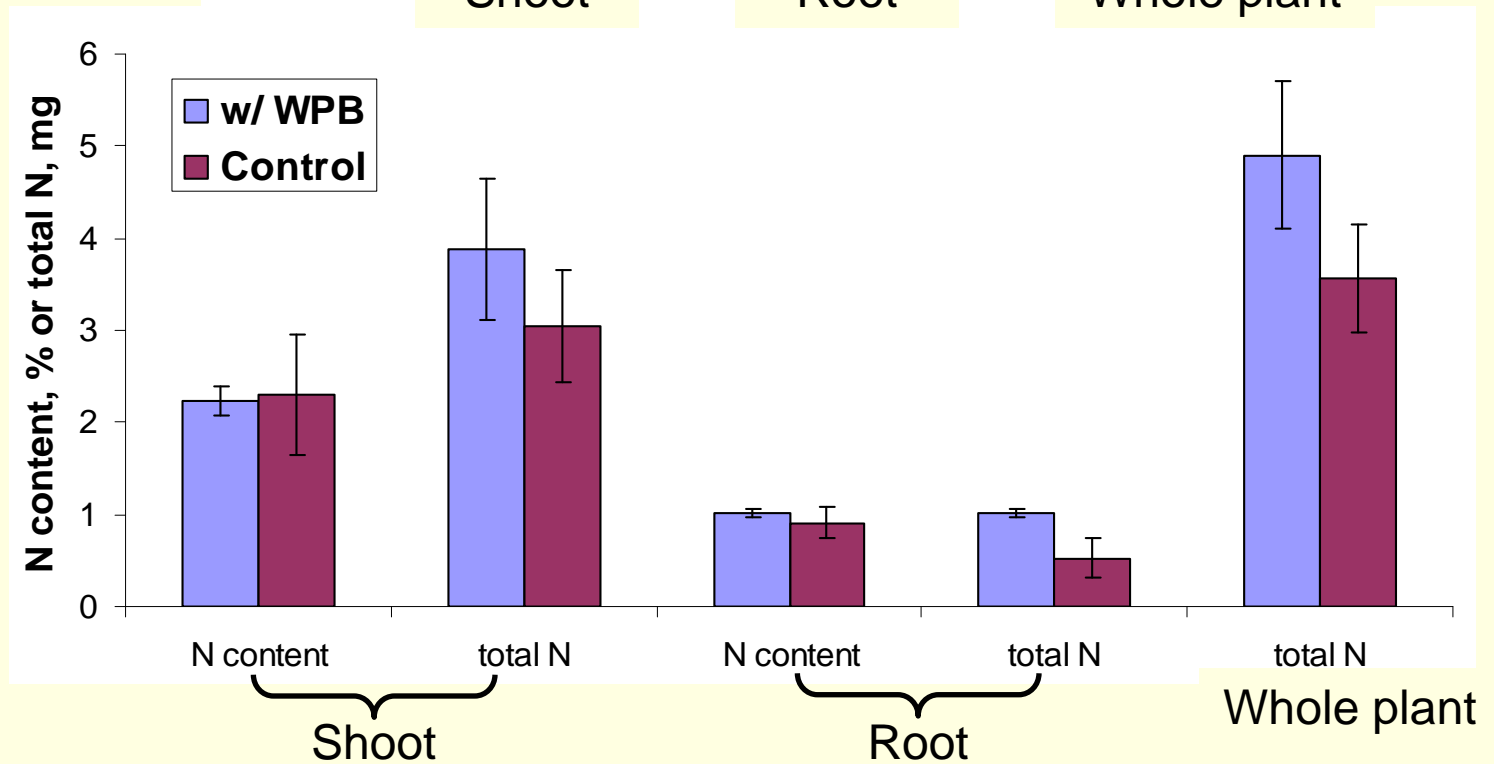
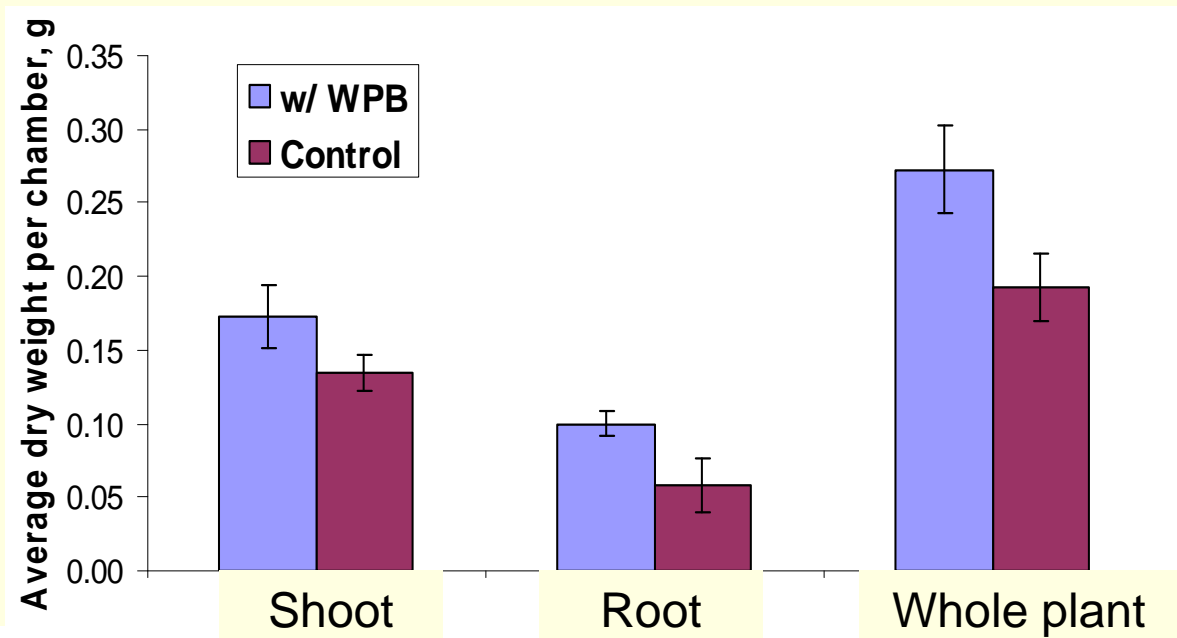
*Plant Growth Promotion by
Endophytes of Poplar*

Grass growth promotion assay



**42% biomass
increase with
WPB inoculation**

**37% total N
increase with
WPB inoculation**



WPB enhances the growth of rice under nitrogen limitation



Expt. Performed in Dr. Rodriguez's lab

Summary of Endophyte Characterization

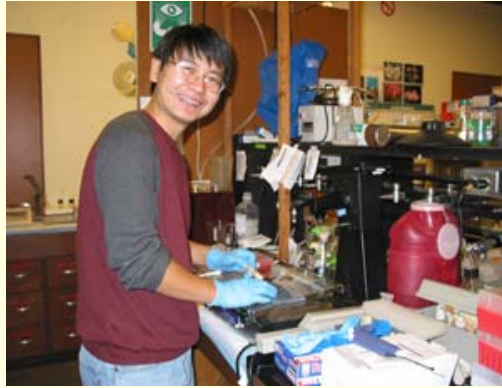
- Initially focused on Burkholderia strain, WPB.
- WPB shares high *nifHDK* gene similarity with known *B. vietnamiensis* strains.
- High nitrogenase activity was determined using both the $^{15}\text{N}_2$ incorporation assay and the Acetylene Reduction Assay.
- WPB can significantly promote growth of Kentucky bluegrass, crabgrass, and rice. Similar experiments with poplar and corn are underway.

Current Research

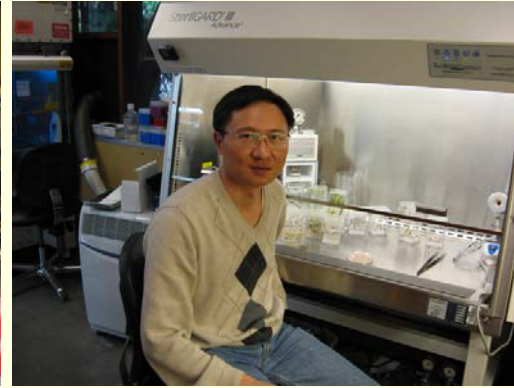
- Propagation of internally-sterile poplar
 - Growth effects
 - Microarrays
- Analysis of willow endophytes



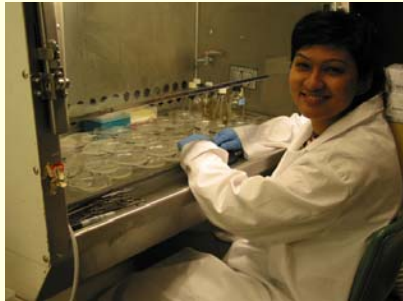
Acknowledgements for Endophyte Research



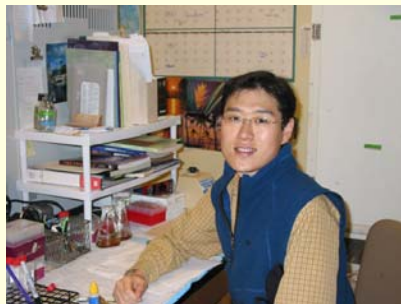
Gang Xin:
WPB studies



Gengyun (George)
Zhang: Turfgrass
studies



Helped with acetylene reduction assays or strain identification: Z. Khan, A. Vajzovic, G. Singleton, and J. W. Kang



Rice studies:

**Dr. Regina Redman and Dr.
Rusty Rodriguez**

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