NIEHS
National Institute of Environmental Health Sciences

RISK eLearning

Phytoremediation

November 12th, 2008 Session 2:
"Phytoremediation of Organics"

Ari Ferro, Principal Environmental Scientist, URS Corporation,
Phytoremediation of Groundwater

Stuart Strand, SBRP-University of Washington,
Progress in Transgenic Plants for Degradation of Organic Pollutants,
Mammalian P450 2E1 in Plants

sbrp Superfund Basic Research Program
Balancing Scientific Excellence with Research Relevance

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Services

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– **Presentation for Risk e Learning Web Seminar**

– **Ari M. Ferro, PhD, URS Corporation**

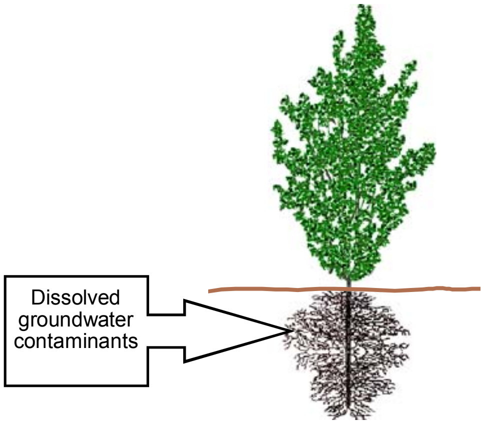
– **November 12, 2008**

Phytoremediation: **The use of plants to remove or stabilize contaminants in soils, wastewater streams, or groundwater**

Phytoremediation of Groundwater:

- Plants: trees
- Contaminants: dissolved organic chemicals, such as, petroleum hydrocarbons, chlorinated solvents, 1,4 - dioxane

Phytoremediation of Groundwater Containing Dissolved Organic Chemical Contaminants



- Contaminants are removed via various phytoremediation processes
- The trees use the water via transpiration

Phytoremediation of Contaminated Groundwater: Outline of Presentation

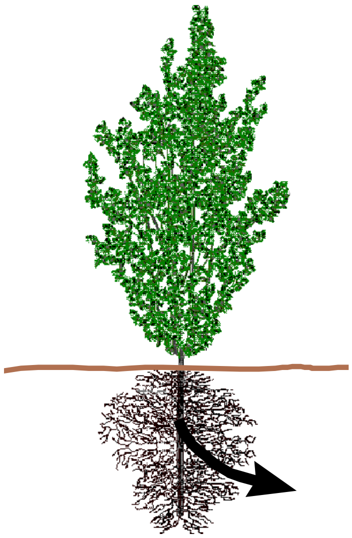
- Phytoremediation processes that enhance the rate of contaminant removal
- Estimating and measuring rates of water use for tree stands
- Three common applications of the technology (and case studies)
  - Biological “pumping and treatment” (Southington, CT)
  - Irrigation with recovered groundwater (High Point, NC)
  - Hydraulic control of groundwater contaminant plumes (Raleigh, NC)

Phytoremediation Processes that Enhance the Rate of Contaminant Removal

- Rhizosphere degradation
- Plant uptake
  - Plant metabolism
  - Phytovolatilization
- Immobilization in root-zone

Contaminant Removal Processes

Rhizosphere: Zone of Soil Influenced by Plant Roots



- Plant root exudates – a food source for microbes
  - sugars, organic acids, nucleotides, flavonoids, enzymes
  - sloughed-off cells, mucilagenous material
- General increase in microbial cell numbers
  - 100 to 1000-fold greater than bulk soil
  - mycorrhizal fungi
- Diverse species of metabolically active microbes brought together at high population density

Contaminant Removal Processes

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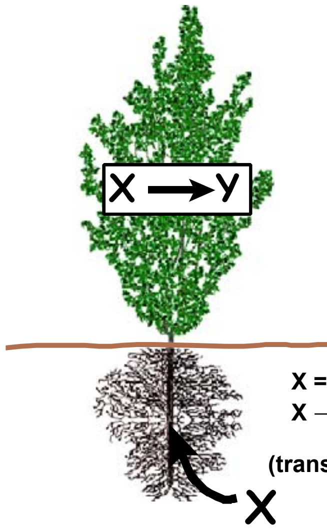
Plant Uptake and Metabolism of Organic Contaminants



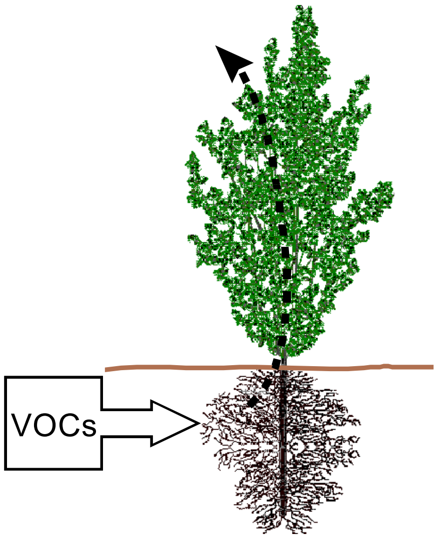
- Plants take up moderately hydrophobic compounds
- Plants contain enzymes which metabolize a wide range of organic chemical contaminants

X = contaminant
X → y = plant metabolism

(transformation, conjugation, and compartmentation)

Contaminant Removal Processes

Phytovolatilization



- Plant uptake of volatile organic compound, translocation to shoots, and exit to atmosphere
- 1,4 - Dioxane is one compound for which phytovolatilization is very effective
- Phytovolatilization of dioxane will be discussed in Case Study 2 (High Point, NC)

Contaminant Immobilization in the Root-zone

- Sorption of hydrophobic contaminants to plant roots (“phytostabilization”)

- Formation of “bound residues” in plant roots
  - plant uptake, followed by enzymatic transformation
  - metabolite is covalently bound to lignin

- “Humification” – formation of covalent (non-extractable) complexes with humus
  - for example, nucleophilic addition of aromatic amines to quinoidal sites in humus
  - one main source of humic material is microbial transformation of dead plant roots

Estimating the Rate of Water use for a Tree Stand

$$V_T = ET_O \cdot \theta \cdot LAI \cdot A$$

V_T = volumetric rate of water use by the stand

ET_O = reference evapotranspiration: rate of transpiration by a well-watered 15-cm tall fescue turf

θ = water use multiplier for the trees within the stand: rate of water use per unit leaf area as a percentage of ET_O

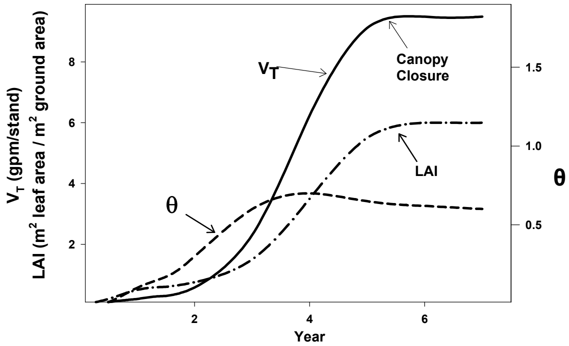
LAI = leaf area index: the leaf area per unit area of ground surface

A = area of the stand

Estimating Water Use

Parameters that Effect V_T

$$V_T = ET_O \cdot \theta \cdot LAI \cdot A$$



Parameters for a densely-planted 0.8 acre stand of willows at the SRSNE Superfund Site
(Case Study 1)

- V_T increases as the stand matures
- Maximum V_T occurs when the canopy closes
- Time required for canopy closure depends on species and planting density
- θ factor depends on tree species and plant stress

Measuring Rates of Water Use

TDPs used to measure water use on a tree at the SRSNE site (*Case Study 1*)

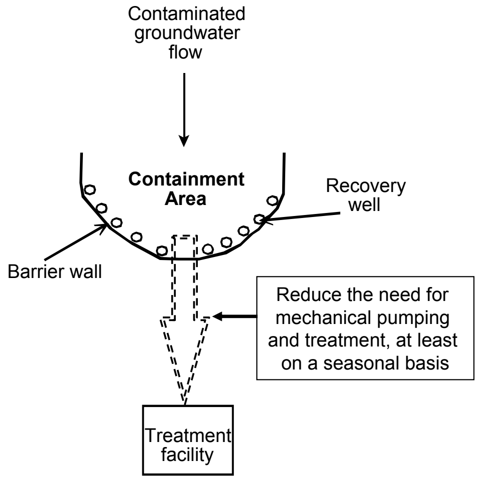
- Thermal dissipation probes (TDPs) are used to measure sap velocity (cm/h)
  - Two needle-like sensors are inserted into holes drilled in the xylem
  - Upper needle is heated, and the temperature difference between the two needles (ΔT) is measured
  - When sap velocity is high, heat in the upper needle is dissipated, and ΔT is reduced
- Values for ΔT and sap velocity are empirically related (Granier, 1985)
- The product of sap velocity (cm/h) and cross sectional area of the sapwood (cm^2) yields sap flow (cm^3/h)

Three Common Applications of the Technology

- Biological "pumping and treatment" system
- Irrigation of tree stands with recovered groundwater
- Hydraulic control of groundwater contaminant plumes using stands of deep-rooted trees

Technology Applications

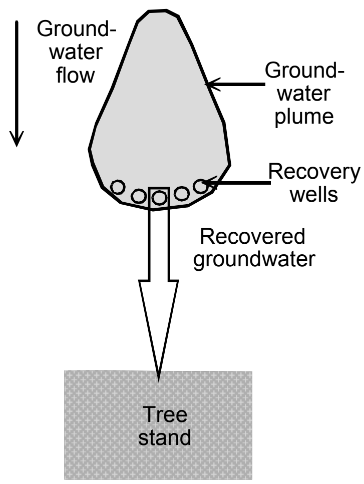
Biological "Pumping and Treatment" System



- Tree stand established in a containment area
- Water is used by trees; contaminants removed by various phytoremediation processes
- Example: SRSNE Site (*Case Study 1*)

Technology Applications

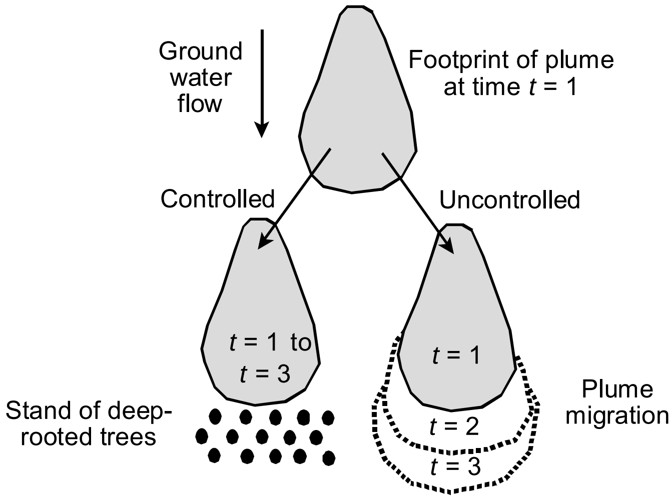
Irrigation with Recovered Groundwater



- Mechanical groundwater recovery wells hydraulically control plume migration
- Recovered groundwater used to irrigate a tree stand
  - Trees use the water
  - Contaminants removed by various phytoremediation processes
- Example: High Point, NC Site (*Case Study 2*)

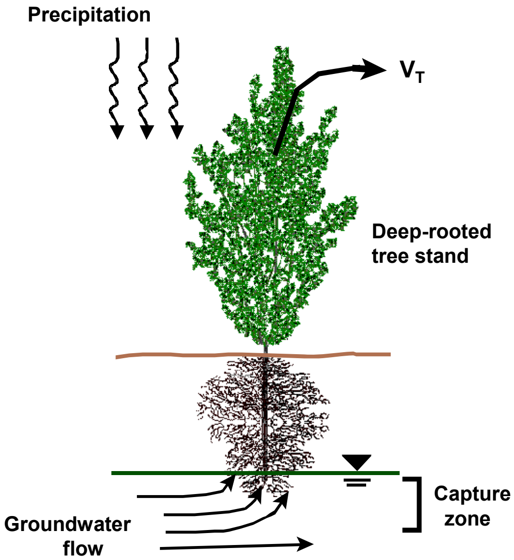
Technology Applications

Hydraulic Control of Groundwater Contaminant Plumes Using Deep-rooted Trees



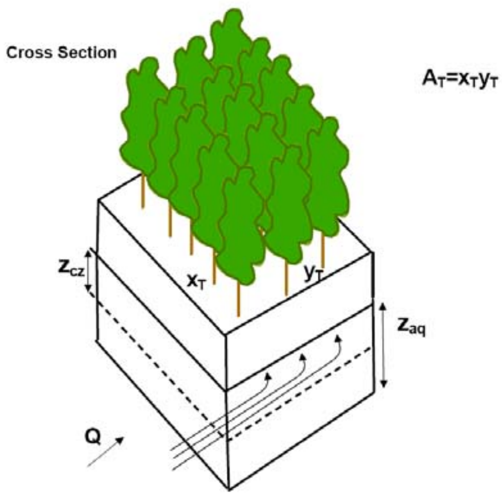
Example: Raleigh, NC Site *(Case Study 3)*

A Stand of Deep Rooted Trees Can Create a Capture Zone



- Special cultural practices used to obtain “deep-rooted” trees
- If V_T for the stand is greater than precipitation, then the trees can use groundwater at a certain rate
- A ***capture zone*** is a specific *thickness* of the saturated zone in which groundwater is taken up by the tree stand
- Dissolved groundwater contaminants removed via phytoremediation processes

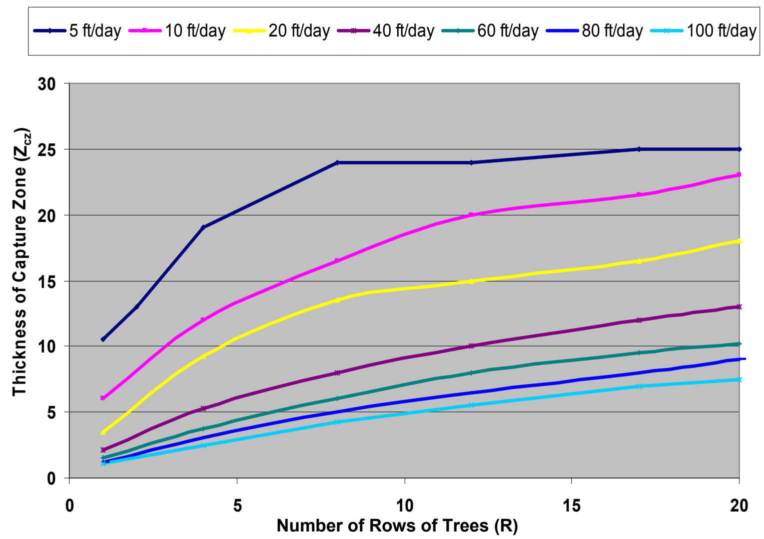
Modeling Capture-Zone Thickness Using MODFLOW



Parameters

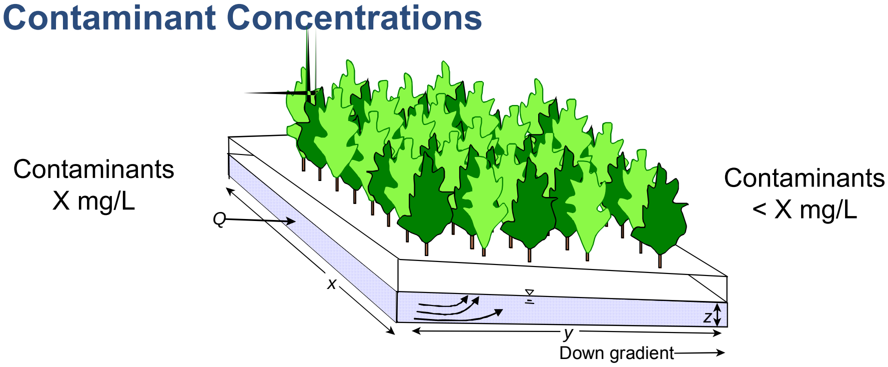
- Q, rate of groundwater flow beneath the root-zone, calculated by Darcy's law: $Q = X_T Z_{AQ} KI$
- R, number of tree rows (y_T)
- Z_{cz}, thickness of the capture zone
- *Thibodeau & Ferro (2007)*

MODFLOW Results: Capture zone thickness (Z_{cz}) is a function of the number of rows of trees (R) and the hydraulic conductivity of the aquifer (K)



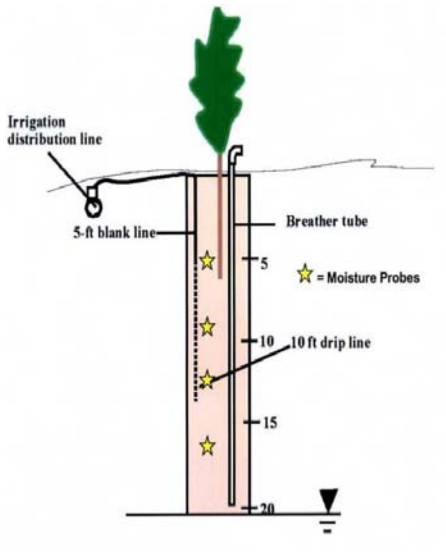
Hydraulic Control

Expected Effects of Phytoremediation on Groundwater Contaminant Concentrations



- In the capture zone, groundwater is taken up by the tree stand
- Dissolved contaminants in the capture zone are removed
- Clean “make-up” water flows into the phytoremediation system
- Therefore, down-gradient of the phytoremediation system, the groundwater contaminant concentrations are reduced

Special Cultural Practices Used to Obtain Deep-Rooted Trees



- Remove obstacles to roots (*drilling/backfilling boreholes*)
- Provide optimal conditions for root growth
  - moisture/nutrients (*vertical subsurface drip lines*)
  - air (*"breather tubes"*)
- Deep planting (pole planting) possible for poplars and willows
- Example: Raleigh, NC Site (*Case Study 3*)

Case Studies

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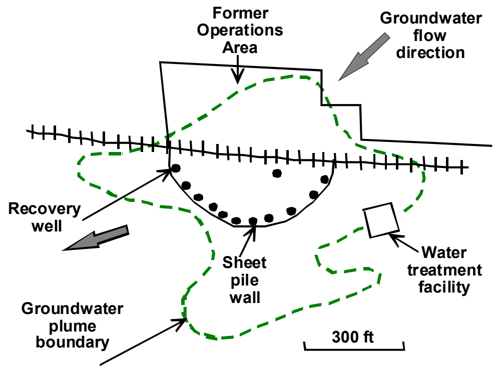
1. Solvents Recovery Service of New England (SRSNE) Superfund Site in Southington, Connecticut (*contaminants: chlorinated solvents*)
   - biological "pumping and treatment" system
   - tree stand established in a containment area

2. High Point, North Carolina (*contaminant: 1,4 - dioxane*)
   - irrigation with recovered groundwater
   - phytovolatilization of 1,4 - dioxane

3. Raleigh, North Carolina (*contaminants: TPHs*)
   - hydraulic control of a groundwater contaminant plume
   - deep-rooted willow and poplar trees

Case Study 1

Biological "Pumping and Treatment" System at the SRSNE Superfund Site



Compliance criterion: Inward hydraulic gradient toward the containment area

- A stand of willow trees was established in the containment area
  - 370 trees, 0.8 acre
  - 1999
- Objective: Reduce the need for mechanical pumping and treatment, at least on a seasonal basis

Case Study 1

Containment area at SRSNE site
Depth-to-groundwater: 4 to 5-ft bgs

Trenches were dug in the Containment Area. Willow cuttings were deeply planted in backfilled trenches.

Case Study 1

Summer 2004. The willow stand (370 trees on 0.8 acre) in their sixth growing season.

Case Study 1

Scaling TDP Data to the Stand-level

Mean values for May through September for the 0.8 acre stand of willow trees planted in 1999.

Year	*Sap velocity (cm/h)*	*Basal area (m^2)*	*Stand water use (gpm)*
2000	*27.8*	*n/a*	*--*
2001	*34.7*	*1.4*	*2.1*
2002	*16.5*	*3.0*	*2.2*
2003	*27.6*	*3.7*	*4.5*
2004	26.7*	*6.8*	*8.0*

*mean value for sap velocity, 2000 to 2003

Case Study 1

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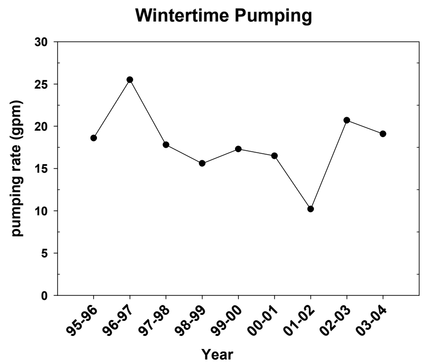
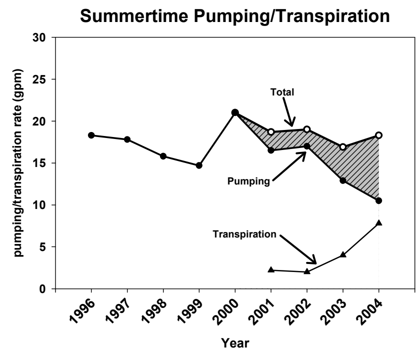
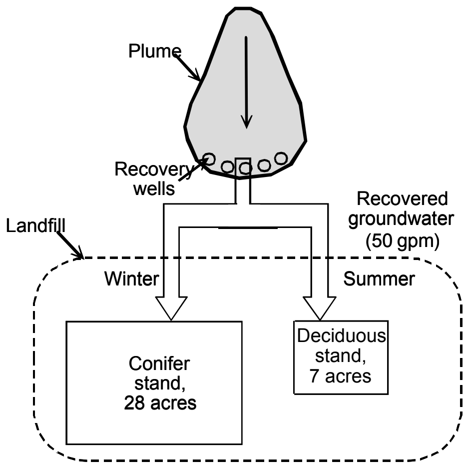




- Objective: Reduce the need for mechanical pumping and treatment on a seasonal basis
- Mean summertime stand transpiration probably reached plateau in 2005 at 9 gpm
- Cost for conventional pumping and treatment = $0.05/gal
- By 2010, the phytoremediation stand will result in a cumulative cost savings of $750,000

Case Study 2

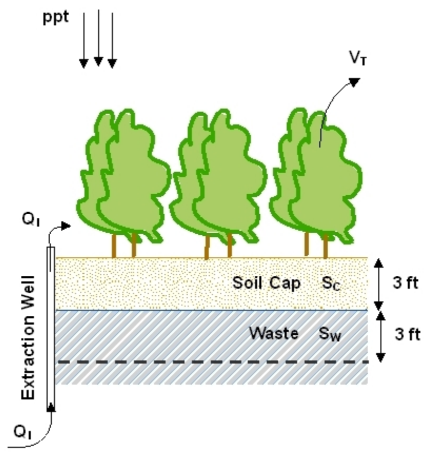
Irrigation of Tree Stands with Recovered Groundwater at Site in High Point, North Carolina



- Groundwater contaminant plume contains 1,4 – dioxane (< 10 mg/L)
- Recovery wells for plume control (50 gpm year-round)
- Recovered groundwater will be used to irrigate stands of trees established on an adjacent closed municipal landfill
- Contaminant treatment: phytovolatilization

Case Study 2

Performance Requirements for the Phytoremediation System on the Landfill



- Rate of landfill leachate production (gallons/month) will not be increased
- Phytovolatilization of 1,4 - dioxane will be effective (no leaching of dioxane below the root-zone)
- Transpiration rates must be sufficient to use precipitation plus irrigation water year-round

Case Study 2

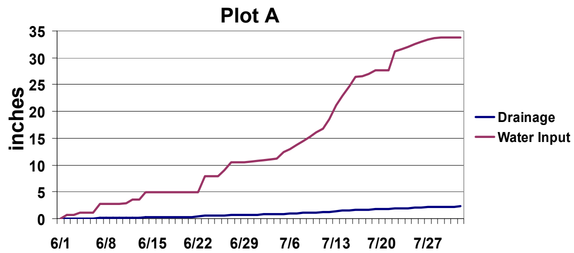
Pilot-scale Project on the Landfill (*May 2004*)

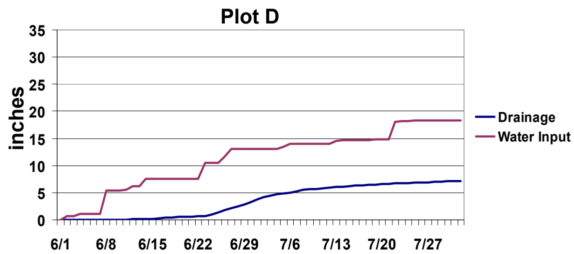
Plot D (control)- 2006

- Objective: To demonstrate that trees established on landfill cap can be irrigated without excessive drainage
- Experimental plots (24 x 36 ft):
  - Plot A, 24 hybrid poplars (with drip irrigation system)
  - Plot D, control, *Lespideza sericea*
- Instrumentation:
  - drain gauges
  - water meters
  - moisture probes
  - rain bucket
  - data logger

Case Study 2

Performance of the Pilot Plots, Mid-summer 2006

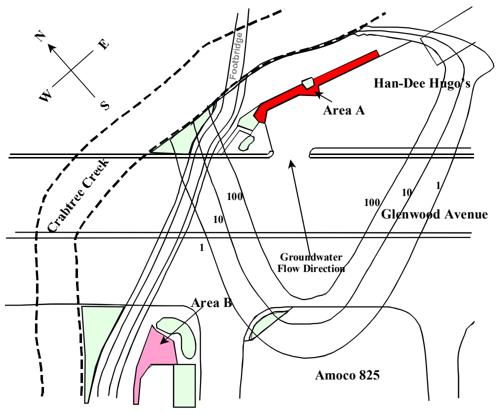




- Data show water input vs. drainage for Plot A poplars and Plot D control
- Conclusions
  - the landfill cap is suitable for the establishment of tree stands
  - Plot A, scaled to 7 acres, would be adequate for a full-scale system
- Upcoming investigation
  - irrigation of pilot stands with recovered groundwater
  - assess fate of dioxane

Case Study 3

Hydraulic Control of Groundwater Contaminant Plume Using Deep-rooted Willow and Poplar Trees

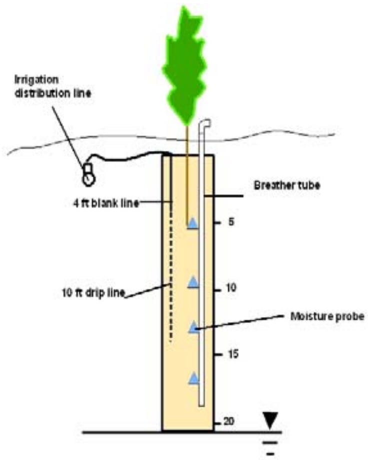


- Two adjacent BP retail outlets in Raleigh, NC
- Groundwater plume contains dissolved TPHs (saturated zone ~20ft below ground surface)
- Trees planted in 2003
  - Area A (contaminated) poplars and willows (25 trees)
  - Area B (uncontaminated) 20 poplars

Case Study 3

Planting Methods and Cultural Practices

(to obtain trees with deep-roots)



- Planting methods
  - boreholes, backfilled with sand/compost
  - vertical drip lines
  - breather tubes
  - moisture probes
  - poplar/willow poles deeply planted
- Subsurface irrigation
  - 2003 – 2006
  - no irrigation in 2007
  - using nutrient solutions in spring

Case Study 3

Tree Stands in Summer, 2007

Area A (contaminated):
Salix alba;
poplar hybrids DN-34 and NM-6

Area B (uncontaminated): Poplar hybrids DN-34 and NM-6

(Tree diameters, 15 – 19 cm, 3 ft above ground surface)

Case Study 3

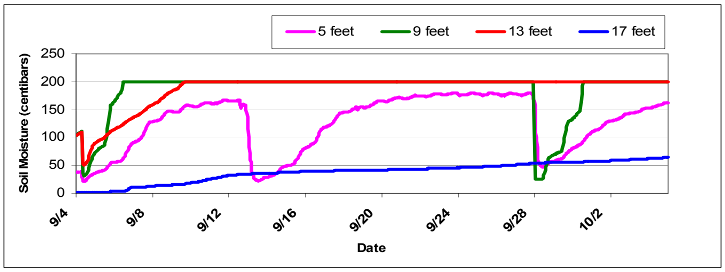
Monitoring Data for Trees in Areas A & B

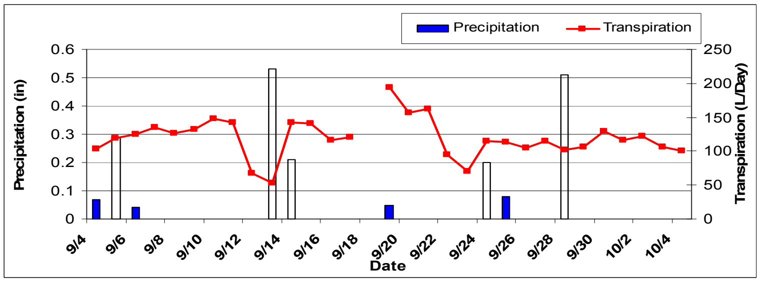
1. Data for rooting depth using moisture probes installed in the back fill
2. TDP data for sap flow (L/d)
3. TDP data for sap velocity (cm/h). These data were used to evaluate potential TPH phytotoxicity

Case Study 3

Data for Rooting Depth

(Poplar NM-6 in Area B, 2006; diameter 17 cm)

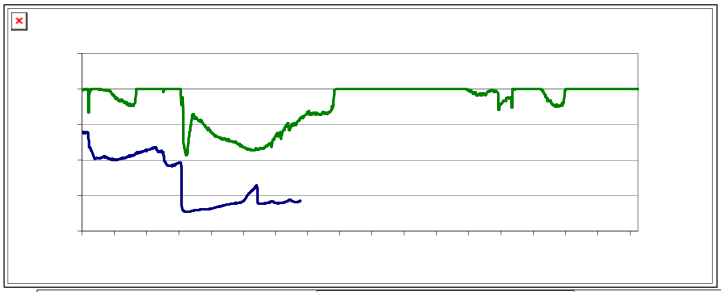


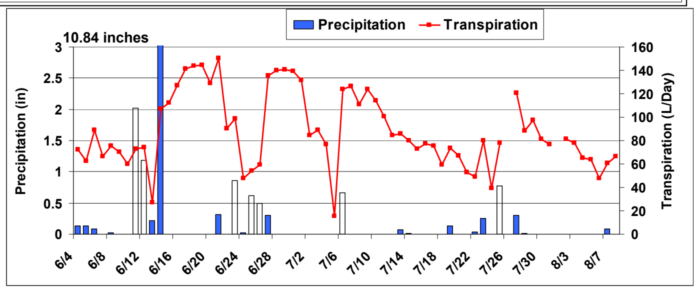


Case Study 3

Data for Rooting Depth

(Willow in Area A, 2006, diameter 15.9 cm)

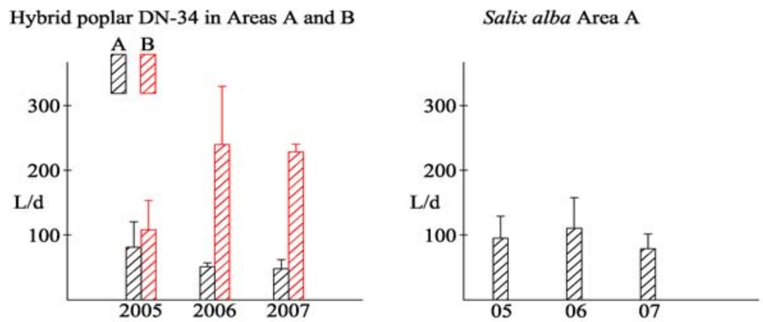




Case Study 3

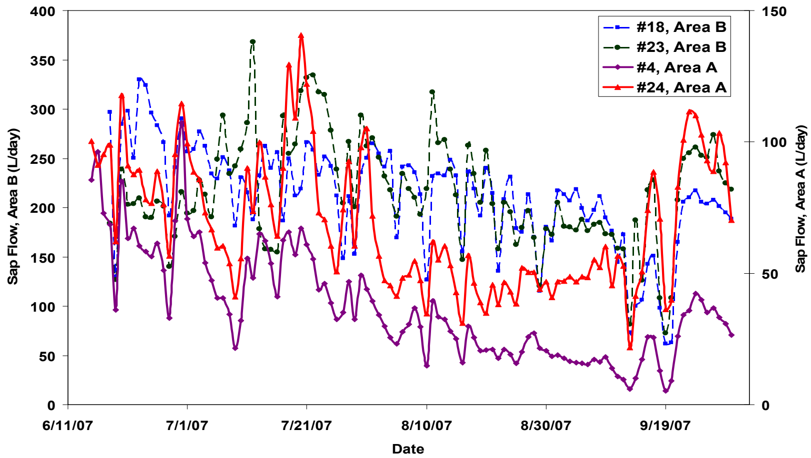
Sap flow data

(water-balance data suggested that plant-available moisture in the vadose zone was depleted in late summer, 2007)



Case Study 3

Sap flow data for Poplar Hybrid DN-34
Area A (*contaminated*) and Area B (*uncontaminated*)



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Comparison of "Early" & "Late" Sap Velocity, 2007

(early, July 15 – August 12; late, August 26 – September 23)

Area	Species	Early *(cm/h)*	Late *(cm/h)*	E/L	Estimated Benzene Conc.
A	poplar, *DN-34*	6.0	2.8	2.1	>100
	willow	18.4	4.0	4.6	>100
	willow	11.5	3.8	3.0	>100
	willow	19.2	10.6	1.8	≤100
	poplar, *DN-34*	13.0	9.2	1.4	>1
B	poplar, *NM-6*	9.4	9.8	0.96	ND
	poplar, *NM-6*	13.8	9.6	1.4	ND
	poplar, *DN-34*	30.2	22.6	1.3	ND
	poplar, *DN-34*	35.6	25.0	1.4	ND

Case Study 3

Conclusions

- Planting methods/cultural practices were effective for establishment of deep-rooted trees
- Plant-available moisture in vadose zone was depleted in late summer, 2007; trees were probably taking up groundwater
- Transpiration rates sharply reduced in for trees in Area A, especially in late summer
  - data suggested TPH phytotoxicity
  - preliminary MODFLOW modeling suggested some degree of hydraulic control for TPH plume in Area A

Questions?

- Treatment processes (rhizosphere degradation, plant uptake/volatilization, immobilization)
- Estimating/measuring rates of water use
- Applications/case studies
  - SRSNE Site: Biological “pumping and treatment” system
  - High Point, NC: Irrigation with recovered groundwater
  - Raleigh, NC: Plume control
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Progress in Transgenic Plants for Degradation of Organic Pollutants, Mammalian P450 2E1 in Plants

Stuart Strand

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University of Washington, Seattle WA

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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

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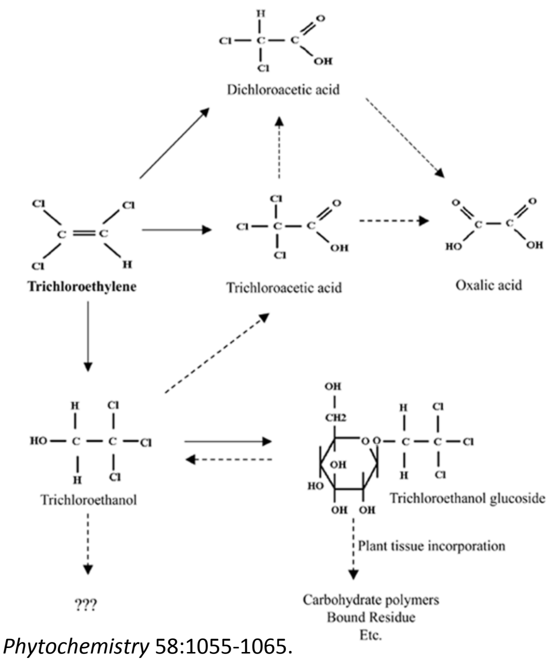
Proposed Fate of TCE in Plants



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

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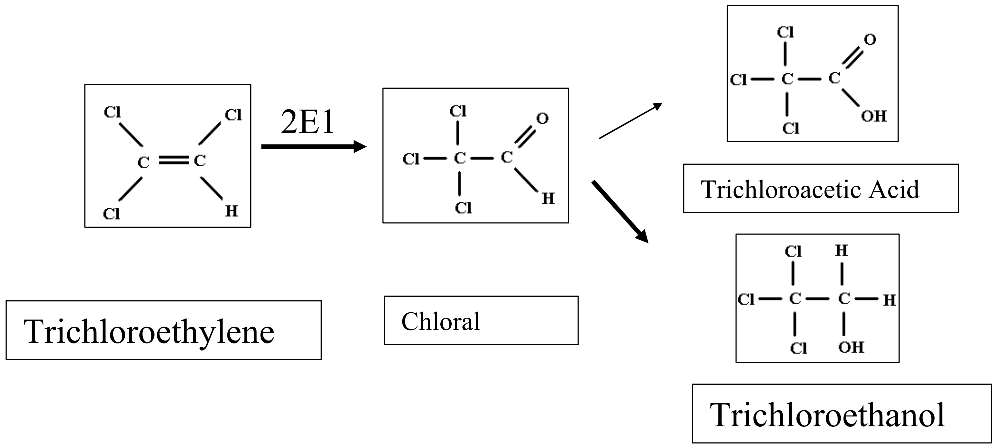
Mammalian Cytochrome P450 2E1 Catalyzes TCE Metabolism



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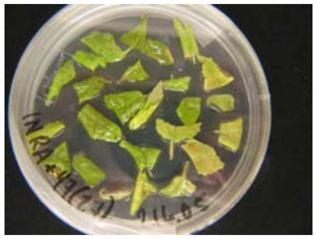
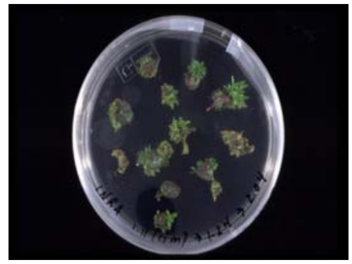
Transformation of Tobacco and Poplar (*P. tremula* x *alba* N717-1B4) using *A. tumefaciens*

Tobacco transformed with cDNA for human cytochrome P450 2E1 (h2E1)
Poplar with rabbit cytochrome P450 2E1 (r2E1)





Uptake of VOCs by Tobacco and Poplar Genetically Modified with h450 2E1

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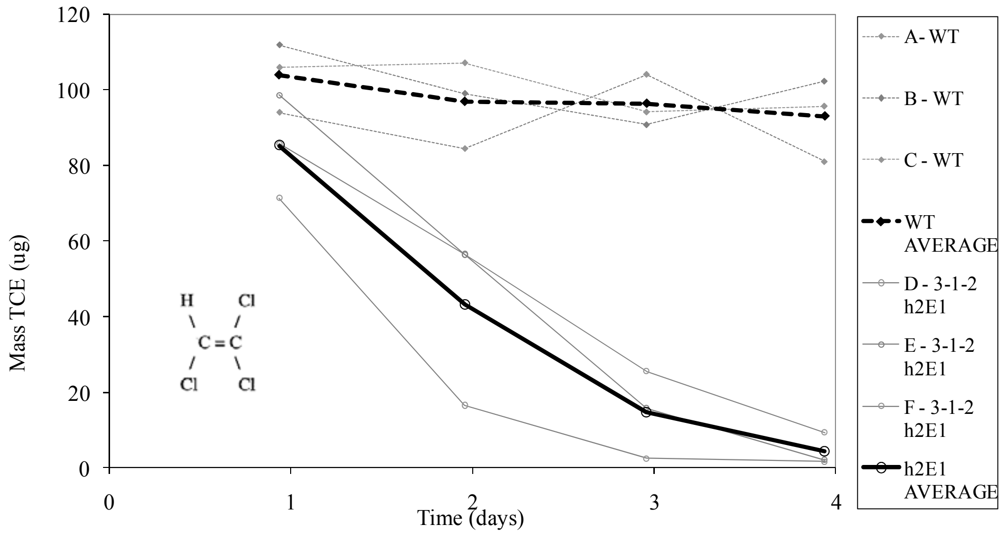
TCE Removal by Tobacco Transformed with h2E1



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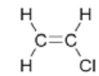
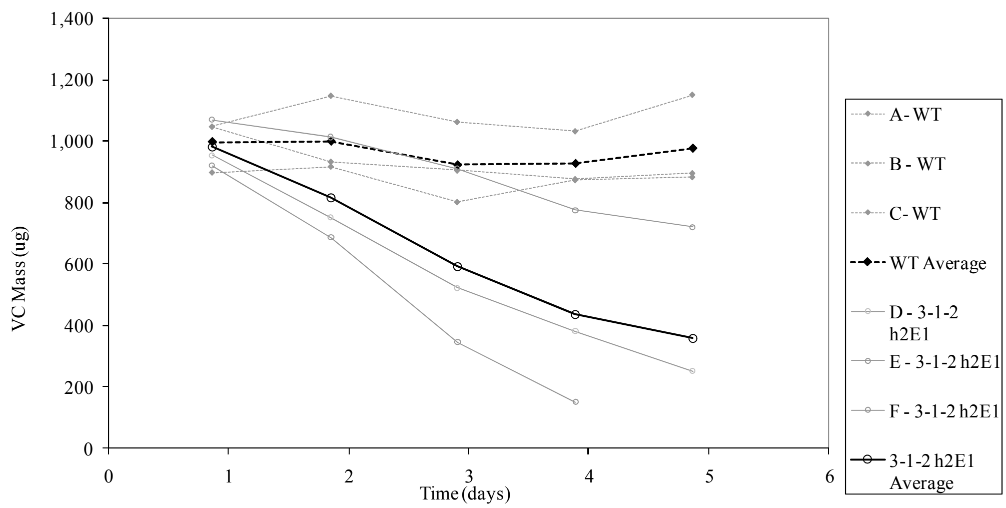
Vinyl Chloride Removal by Tobacco Transformed with h2E1





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h2E1 Transformed Tobacco Summary

PCE	**No increase**
TCE	+
cis-DCE	+**?**
VC	+
Methyl Chloroform	**No increase**
Chloroform	+
Carbon Tetrachloride	+
Benzene	+
Toluene	+

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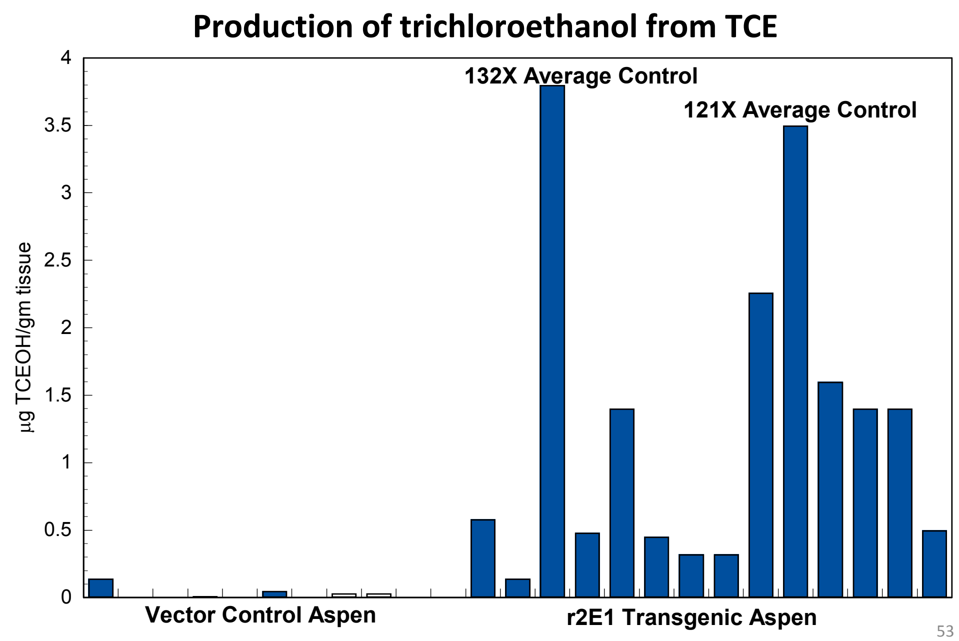
Increased Metabolism of TCE in CYP2E1 Transgenic Poplar



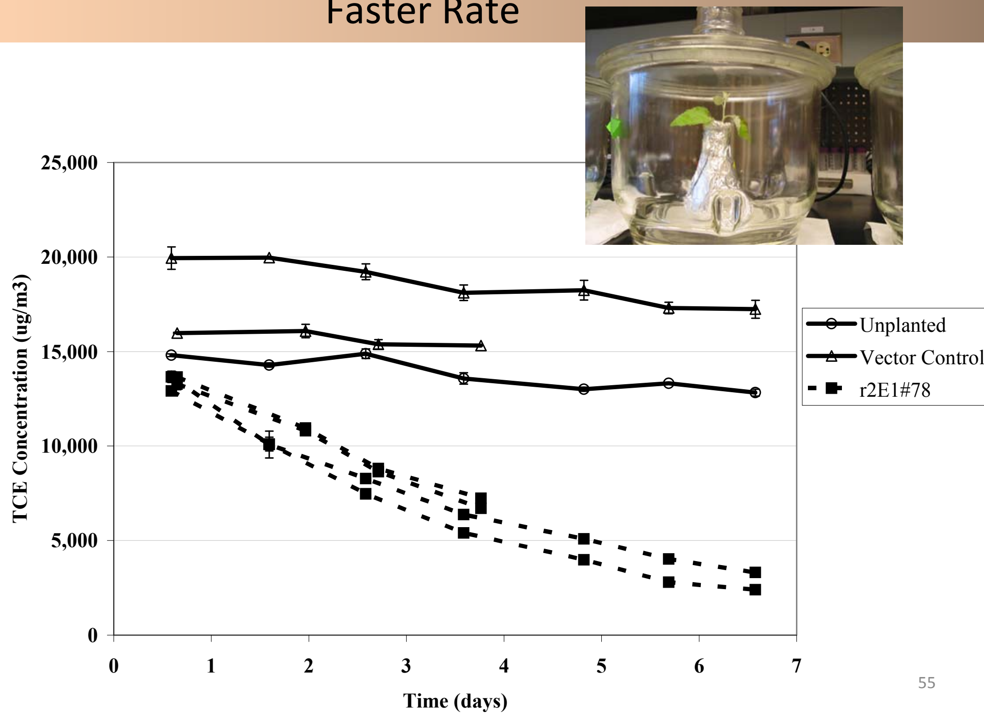
r2E1 Transgenic Poplar Removed TCE from Water at a Faster Rate

Transgenic Plant	% Removal	Rate *
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

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

r2E1 Poplar Plants Removed TCE from Air at a Faster Rate



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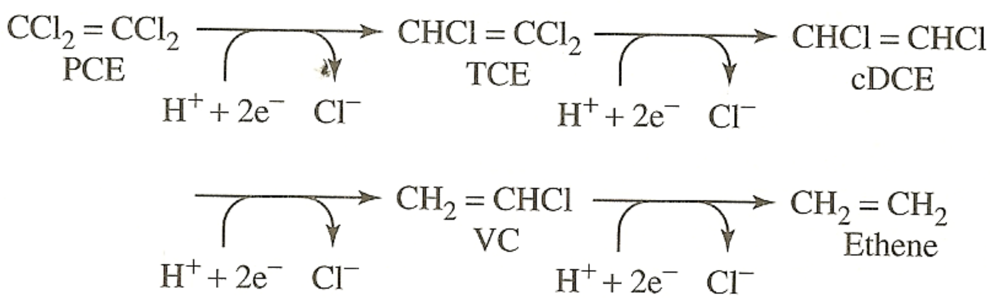
TCE in Groundwater Usually Accompanied by cis-1,2-Dichloroethylene and Vinyl Chloride

Reductive dechlorination of chlorinated ethenes by anaerobic bacteria



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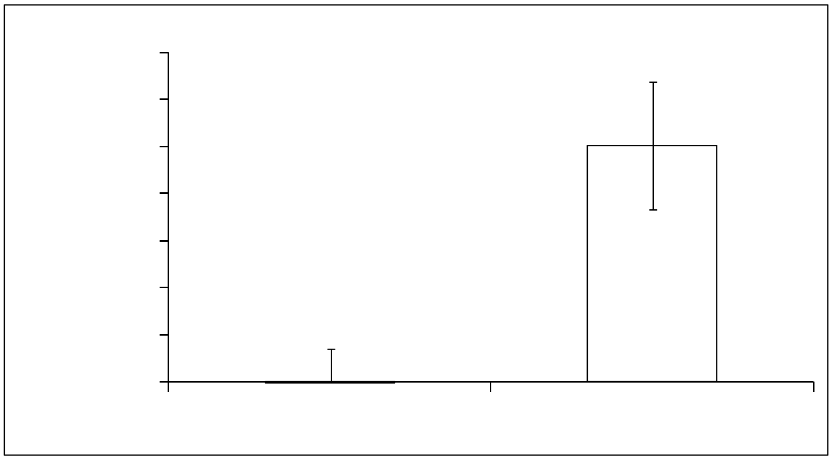
cis-1,2-Dichloroethylene Removal by Poplar Transformed with r2E1



Uptake Rate (ug/(g plant mass*day))
r2E1 #78 = 5.03 ± 1.37
WT = -0.01 ± 0.73

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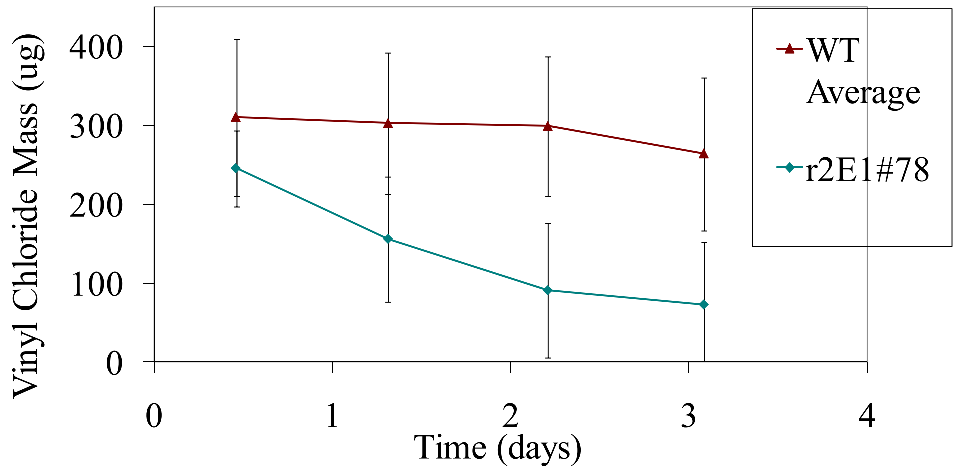
Vinyl Chloride Removal by Poplar Transformed with r2E1



Uptake Rate (ug/(g plant mass*day))
r2E1 #78 = 45.70 ± 8.59
WT = 14.56 ± 3.79

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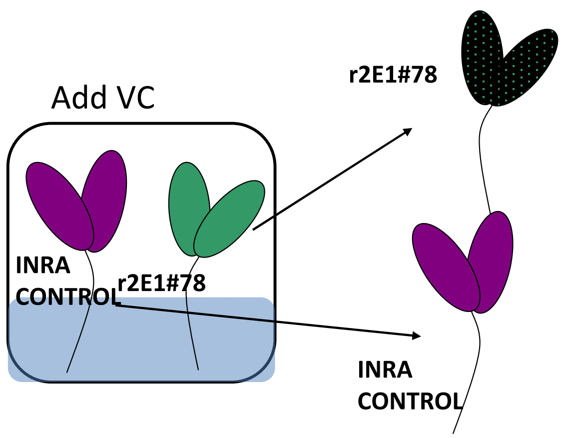
Effect of Vinyl Chloride Transformation on Poplar

CYPP450 2E1 catalyzes oxidation of vinyl chloride into transient reactive metabolites such as <u>chloroethylene oxide</u> and <u>2-chloroacetaldehyde</u>, which can bind protein and DNA



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r2E1 Poplar Summary

PCE	**No increase**
TCE	+
cis-DCE	+
VC	+

Field Trials of Transgenic Poplar Modified with r2E1 for Phytodegradation of TCE in Groundwater

Does enhanced metabolism in laboratory translate into enhanced remediation in the field?

Field Trials of Transgenic Poplar Modified with r2E1 for Phytodegradation of TCE in Groundwater

Biosafety and Regulatory Aspects

- Regulatory approval for field trials by USDA APHIS under industrial and pharmaceutical biotechnology regulations
- Each site separately permitted
- INRA hybrids not sexually compatible with local poplar species
- Females only
- Flowering delayed for 7 to 10 years
  - Except rare single flowers which are monitored and removed
- No growth from wind blown branches
- All plant tissue to be gathered and autoclaved or composted
- Toxicity of plant tissues to herbivores to be tested in following years

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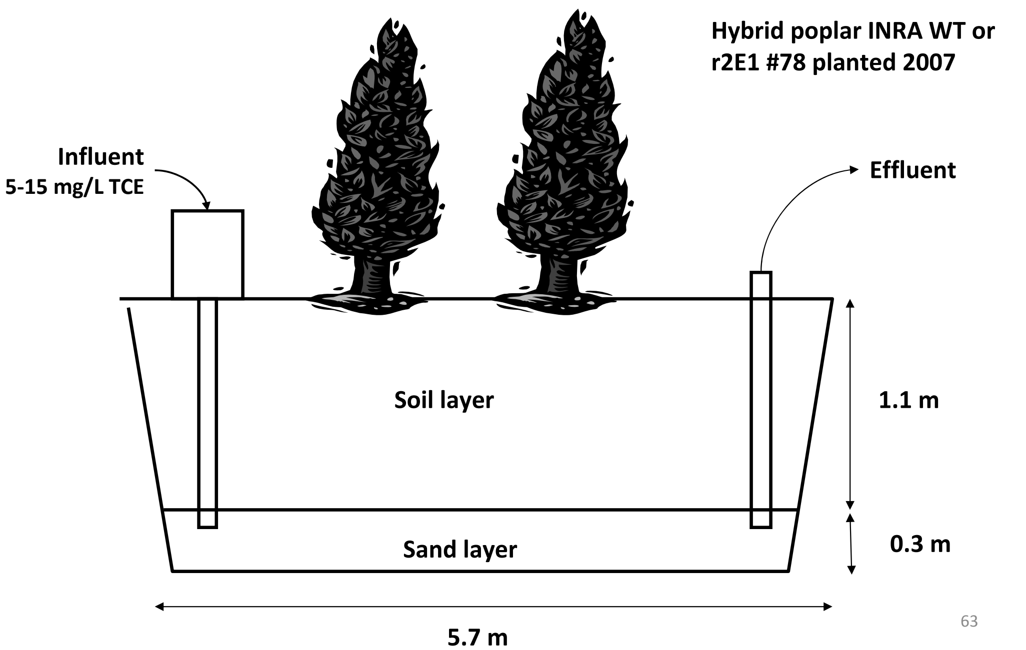
UW Phytoremediation Field Site



UW Phytoremediation Field Site

Bed 3
Unplanted Control

Bed 6
r2E1 #78

Bed 8
INRA 717

1. Trees planted April 14, 2007
2. Dosing with ~ 10 mg/L TCE began June 22, 2007
3. Measure parameters to determine chemical fate
   - Influent and effluent water
   - Soil volatilization
   - Leaf volatilization
   - Soil chloride
   - Leaf, trunk, and root tissue

UW Phytoremediation Field Site
Bed 3 – Unplanted Control

August 2008

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UW Phytoremediation Field Site
Bed 6 - r2E1#78

April 2007

August 2008

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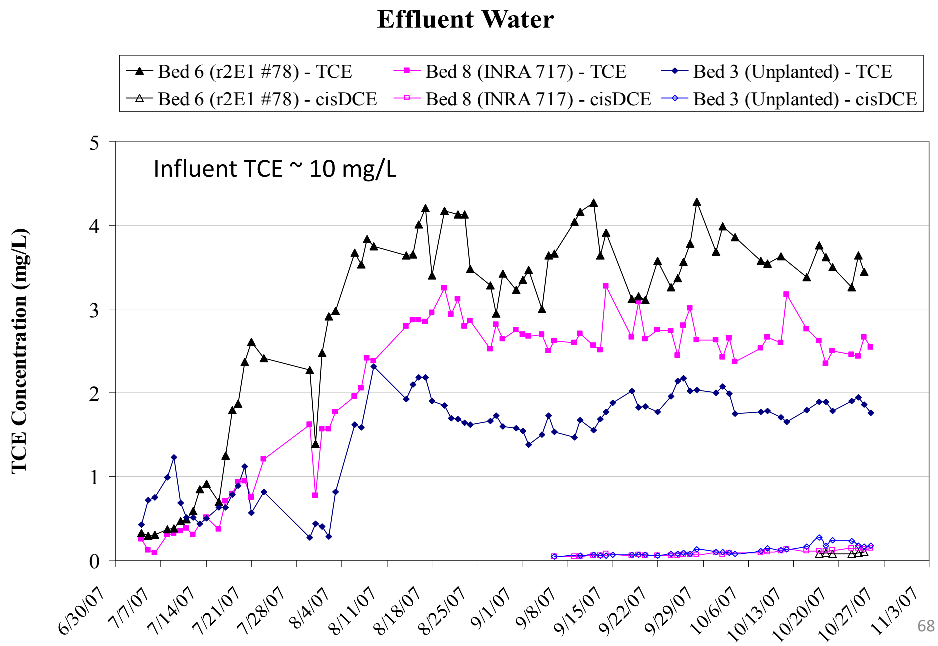
UW Phytoremediation Field Site
Bed 8 – INRA 717 Control

April 2007

August 2008

Comparative TCE Uptake
Summer 2007



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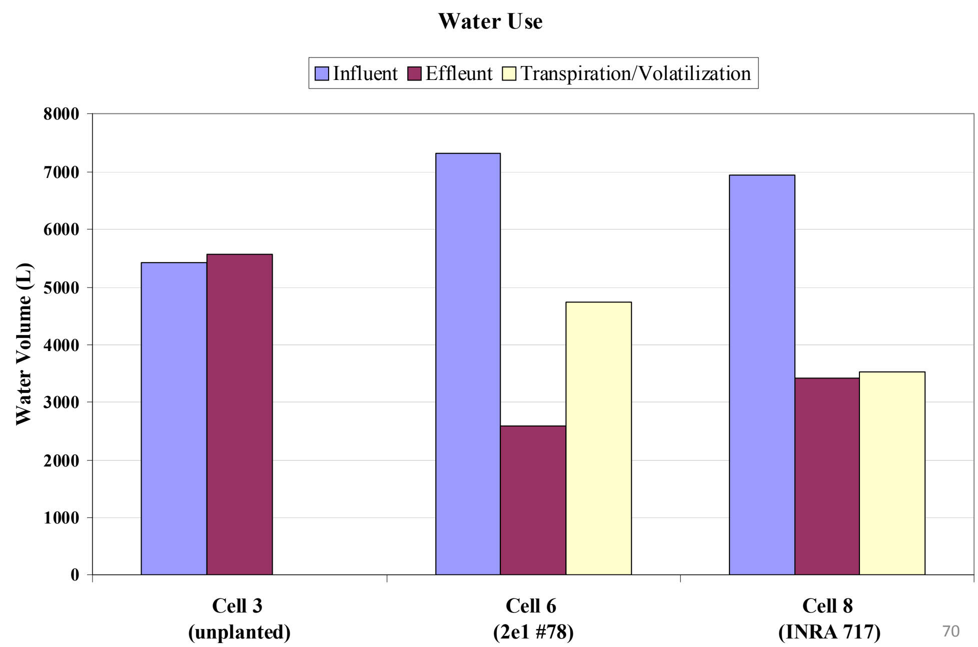
Summer 2007

- After 1st growing season the transgenic trees did not demonstrate increased effectiveness against TCE.
- Perhaps due to limited tree size and water uptake.

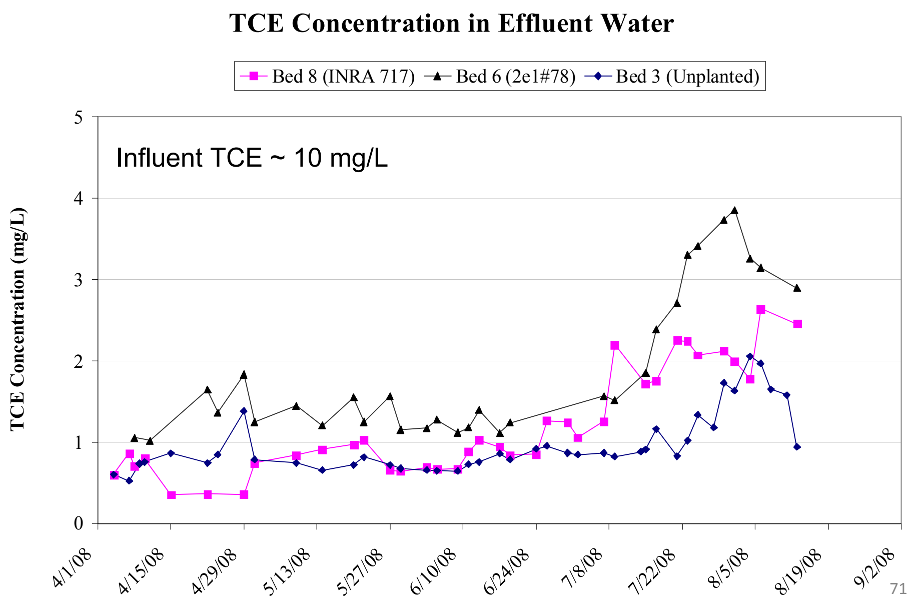
2008 Question #1 – are trees large enough to affect test bed environment?

Summer 2008

Water Uptake (April 1 – Sept 15)



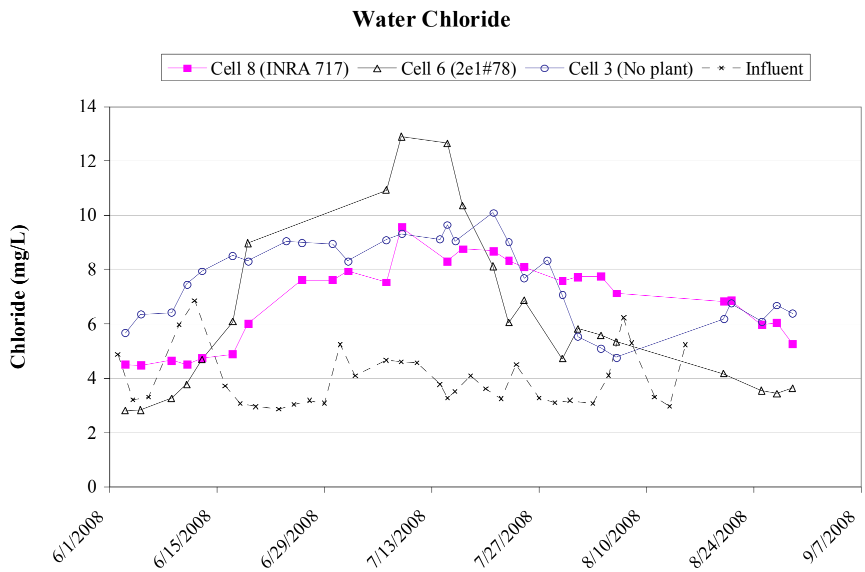
Summer 2008


TCE Concentration in Effluent Water
Bed 8 (INRA 717)
Bed 6 (2e1#78)
Bed 3 (Unplanted)
Influent TCE ~ 10 mg/L
TCE Concentration (mg/L)
0
1
2
3
4
5
4/1/08
4/15/08
4/29/08
5/13/08
5/27/08
6/10/08
6/24/08
7/8/08
7/22/08
8/5/08
8/19/08
9/2/08

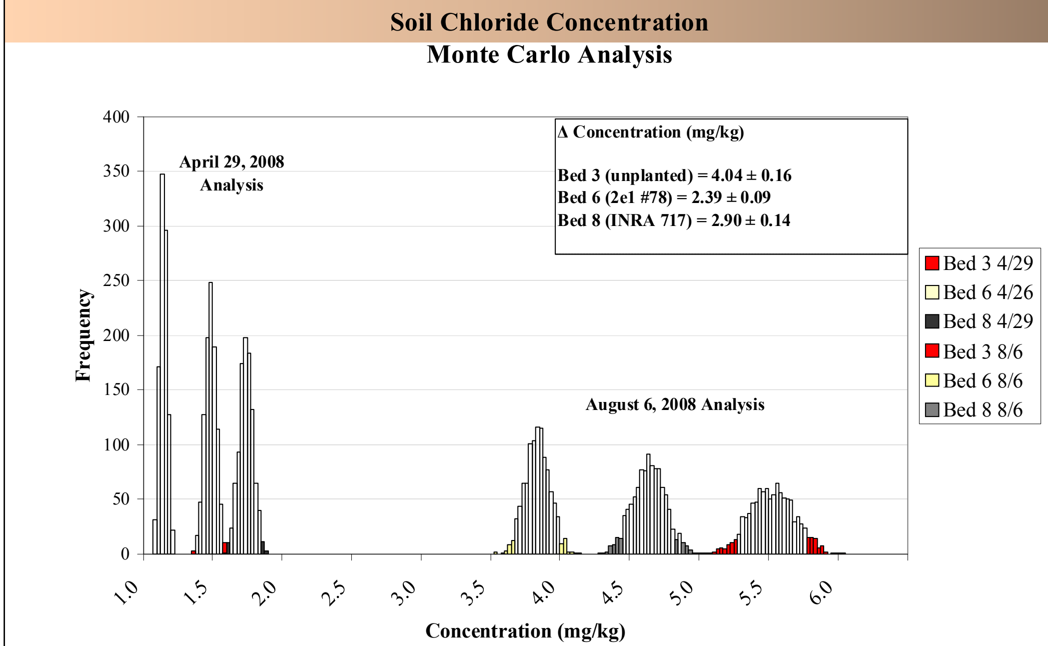
Summer 2008

- Planted beds are taking up significant volumes of water.
- Unplanted beds are not taking up water
  - Relatively saturated
- However, effluent TCE concentration in the planted beds are not significantly different from the unplanted bed.
- Transgenic trees did not significantly affect TCE concentration in groundwater.
- Is it simply due to soil sorption or is change in concentration due to degradation?

Summer 2008
Chloride ion production



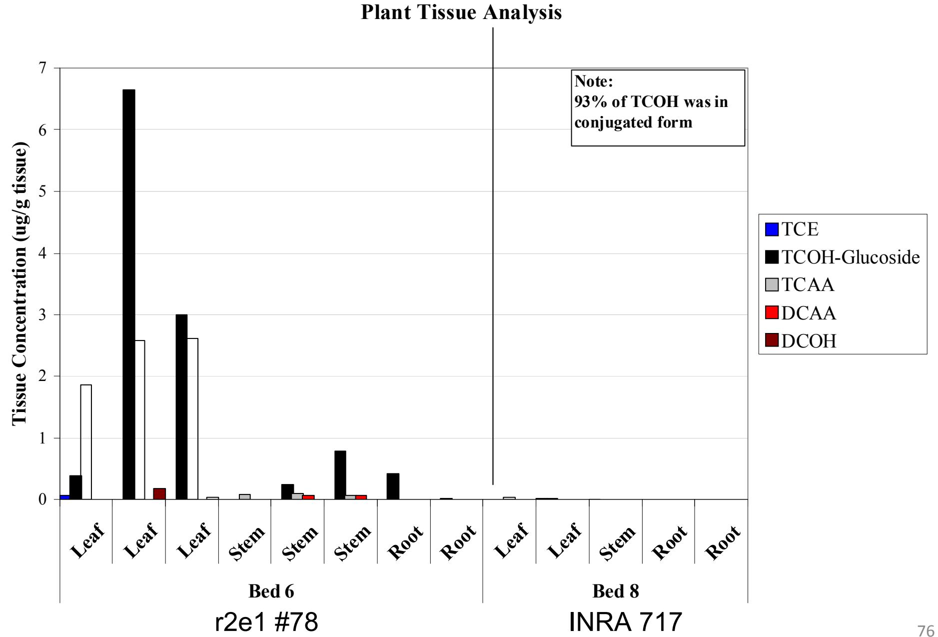
Summer 2008


Soil Chloride Concentration
Monte Carlo Analysis
Δ Concentration (mg/kg)
Bed 3 (unplanted) = 4.04 ± 0.16
Bed 6 (2e1 #78) = 2.39 ± 0.09
Bed 8 (INRA 717) = 2.90 ± 0.14
April 29, 2008 Analysis
August 6, 2008 Analysis
Frequency
Concentration (mg/kg)
Bed 3 4/29
Bed 6 4/26
Bed 8 4/29
Bed 3 8/6
Bed 6 8/6
Bed 8 8/6

Summer 2008

- Soil and water chloride data suggest that degradation is occurring.
- Effluent metabolite data in water and DO levels suggest that unplanted bed is likely reductive dechlorination.
  - Likely will cease when soil organic matter or other electron donor is exhausted.

Summer 2008
Is there evidence of 2e1 activity?



Why aren't the transgenic trees increasing TCE degradation in the test beds?

Hypotheses:

1. Phytoremediation of TCE in planted beds is primarily due to rhizosphere effects.

   Phytodegradation is insignificant in comparison

2. Mass transfer of TCE is limiting degradation.

   Mass transfer from "groundwater" to root

   Mass transport from root to site of phytodegradation (leaves?)

Next Steps

1. Continue field measurements, including tissue analysis
2. Verify expression of CYP450 2E1 through mRNA analysis of root and leaf tissue
3. Repeat selected rhizosphere analysis with soil microcosms
4. Investigate possibility of creating another transformant under control of root-specific promoter.

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Registration is open for the third Phytoremediation web seminar:

"Phytoremediation of Metals" – November 25th

For more information and archives of this and other Risk e Learning web seminars please refer to the Superfund Basic Research Program Risk e Learning web page:

http://tools.niehs.nih.gov/sbrp/risk_elearning/

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