Successful Field and Lab Tests of a Multi-Process Phytoremediation System for Decontamination of Petroleum and Salt Impacted Soils

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Development and Proof of the Multi-Process Phytoremediation System (MPPS)

1. 6 Years of Lab Studies
   1. Soil spiked with creosote (PAHs)
   2. Total Petrol. Hydrocarbon (TPH) contam. soil
   3. PAH contaminated soil from urban foundry
   4. Plant growth on salt impacted soils
2. 3 Years of Field Studies
   1. TPH, Talisman Energy, Turner Valley, AB 2005-06
   2. TPH, North East Alberta 2005-06
   3. TPH, Imperial Oil Land Farm, Sarnia, ON 2004-06
   4. DDT, Simcoe, ON 2005

Advantages of Phytoremediation

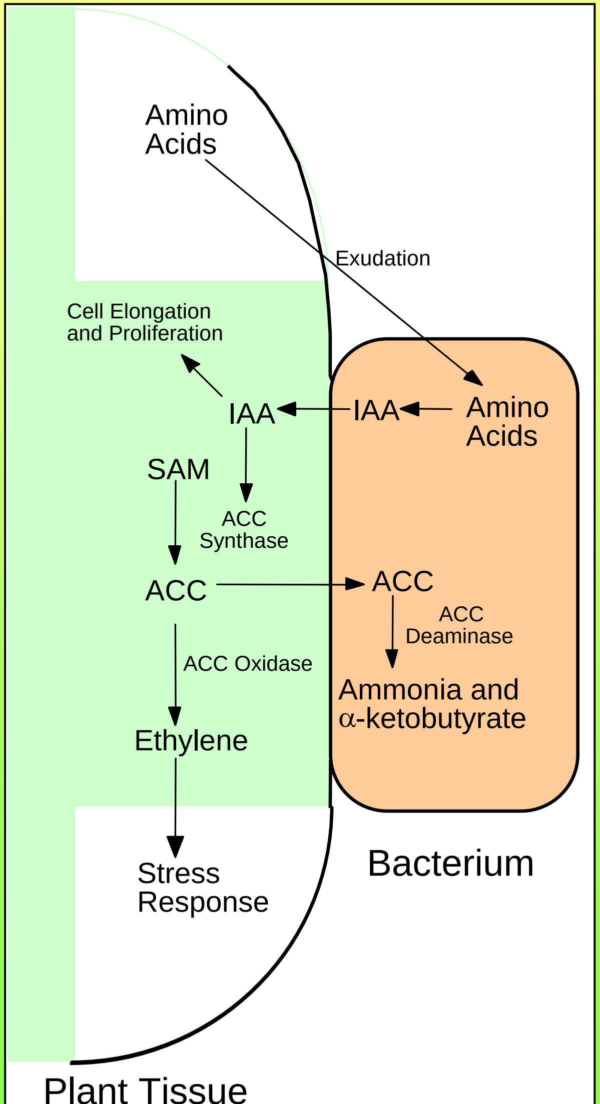
1. Improves the natural structure and texture of soil
2. It is driven by solar energy and suitable to most regions and climates
3. It is low in cost and technically feasible
4. Plants can provide sufficient biomass for rapid remediation; promote high rhizosphere activity
5. Restoration
6. > 30,000 sites in Canada where such technology is needed, > 300,000 sites in the US

Description of the Multi-Process Phytoremediation System (MPPS)

Physical soil treatment:	**Till the soil: exposure to sunlight and air** **Exposure to sunlight photooxidizes contaminants**
Bioremediation:	**Inoculation of PAH/TPH degrading bacteria**
Phytoremediation:	**Growth of plants alone on the soil**
MPPS:	**Land farming the soil for two weeks** **Inoculation of PAH degrading bacteria** **<u>Growth of plants with PGPR</u>**

<u>PGPR:</u> Plant growth promoting rhizobacteria.
Prevents the synthesis of stress ethylene.

Interaction of a PGPR containing ACC deaminase with a plant seed or root

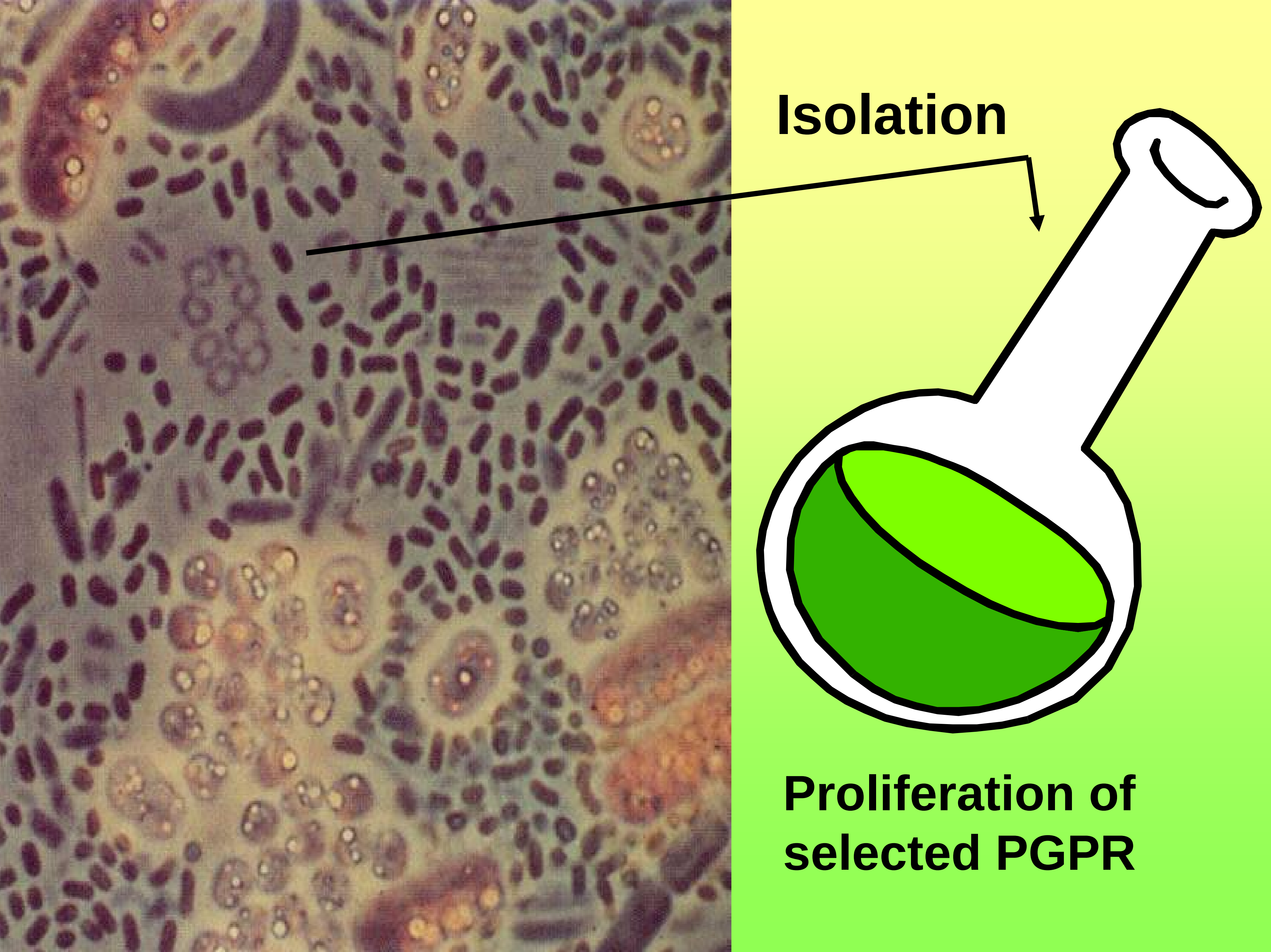


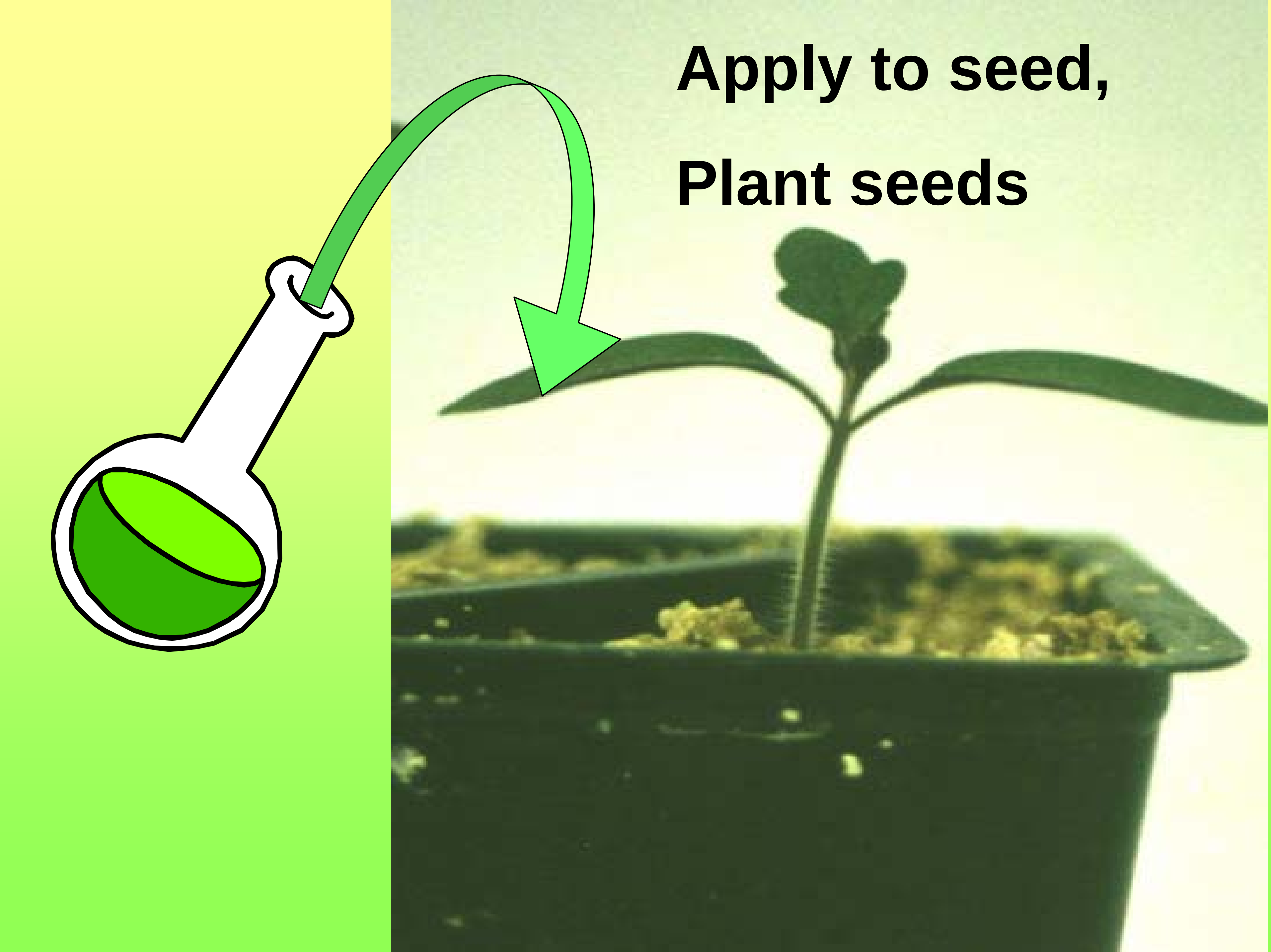
Plant growth promoting rhizobacteria (PGPR)

Two natural, non-pathogenic strains of *Pseudomonas putida* (UW3 and UW4)

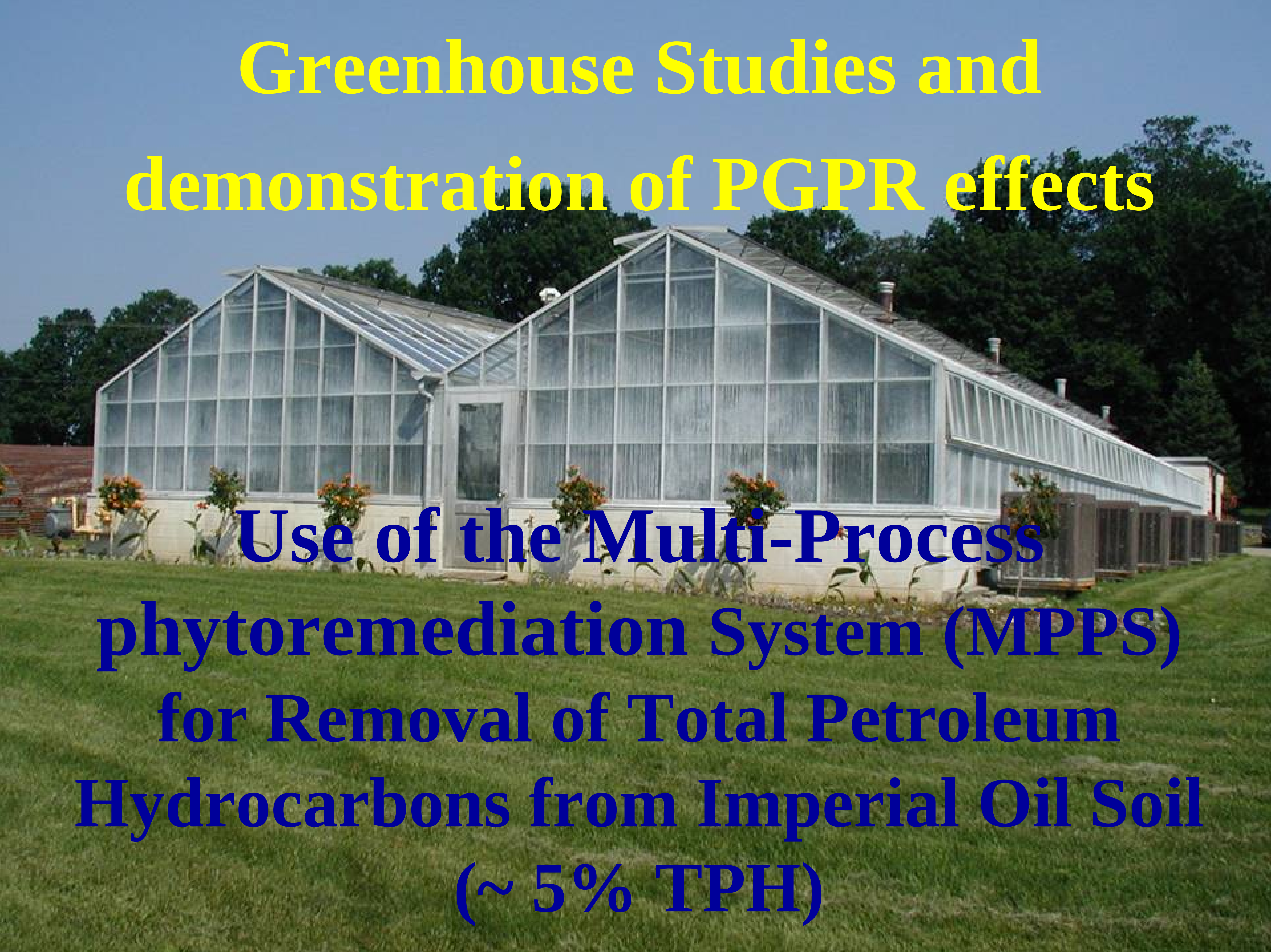
- **One has high ACC Deaminase**
- **One is an auxin producer**

Both PGPR are applied to seeds prior to planting


Isolation
Proliferation of
selected PGPR


Apply to seed,
Plant seeds

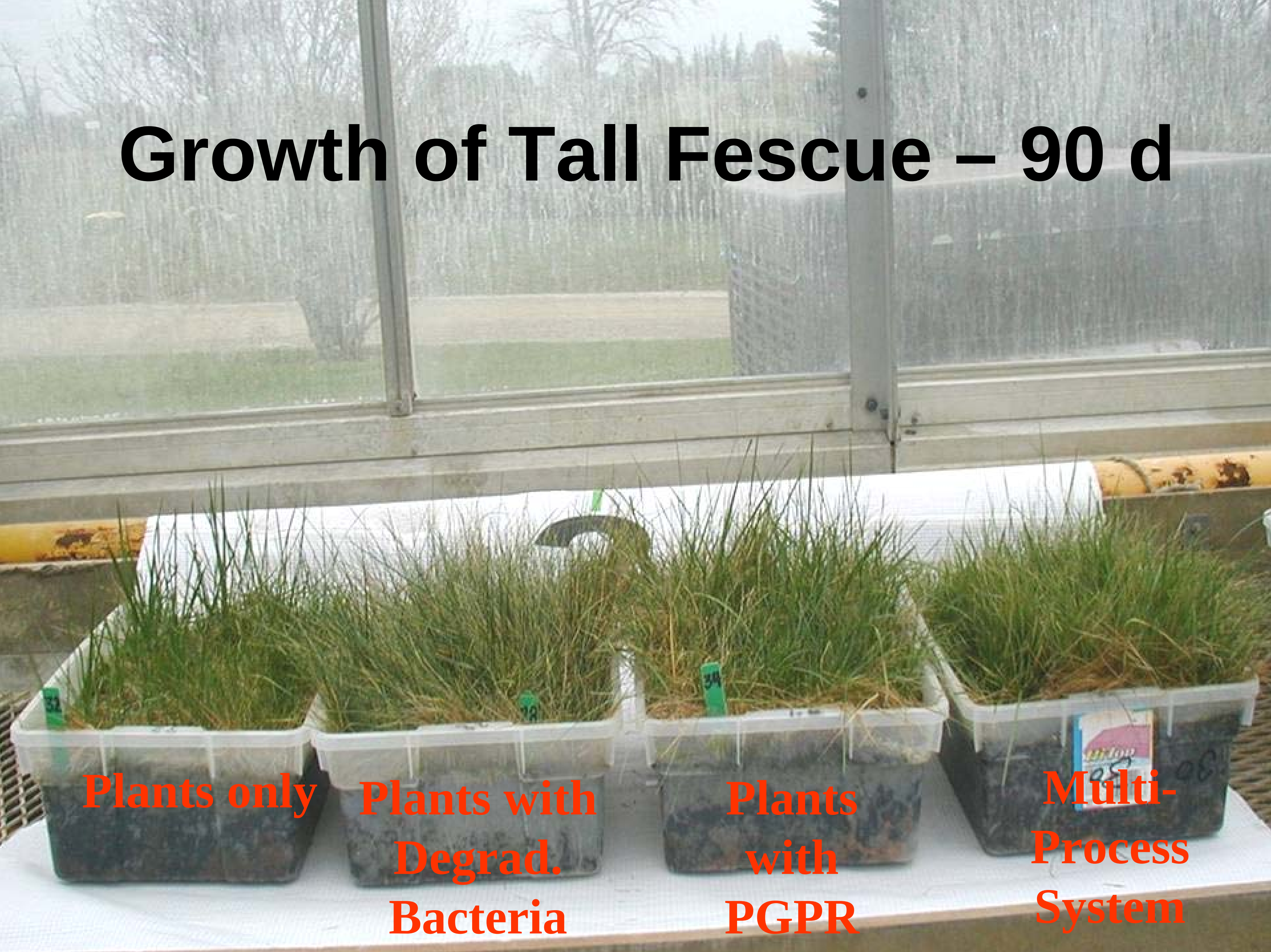
Few days later (Tomato)



Greenhouse Studies and demonstration of PGPR effects

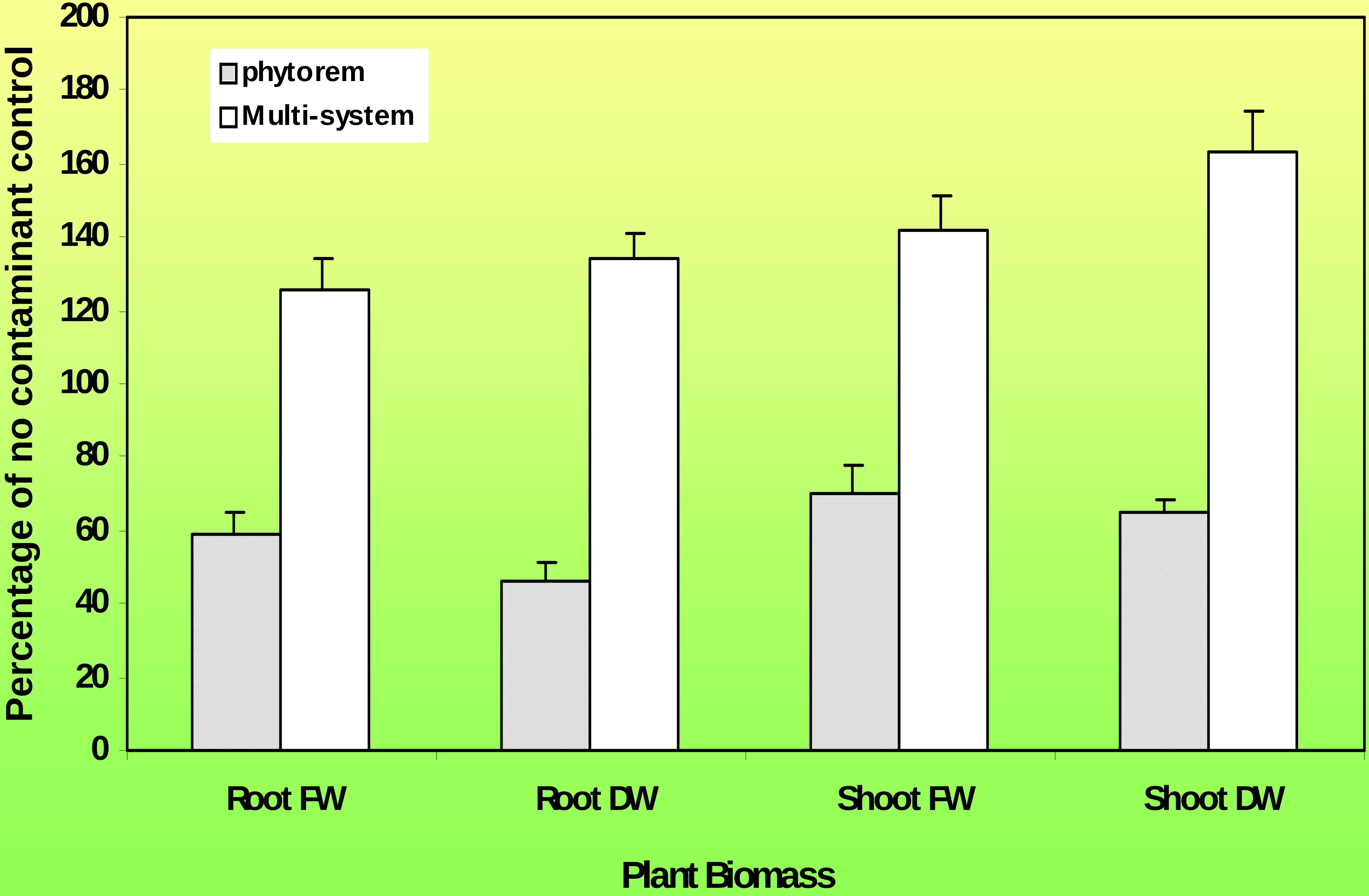
Use of the Multi-Process phytoremediation System (MPPS) for Removal of Total Petroleum Hydrocarbons from Imperial Oil Soil (~ 5% TPH)

Growth of Tall Fescue – 90 d

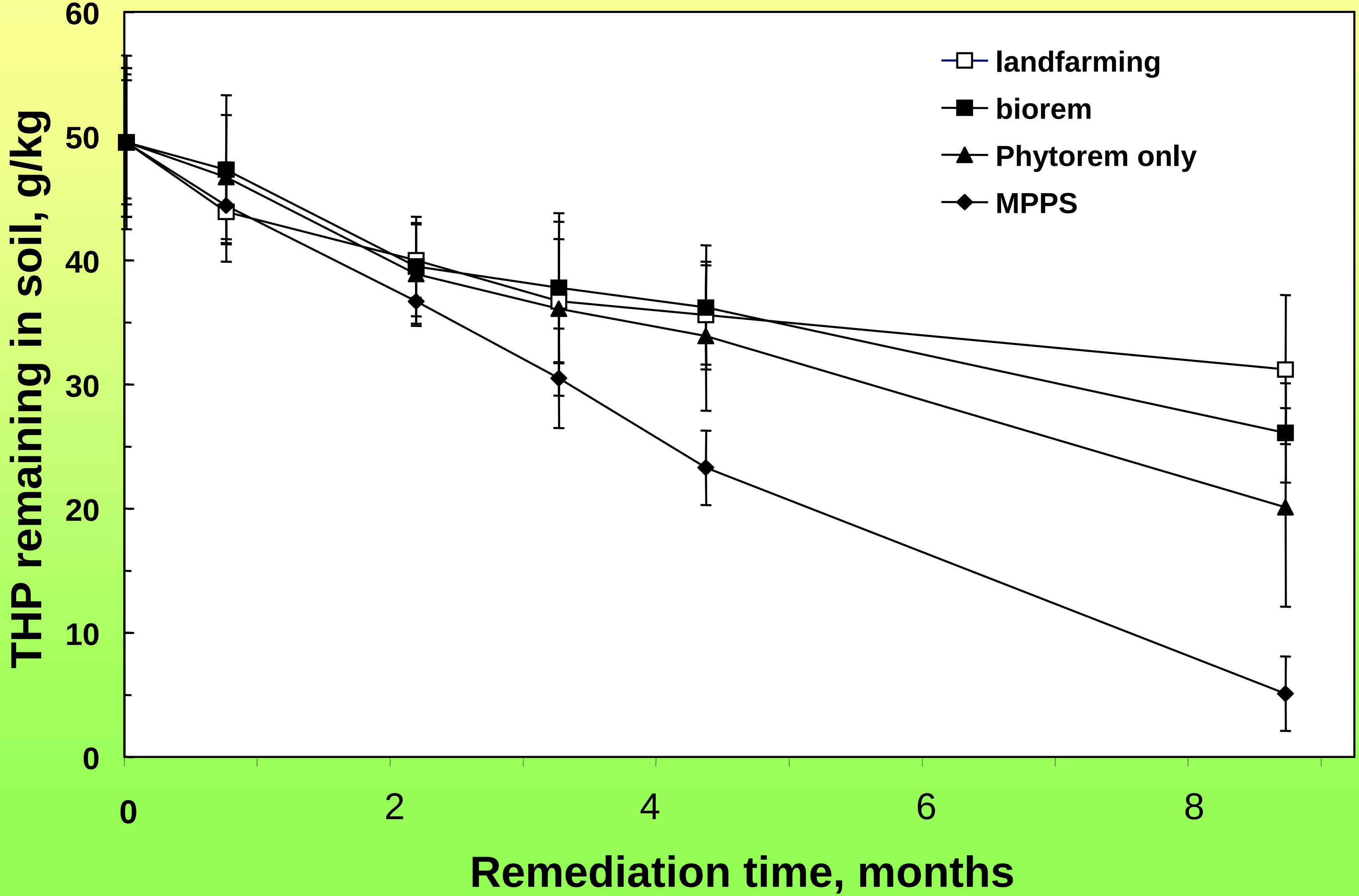


Plants from the MPPS on Land-Farmed Contaminated Soil

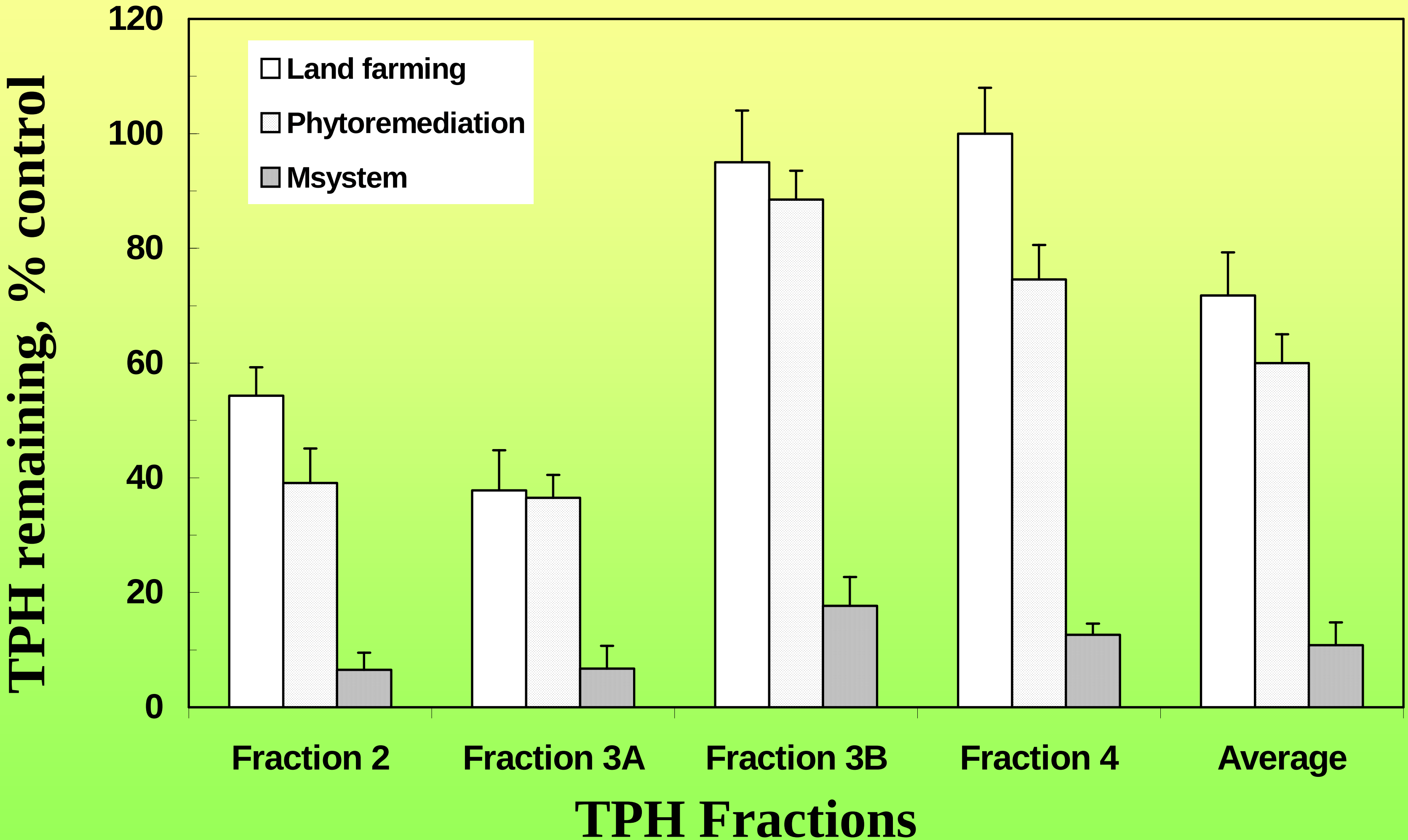
Plant Biomass for Phytoremediation and the MPPS



Remediation Kinetics of Four Methods Tested



Remediation of different fractions of TPH after 2 4-month seasons



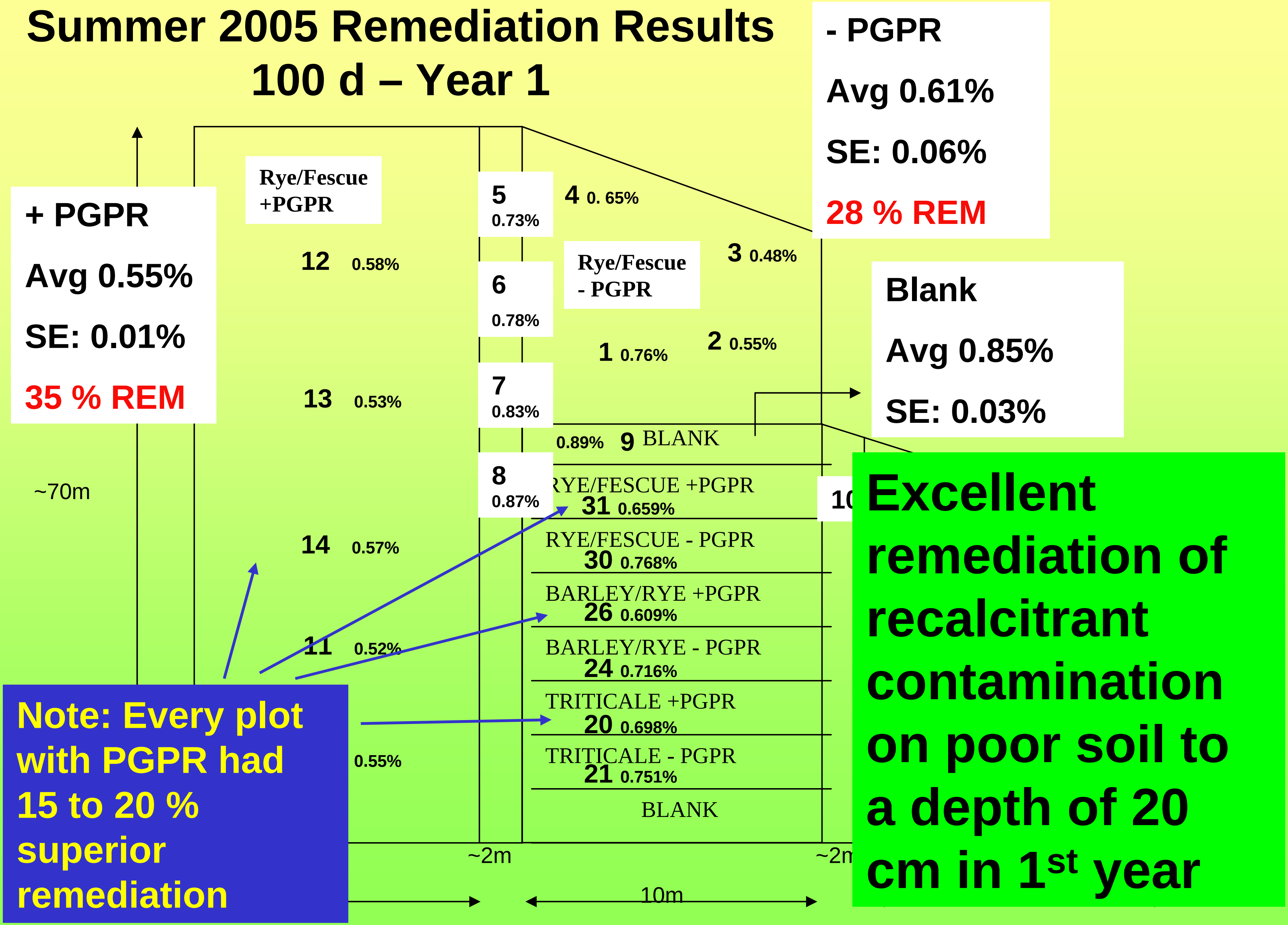
<u>Contaminants metabolized/degraded:</u> ~40g TPH/kg soil removed, < 100g DW plants/kg soil. Plants cannot be 40 % TPH.

Field Test at Talisman Energy Biopile (1 % TPH) Turner Valley Alberta Year 1: Summer 2005 – Rye/Fescue Growth

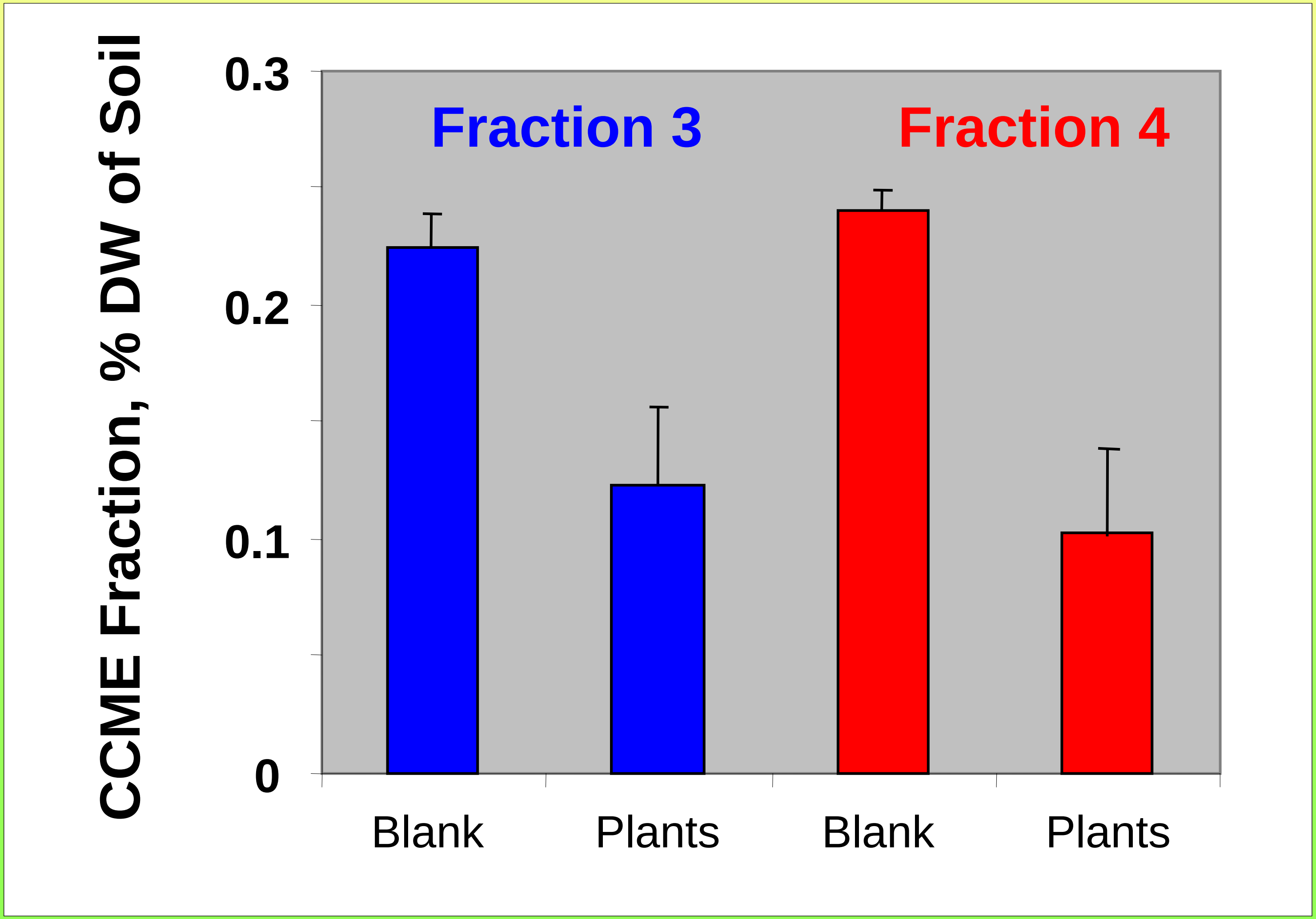
90 d

Note: Weather cool and wet.
Poor, gravelly soil required fertilizer

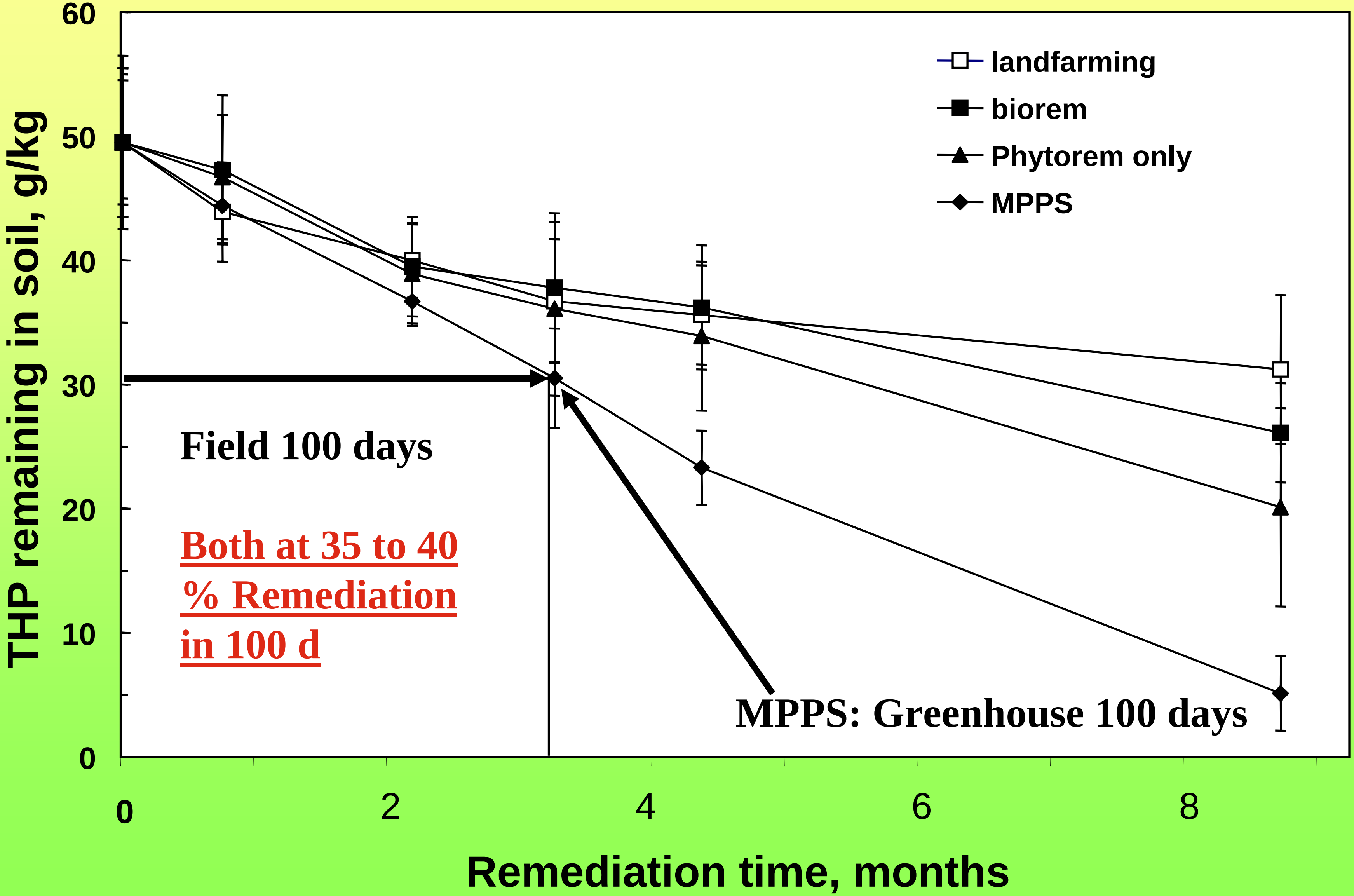
Talisman Energy,Turner Valley Site
Summer 2005 Remediation Results
100 d – Year 1



CCME Fractions remaining in Turner Valley Soil after 100 d – Year 1 (rye/fescue + PGPR)

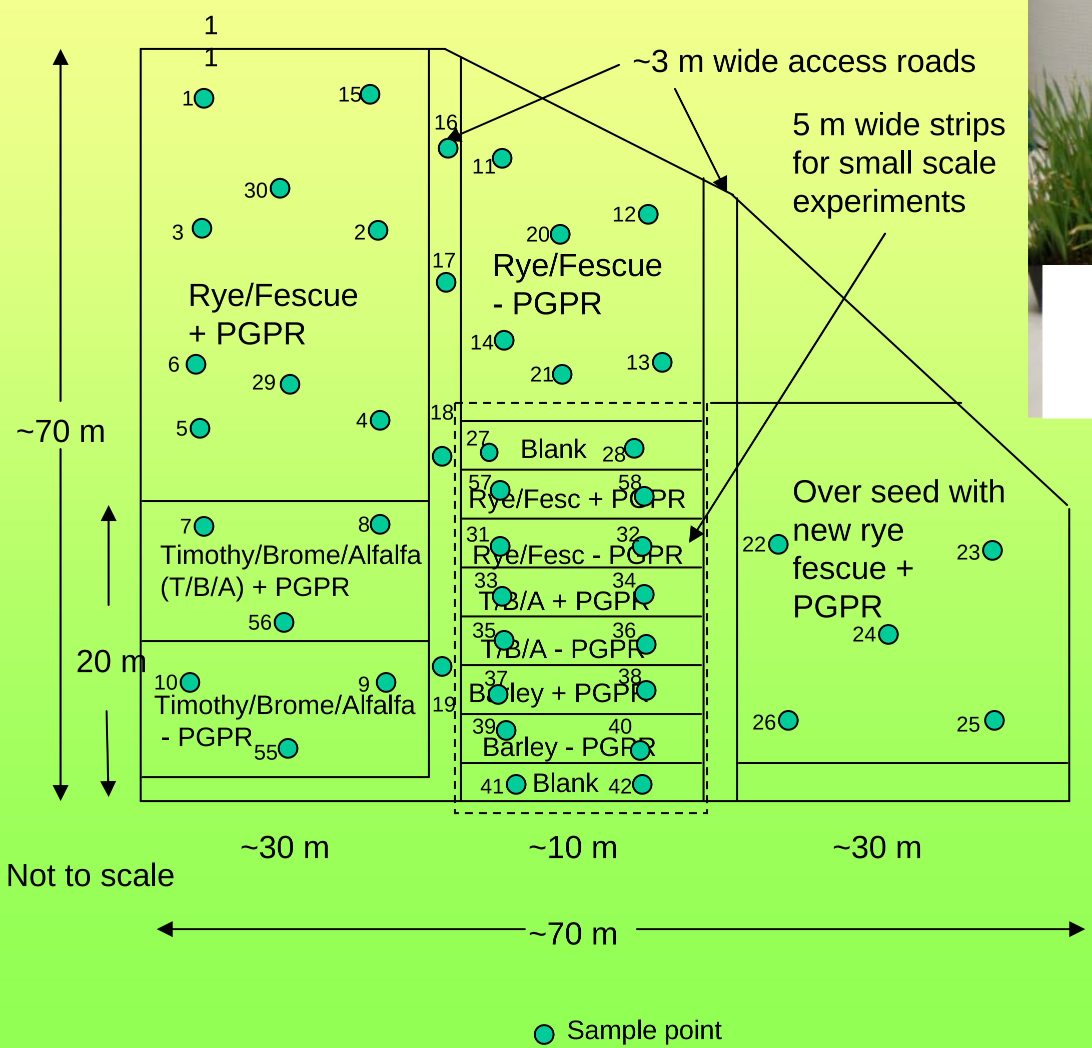


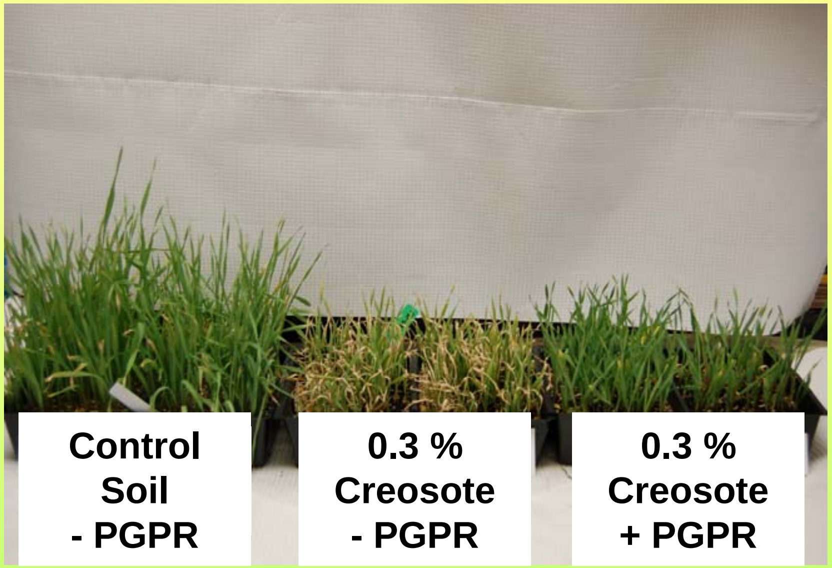
Remediation Kinetics For Turner Valley Soil
Greenhouse vs. Field



Turner Valley Year 2 2006
Planted May 15, 2006

Site plan May 13th, 2006





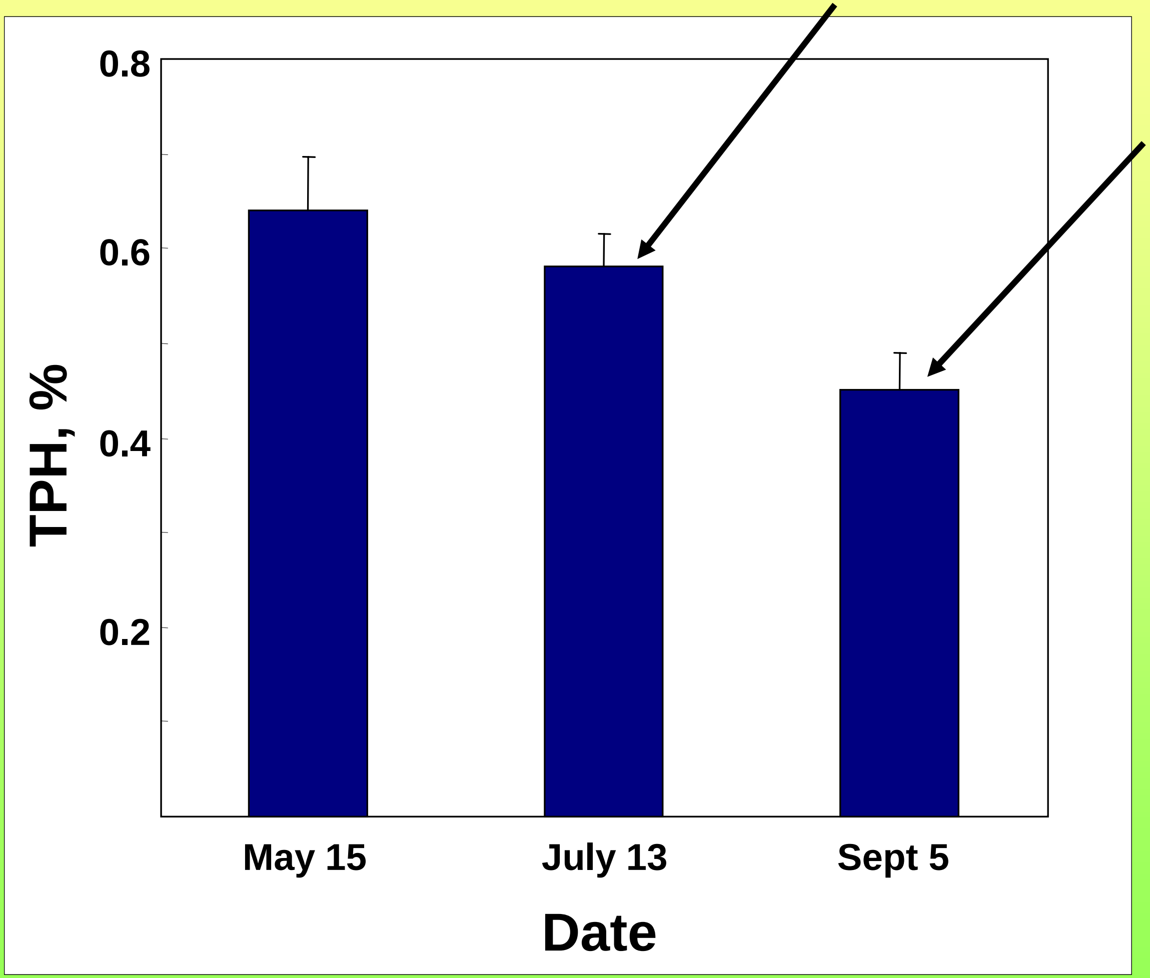
- **Quality control**
- **Ryegrass treated with seed treater**
- **UW3 + UW4 + Me-Celluose**
- **Test of PGPR efficacy on 3 % Creosote**
- **Plants grew well in the field**

Turner Valley, Year 2 2006

- Planted May 15, 2006
- Treated with seed treater
- UW3 + UW4 + Me-Celluose
- Good positive PGPR effect
- Excellent growth

Turner Valley, Year 2 Remediation

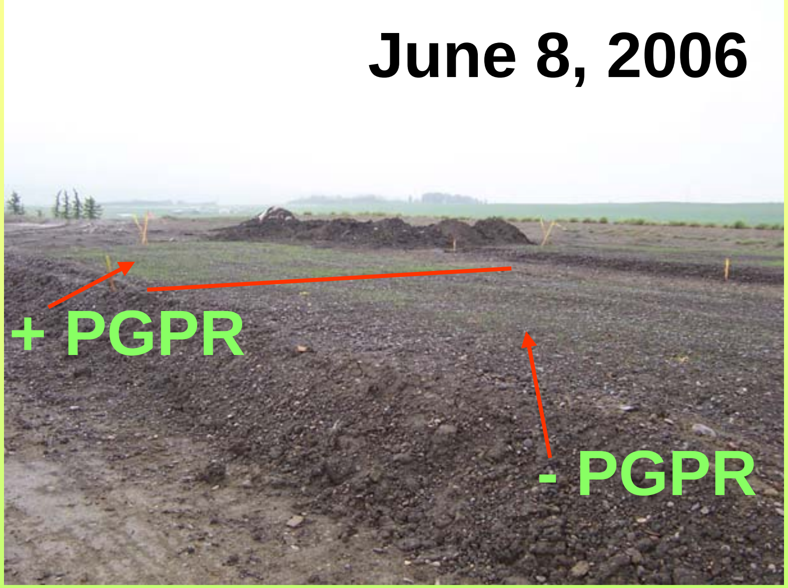
9 % Remediation in 2 months

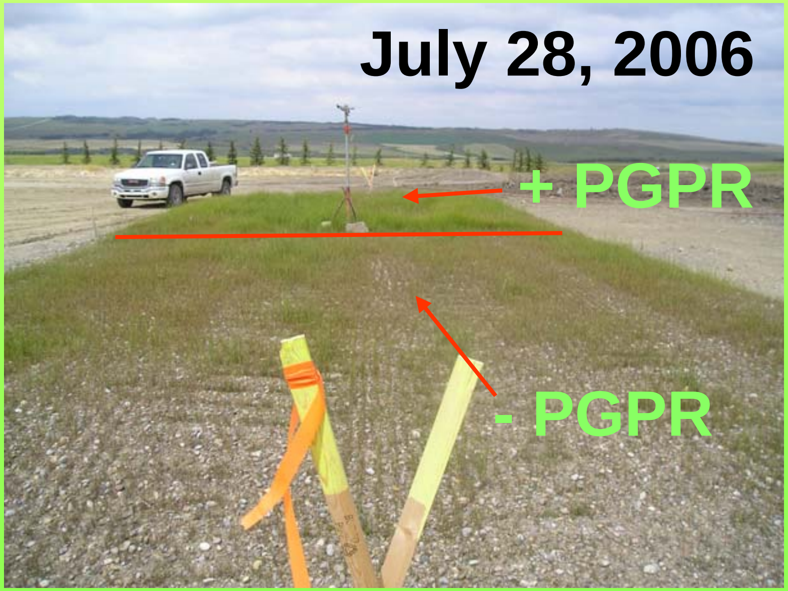


30 % Remediation in 3.5 months

Same as the remediation kinetics observed in previous years

Turner Valley 2006 – 60 cm deep plot on clay pad - Planted May 15, 2006

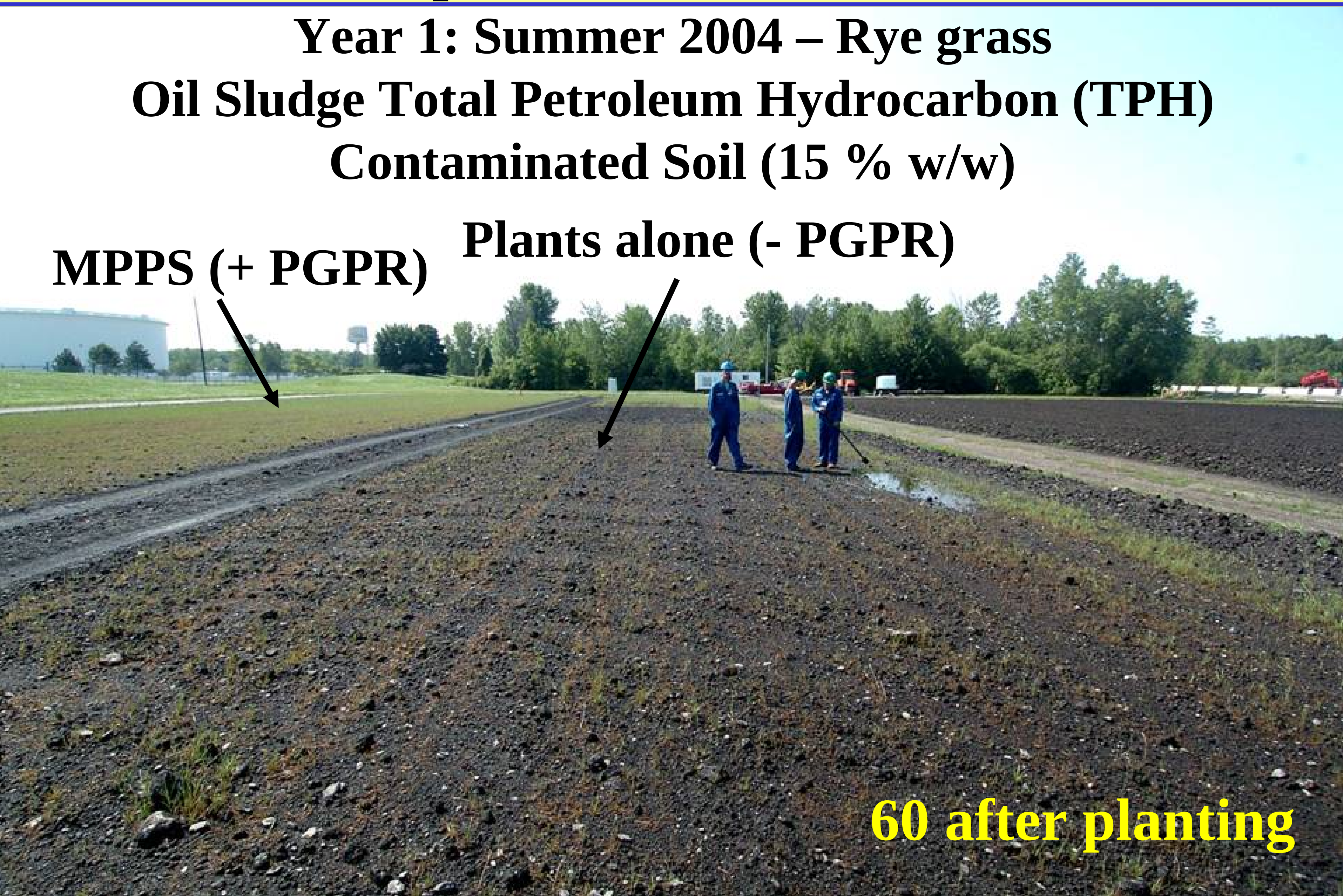




- **Controlled experiment**
- **Test depth of remediation**
- **UW3 + UW4 + Me-Celluose**
- **Spring ryegrass and tall fescue**
- **Plants have grown well**
- **Positive PGPR effect**
- **Remediation Sept 5:**
  15 % w/ PGPR, 6 % w/o PGPR

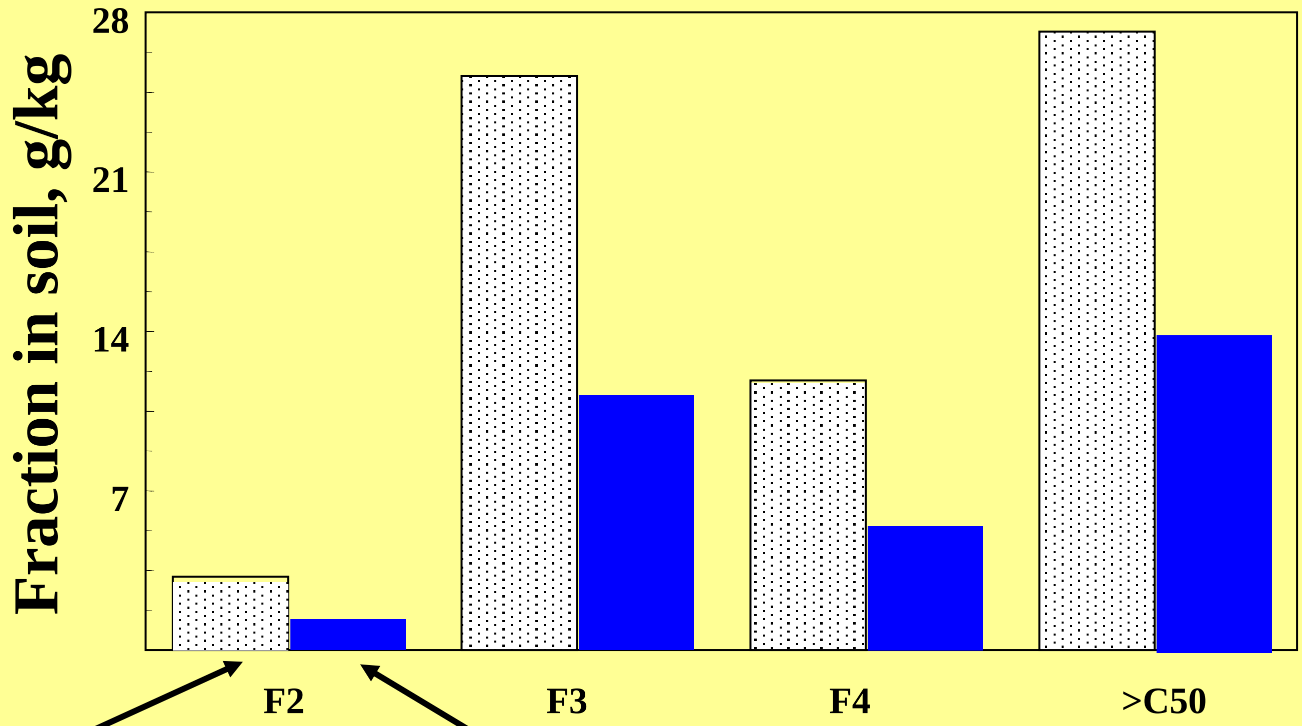
Field Test at Imperial Oil Land Farm, Sarnia, ON

Year 1: Summer 2004 – Rye grass
Oil Sludge Total Petroleum Hydrocarbon (TPH) Contaminated Soil (15 % w/w)



Contaminants metab and/or degraded:

- ~75 g TPH/kg soil removed
- All fractions removed
- 100 g DW plants/kg soil.
- Plants cannot be 75 % TPH.

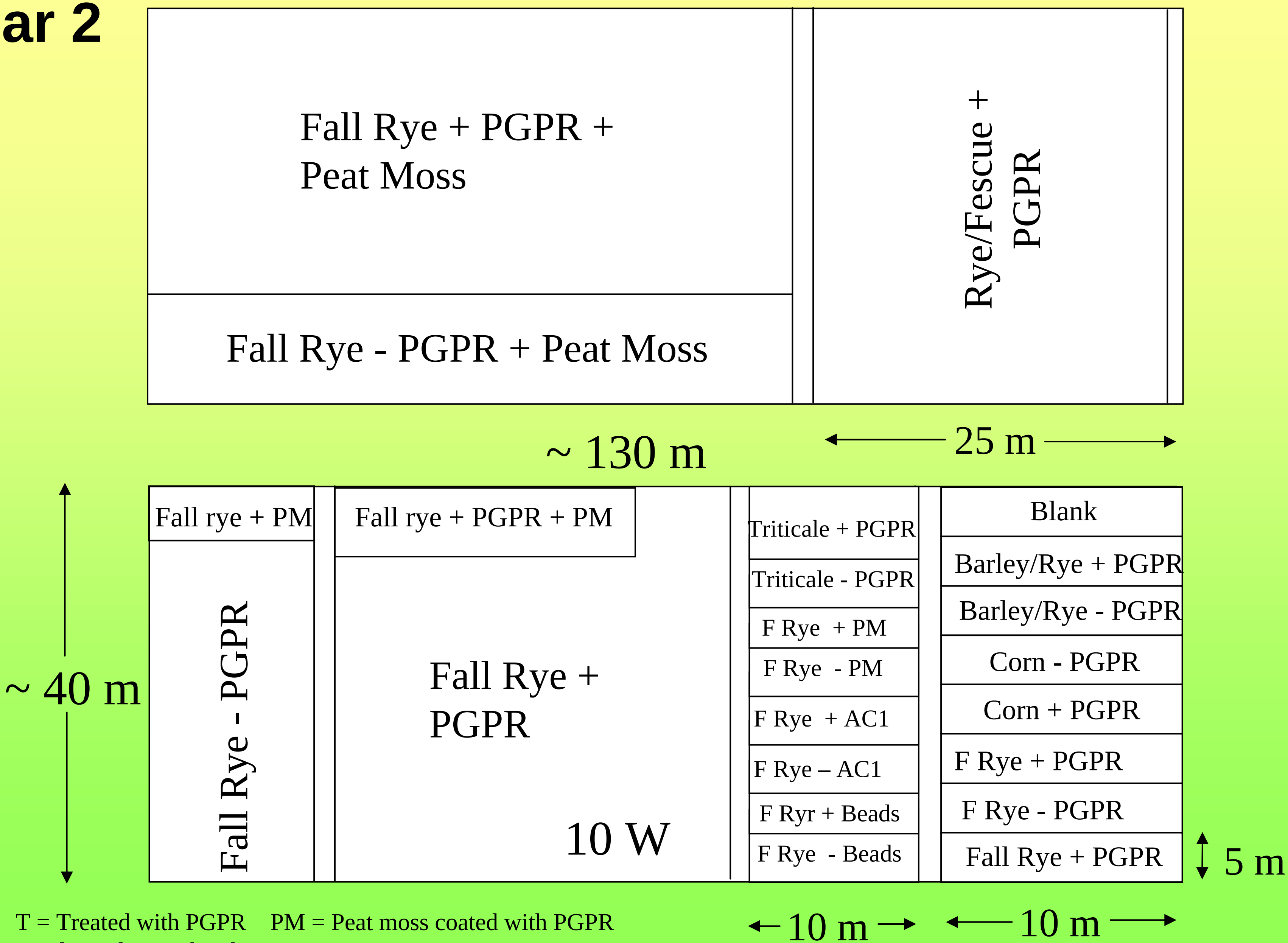


MPPS

Note: Summer 2004 was a cool wet summer

ation of TPHs CCME from Imperial Oil Land Farm, 120 d

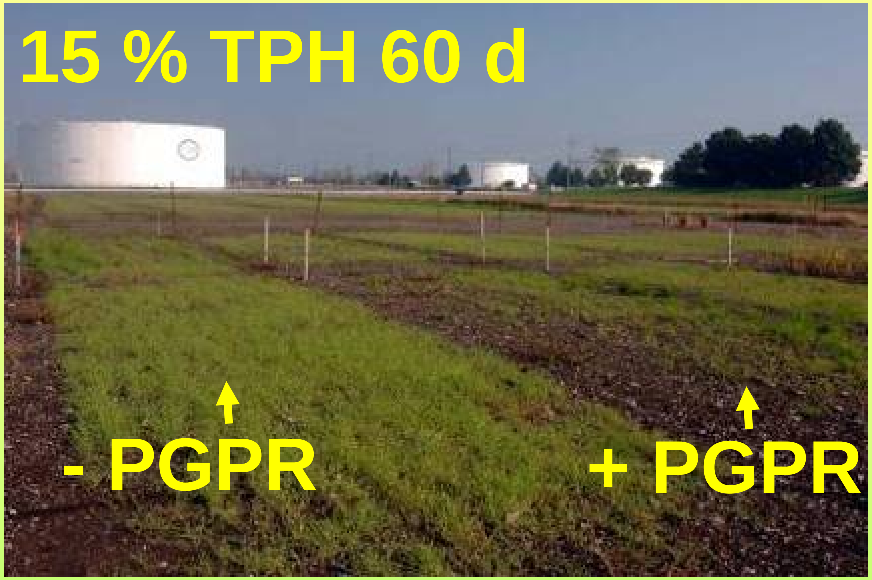
Sarnia Land Farm – 10E and 10 W – 2005 Planting Year 2

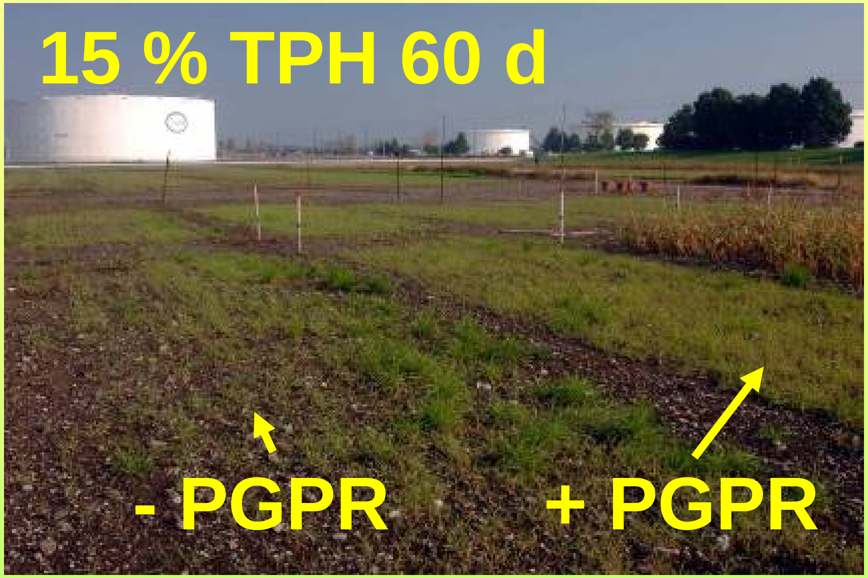


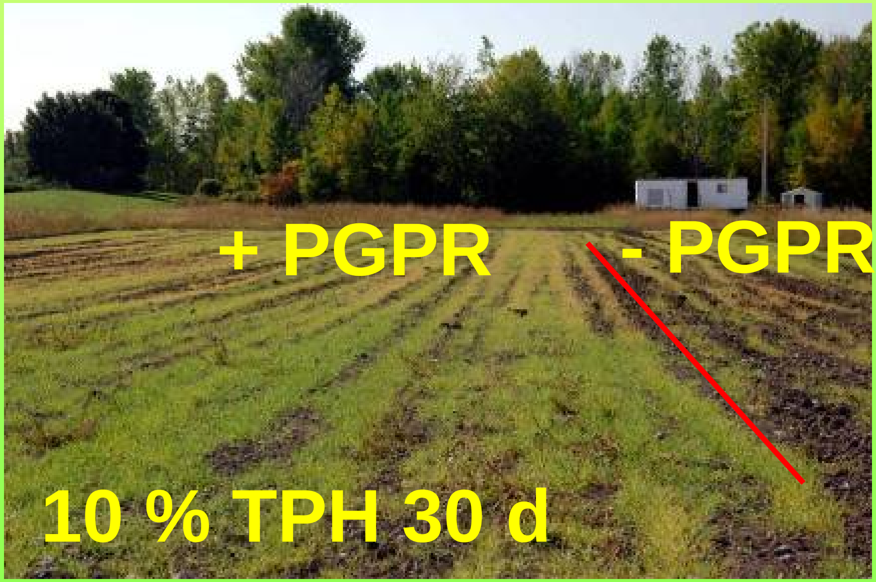
T = Treated with PGPR PM = Peat moss coated with PGPR
Beads = Alginate beads containing PGPR

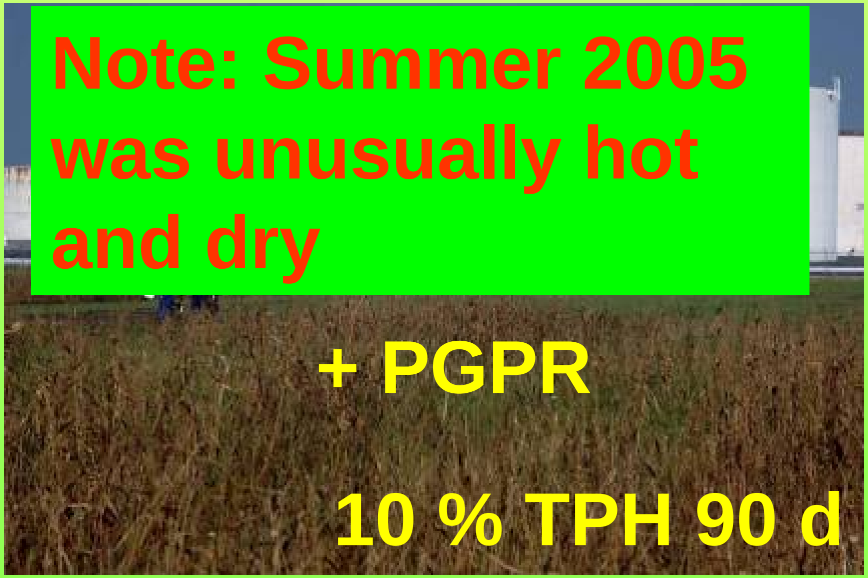
Not to scale

Imperial Oil Land Farm, Sarnia, ON
Year 2: 2005 – Fall Rye Growth









Imperial Oil Land Farm, Sarnia, ON
Summer 2005 – Rye/Fescue
Results

Root depth (+ PGPR) after 90 d was > 40 cm

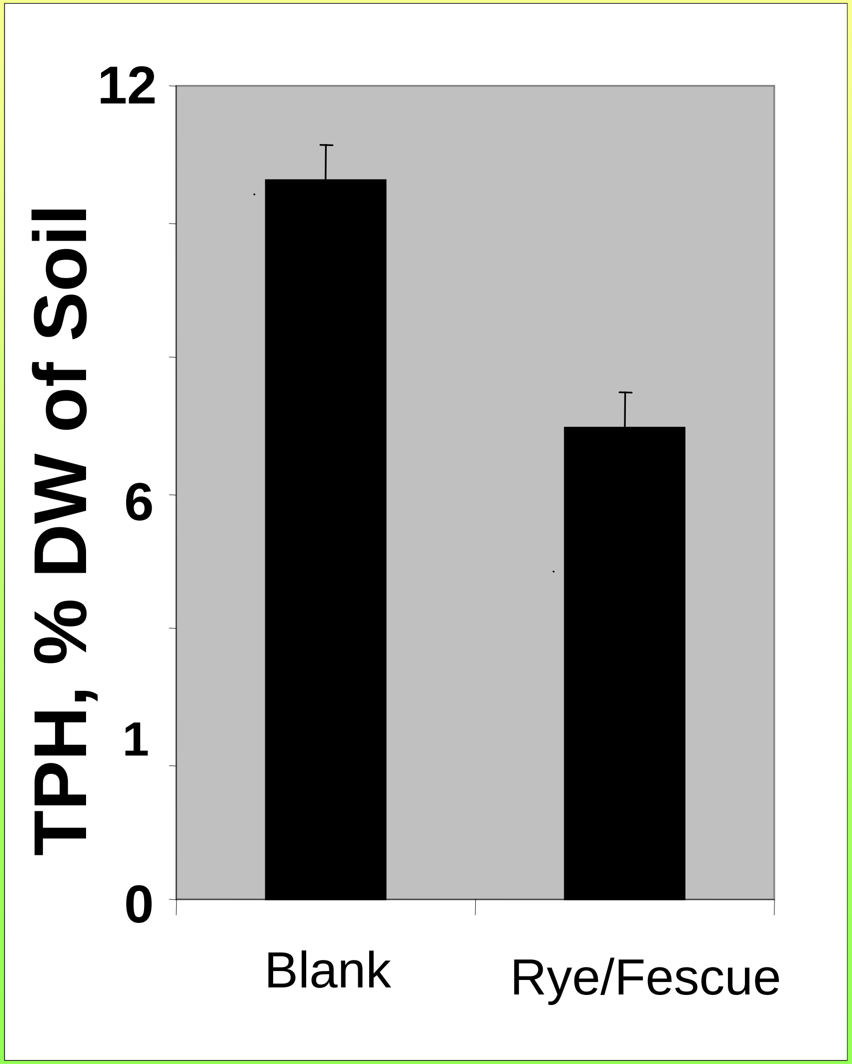
Extent of Remediation

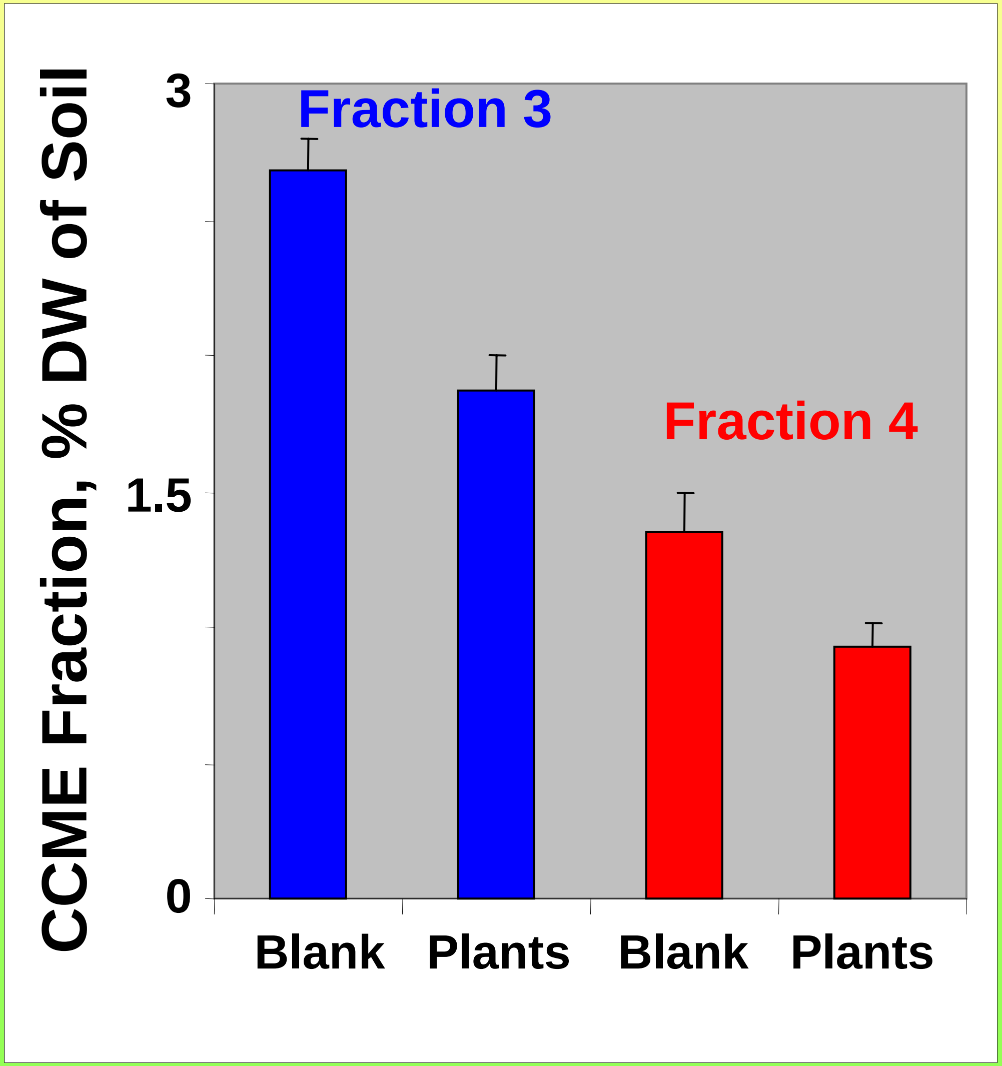
+ PGPR

30 d	9 % ± 1.5
60 d	17 % ± 2
120 d	35 % ± 4

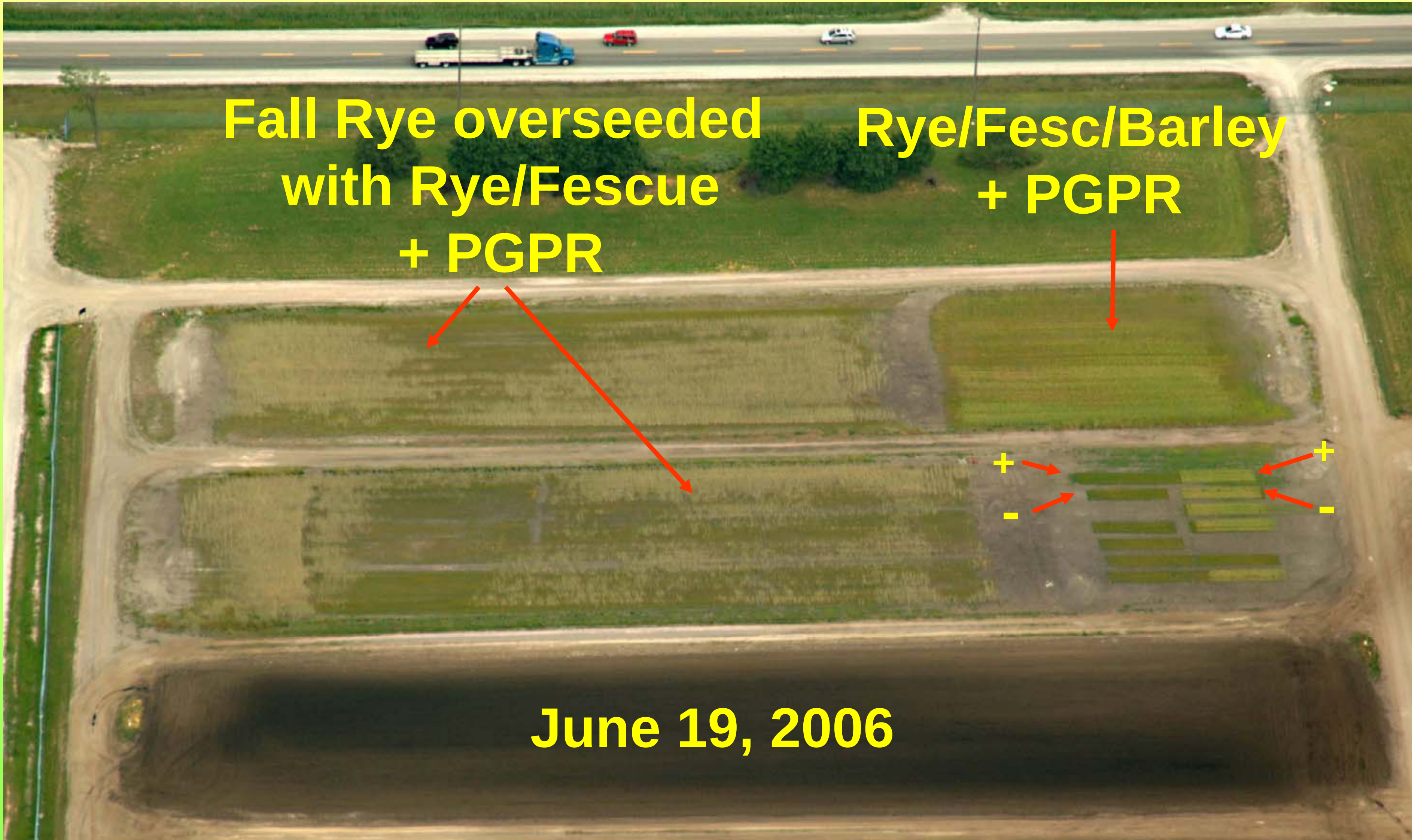
- PGPR remediation was about 50 % slower

TPH Removal from Sarnia Year 2



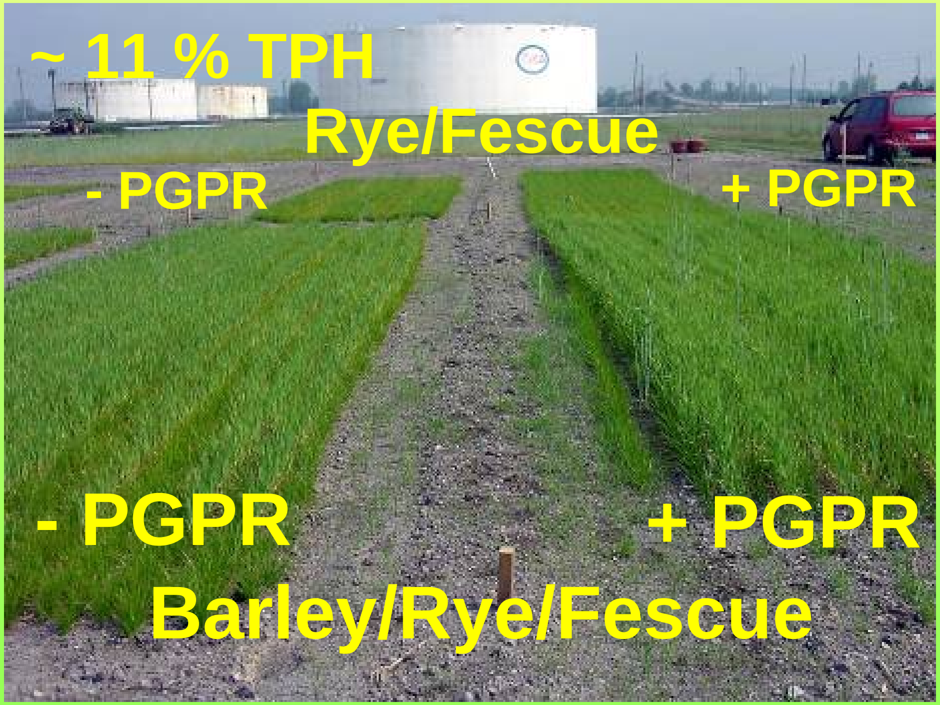


Imperial Oil Sarnia Land Farm Year 3



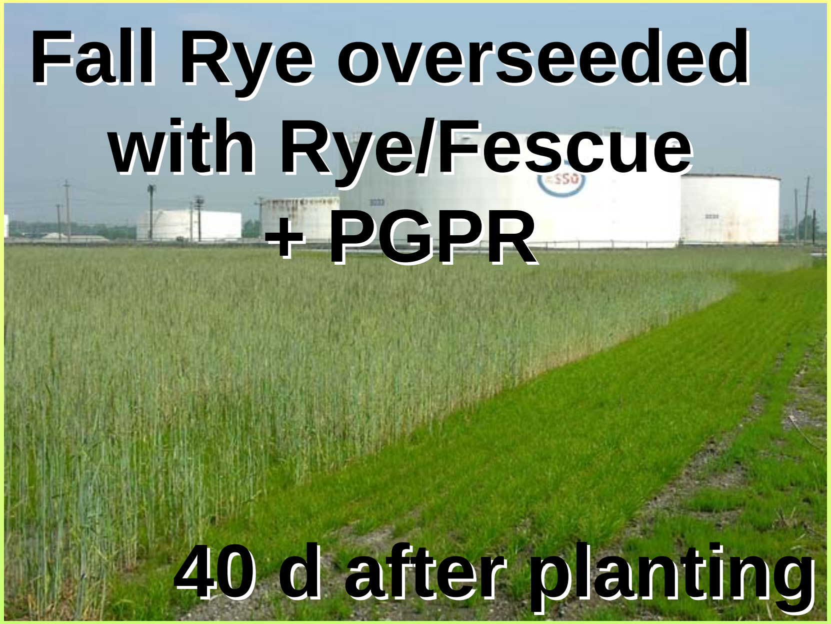
Imperial Oil Sarnia Land Farm
Year 3 (2006)

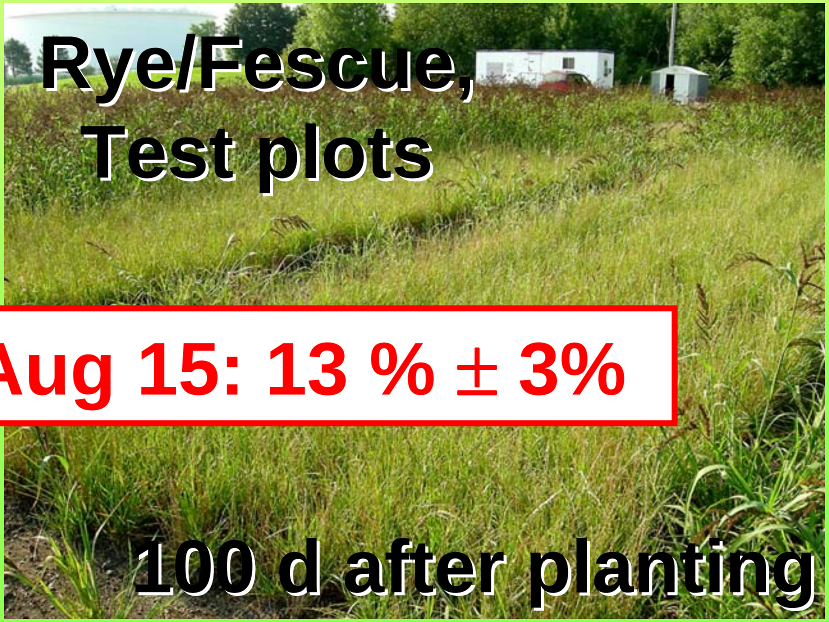
- Planted Barley/Fescue/Rye Grass on April 20, 2006
- Plants were treated with PGPR (UW3 and UW4) using a mechanical seed treater



40 days after planting – Weather good

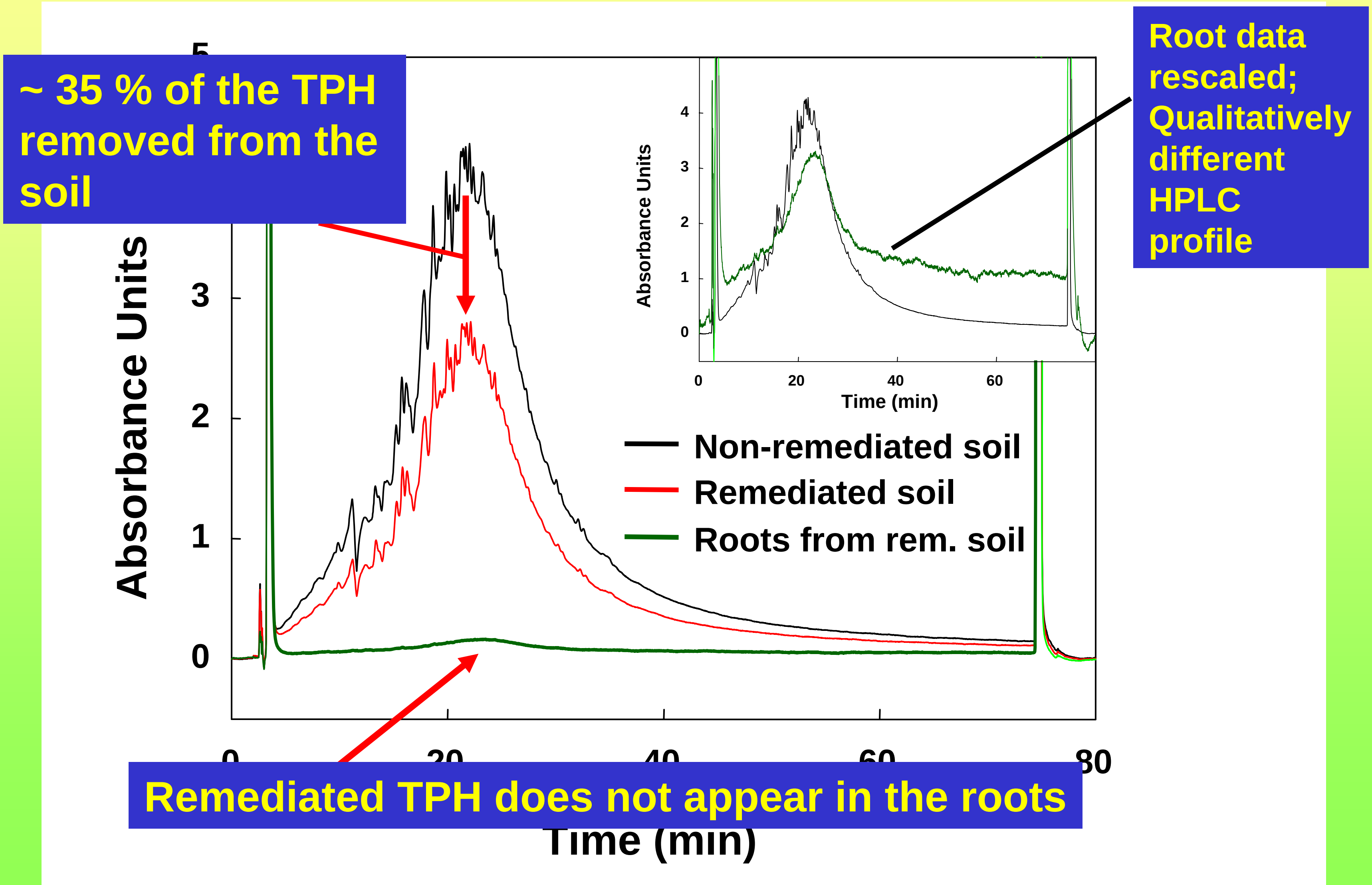
Imperial Oil Sarnia Land Farm Year 3





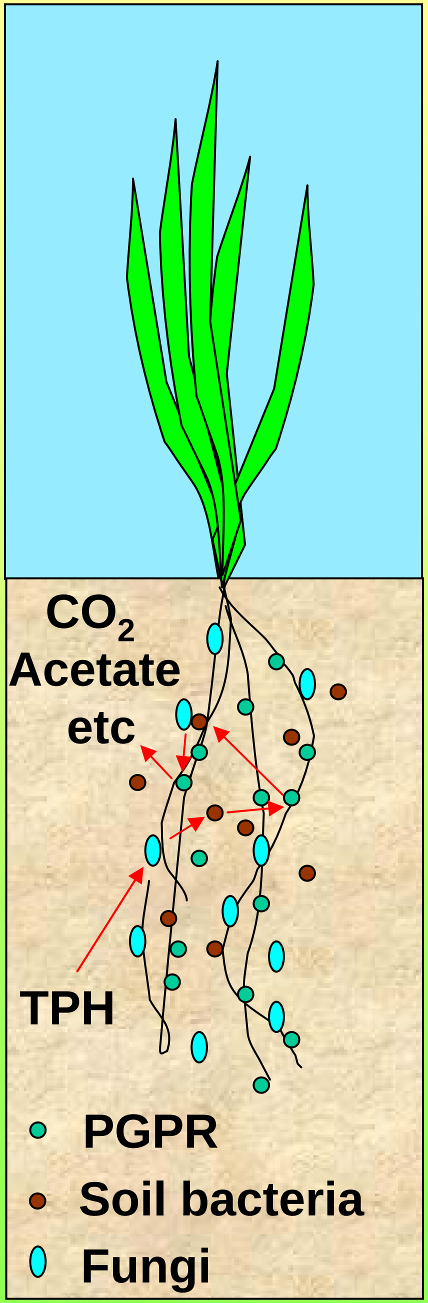
Remediation data on Aug 15: 13 % $\pm$ 3%

HPLC assay for TPH uptake by Rye/Fescue roots



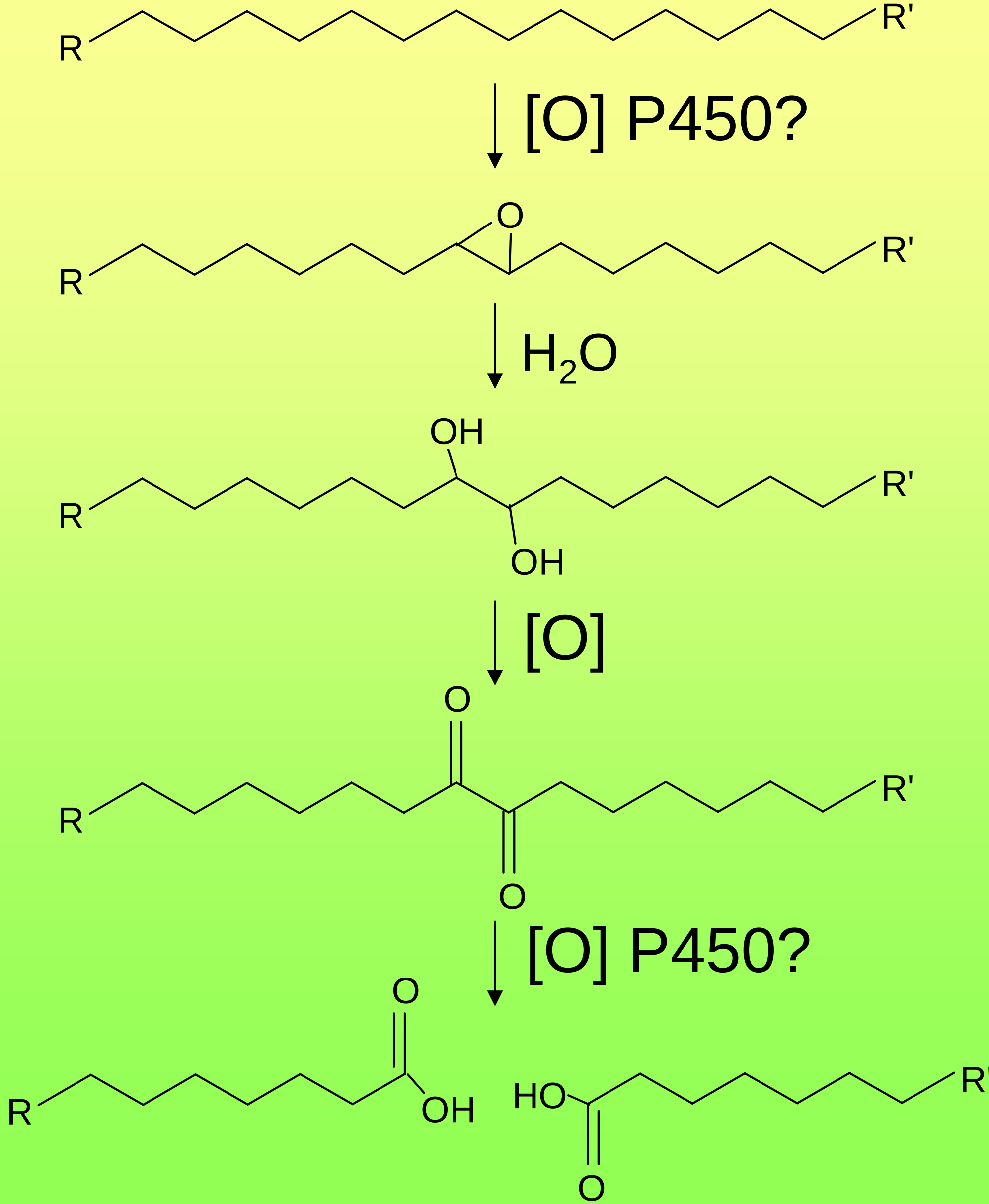
What happened to the TPH?

- TPH removed from the soil, but not in the plant roots
- TPH must have been degraded
- Where was it degraded?
- Perhaps in rhizosphere by bacteria, fungi and roots
- Soil fungi and bacteria have very active and diverse metabolic activities
- In soils with PGPR treated plants, bacteria and fungal counts are 5 to 10 fold higher



Possible degradation pathway of TPH in Soil

Aliphatic hydrocarbon



2 Fatty Acids

PGPR Can Degrade Oil

Saturated TPH in Phos-Buff-Saline

TPH sole reduced carbon source

No Inoculation   Inoculation w/ PGPR

Grow for 12 h on shaker

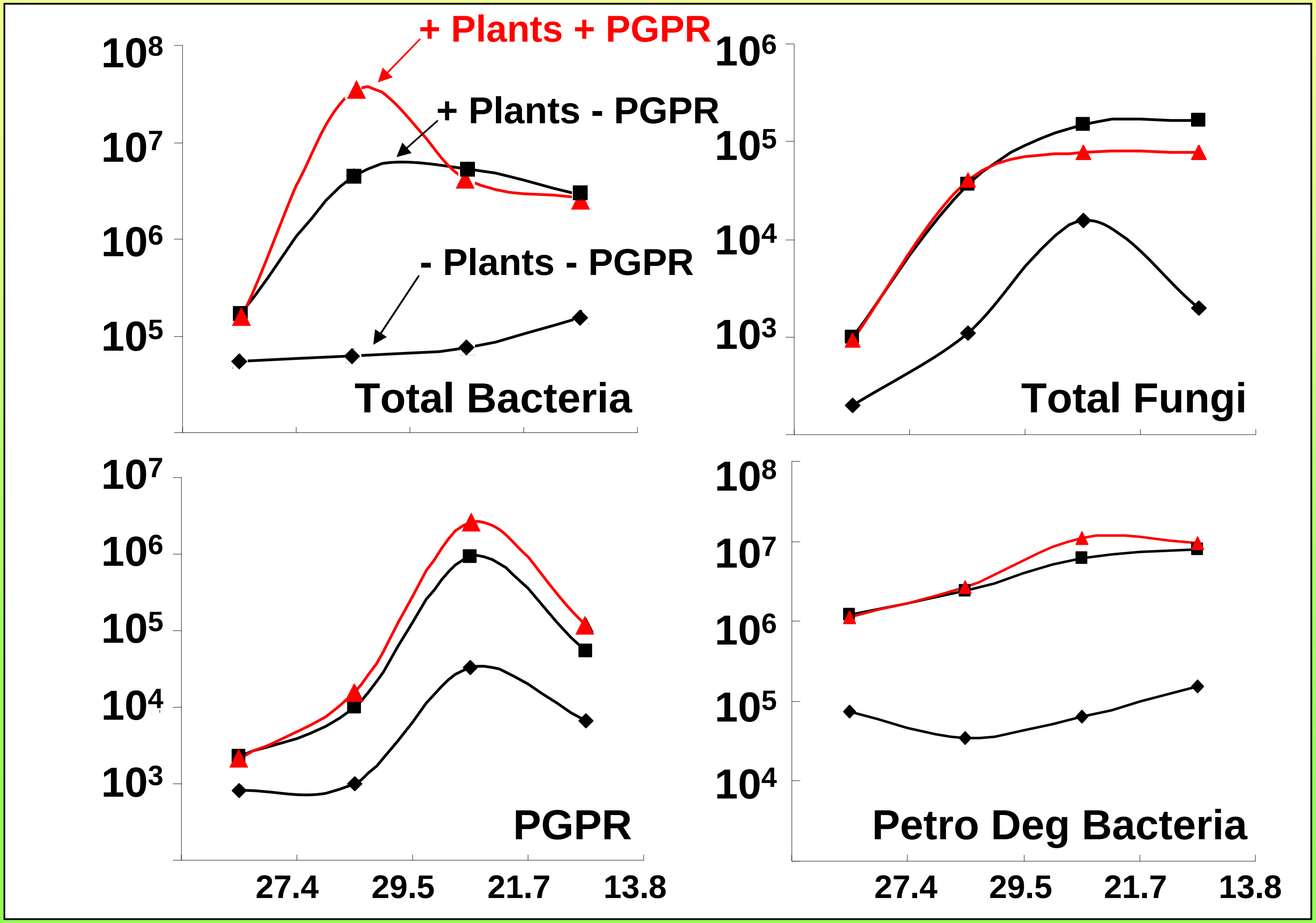
Control   UW3 (PGPR)

Control   UW4 (PGPR)

Control   Oil consuming bact.

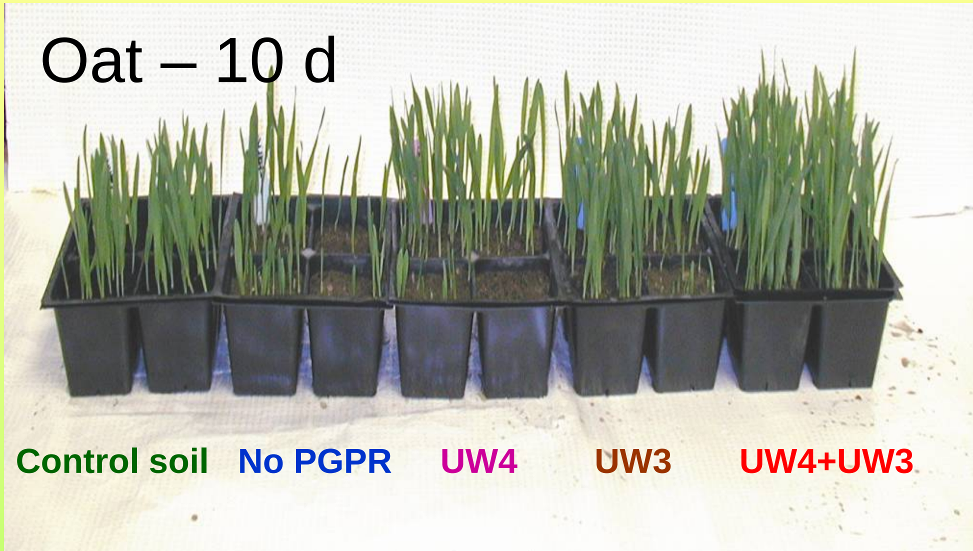
Microbes in Sarnia Land Farm Soil From Remediation Field Trial 2006

Rye Fescue Barley Soils



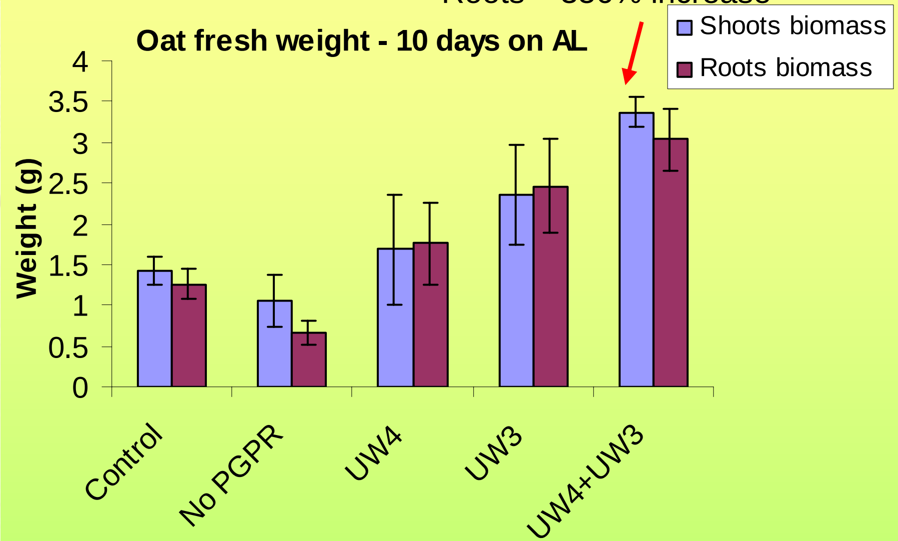
Enhancement of Plant Growth on Salt Contaminated Soils Using PGPR

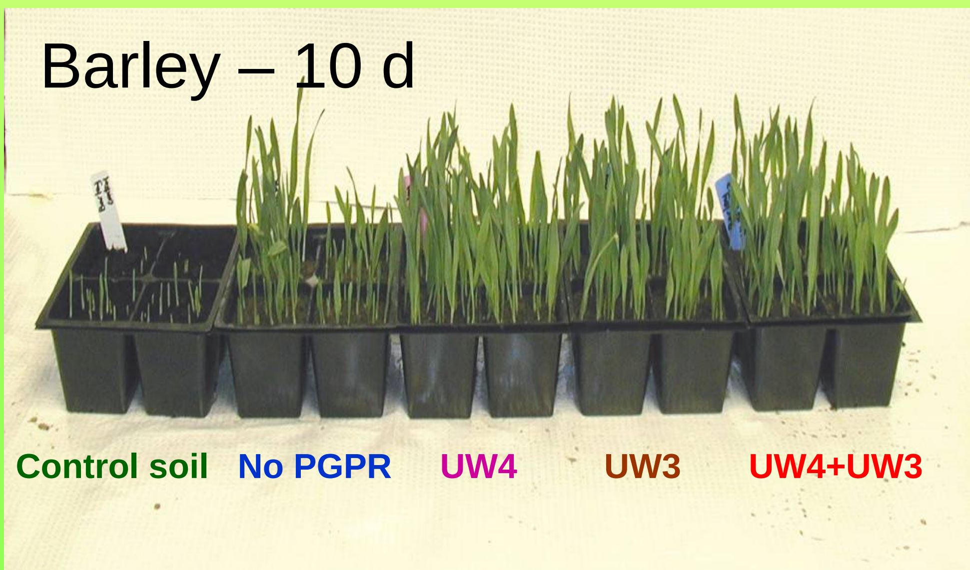
Effect of PGPR on Plant growth in Alberta Salt Impacted Soil
EC = 2.2 dS/m, SAR = 27, Cl = 260 mg/kg



Shoots – 219% increase

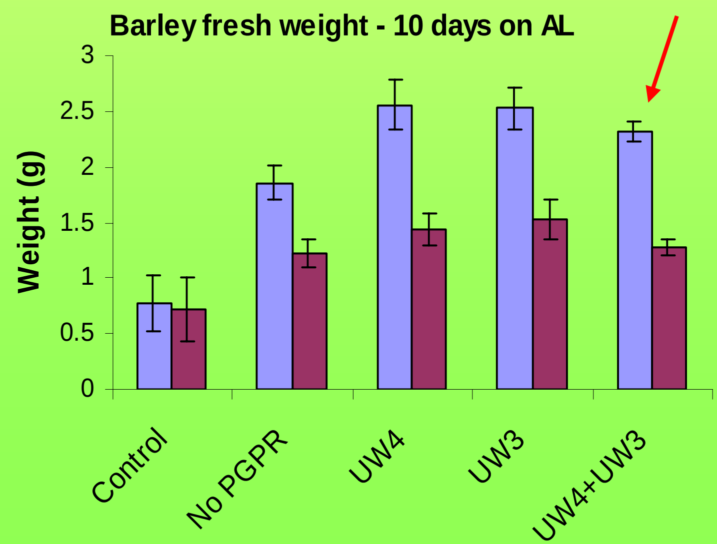
Roots – 356% increase





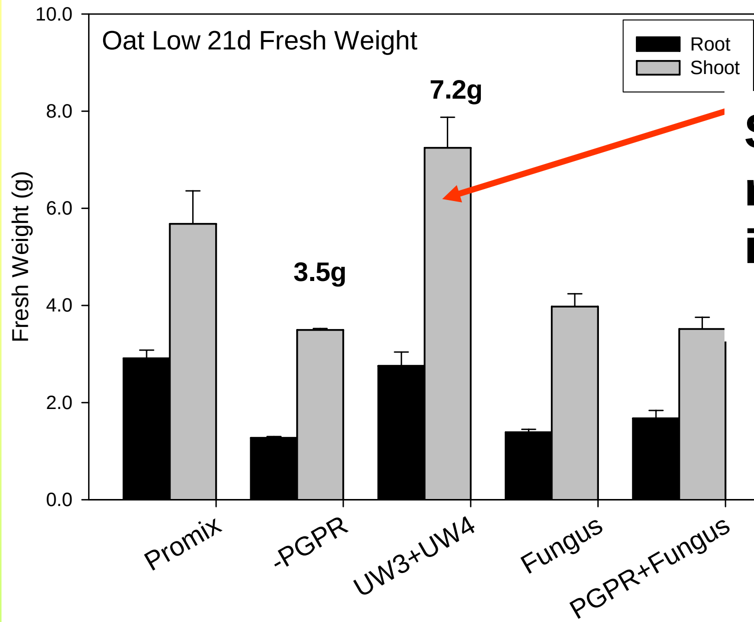
Shoots – 24% increase

Roots – 4.1% increase



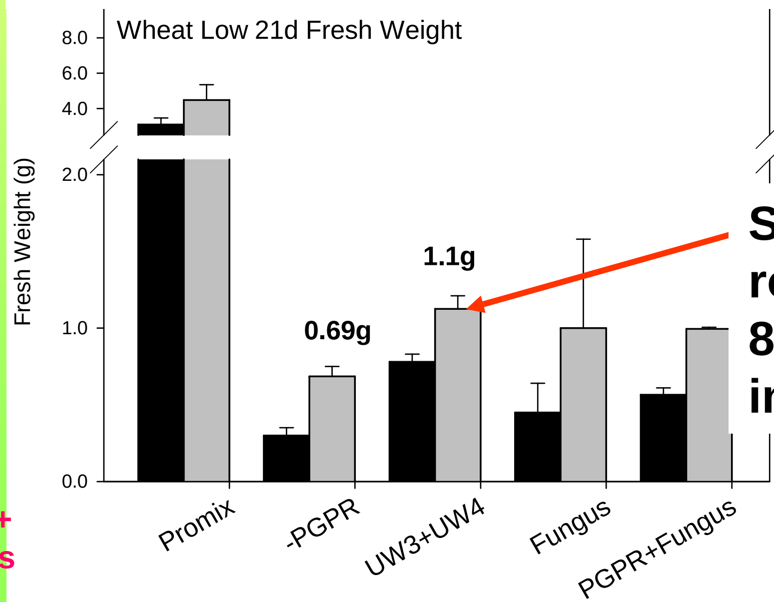
Sask Salt Soil: EC = 14 dS/m , SAR = 11, Cl = 1880 mg/kg

Promix -PGPR UW3+UW4 Fungus PGPR +Fungus



Shoots and roots 100 % improved

Promix -PGPR UW3+UW4 Fungus PGPR+ Fungus



Shoots and roots 60 to 80 % improved

CONCLUSIONS

- **MPPS has great potential for efficient remediation of organic, salt and metal contaminated sites**
- **PGPR is the key: healthy plants with vigorous roots in PAH, TPH, DDT, salt and metal contam. soils**
- **PGPR alleviate stress and promote growth: Low ethylene and high auxin**
- **8 Months in the greenhouse: MPPS removed 90 % of recalcitrant TPHs and PAHs**
- **3 years of fields tests successful: MPPS removed 30 % to 60 % of recalcitrant TPHs and DDT per year**
- **Contaminants metabolized and/or degraded**
- **Great promise for restoration of oil and salt impacted sites, and brownfields**

Future Work

- Continued field testing of the MPPS
- Increasing research on salt remediation
- Ready to deploy at new sites
- Proposals for new sites are being entertained
- TPH sites can be remediated in 2 to 4 years
- Further research on salt and metal remediation underway

Colleagues and partners

- The people that do all the work
  - Karen Gerhardt
  - Yola Gurska
  - Mark Lampi
  - Pearl Chang
  - Wenxi Wang
  - Haitang Wang
  - Aaron Khalid
  - David Isherwood
  - Shan Shan Wu
  - Julie Nykamp
  - Anabel Ueckermann
  - Xiao-Ming Yu
- Collaborators
  - Xiao-Dong Huang
  - Bernie Glick
  - Perry Gerwing
  - George Dixon
  - Ron Pitblado
- Partners
  - D Bristow, L Lawlor, M Young, Imperial Oil
  - J Gordon, Talisman Energy
  - A Hopf, BASF
  - G Surgeoner, OAFT
  - G Stephenson, Stantec
  - G Brooks, CRA
  - D Hoffman, Ginseng Growers
  - Region of Waterloo
  - City of Guelph
  - NSERC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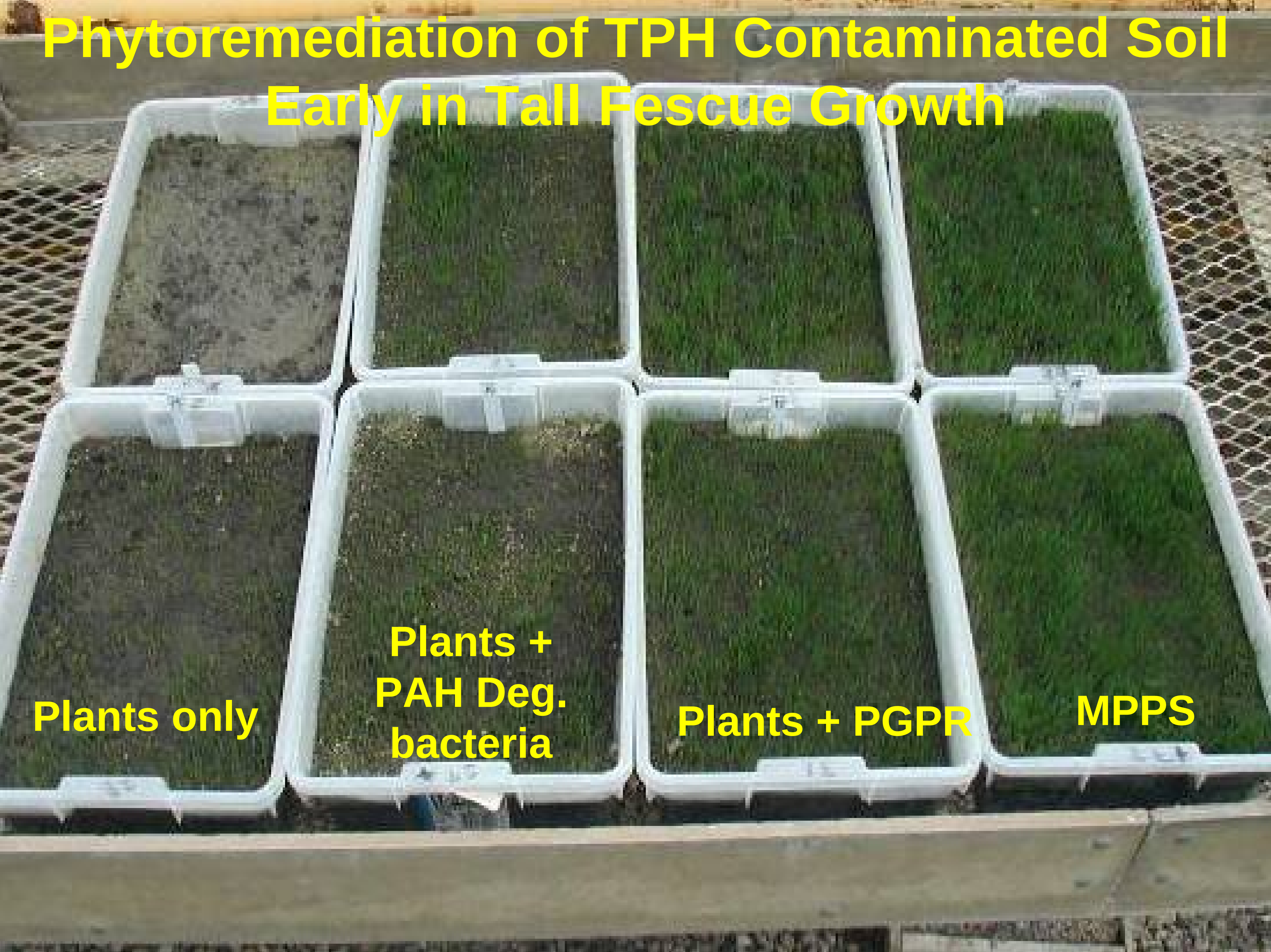
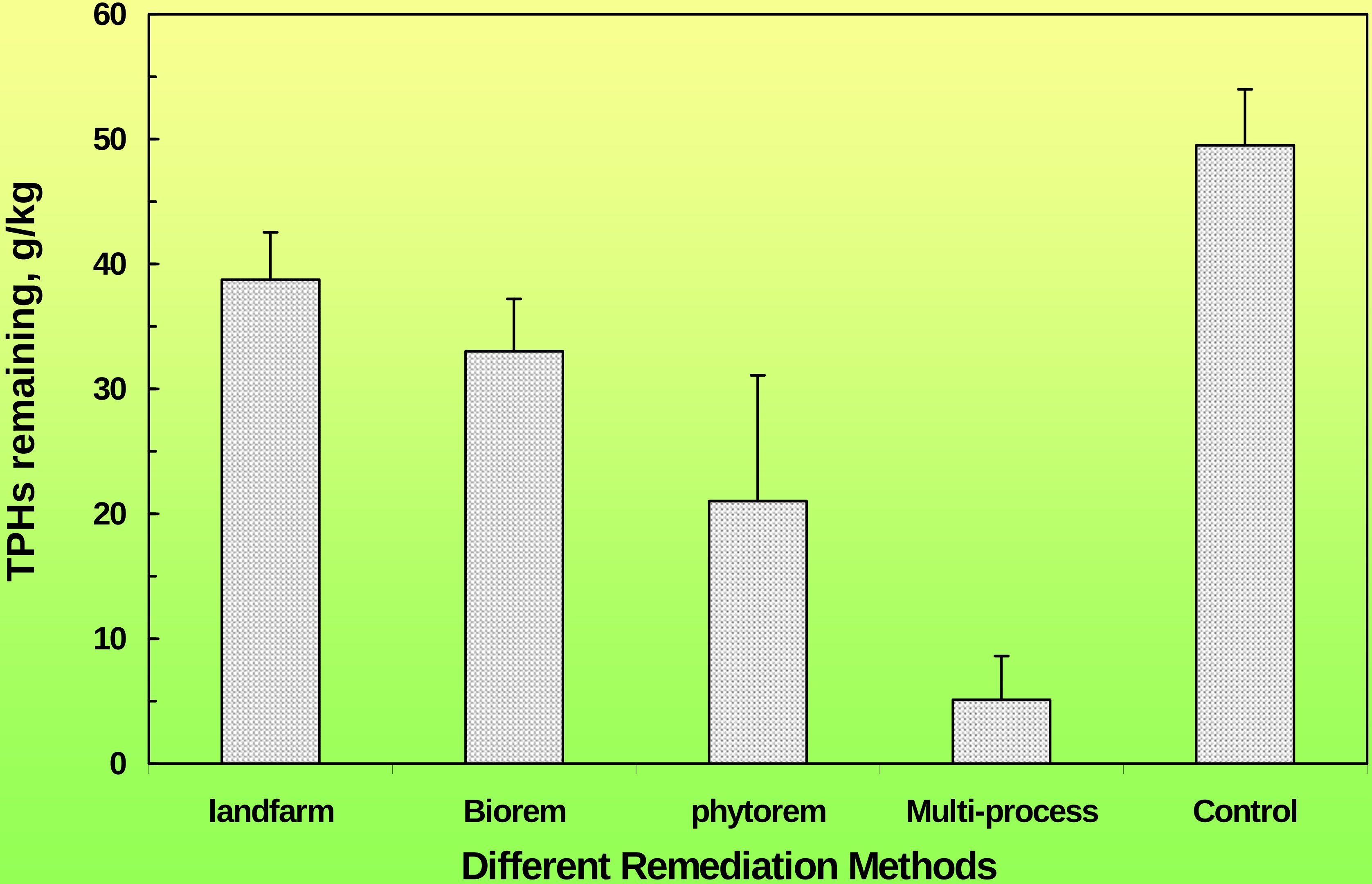
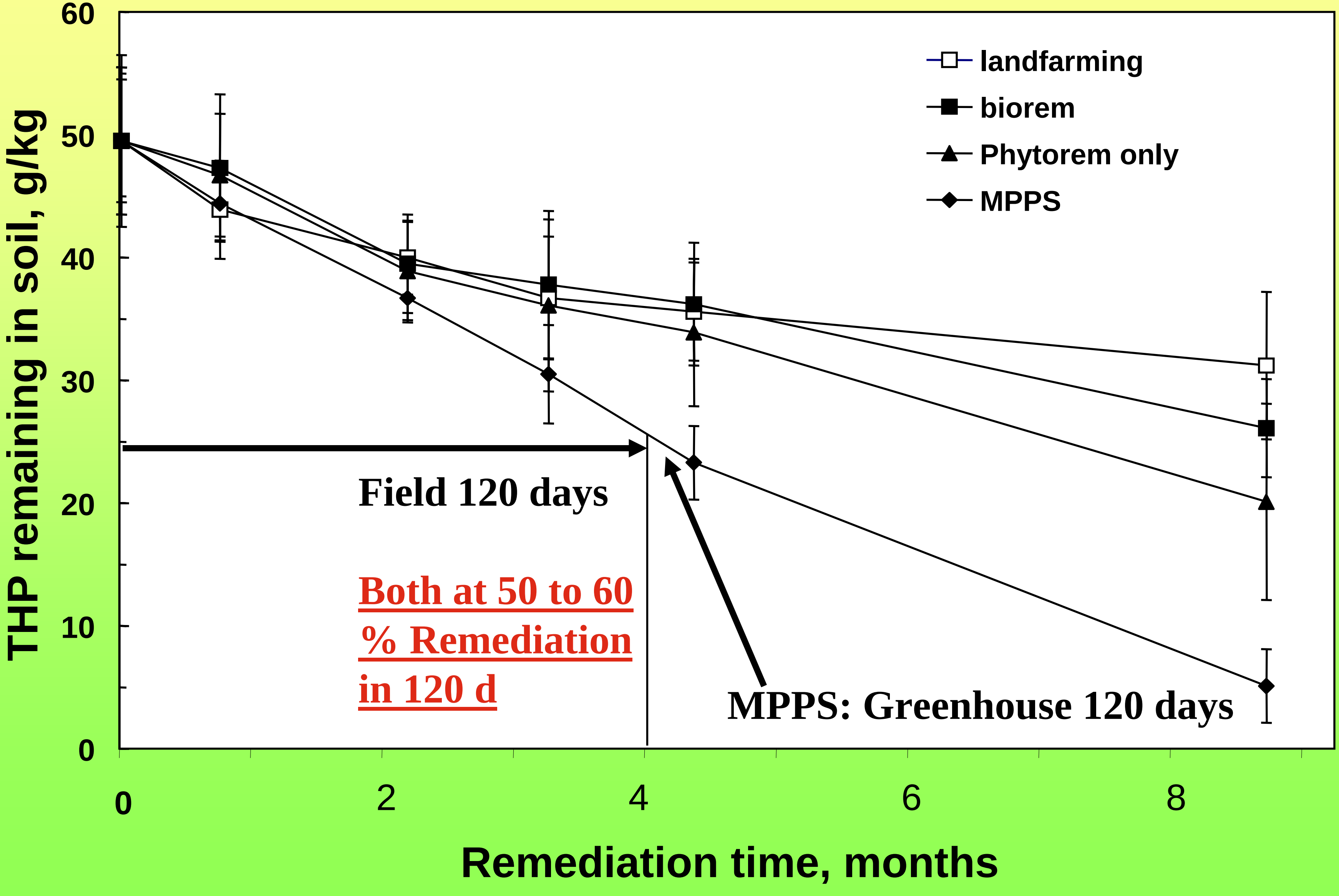

Phytoremediation of TPH Contaminated Soil
Early in Tall Fescue Growth
Plants only
Plants +
PAH Deg.
bacteria
Plants + PGPR
MPPS

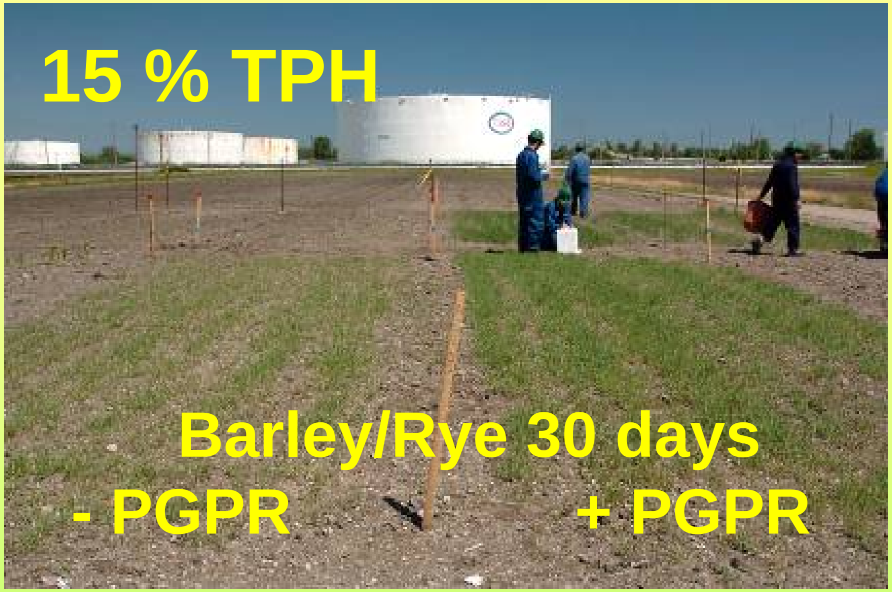
Effectiveness of Different Methods on TPH Remediation - Second 4 month season (i.e., 8 months total)



Remediation Kinetics For Land Farm Soil
Greenhouse vs. Field



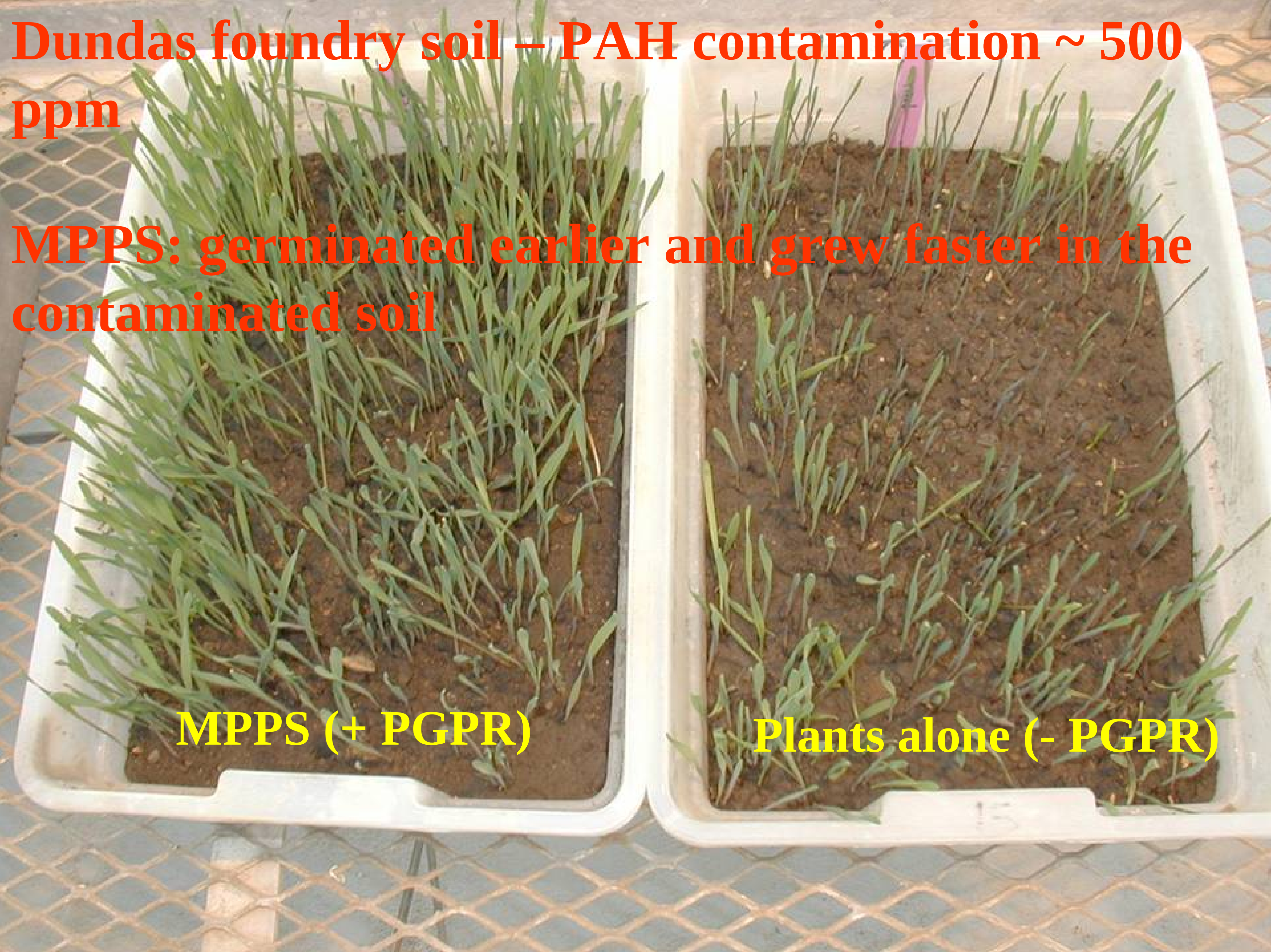
Sarnia Land Farm – 2005

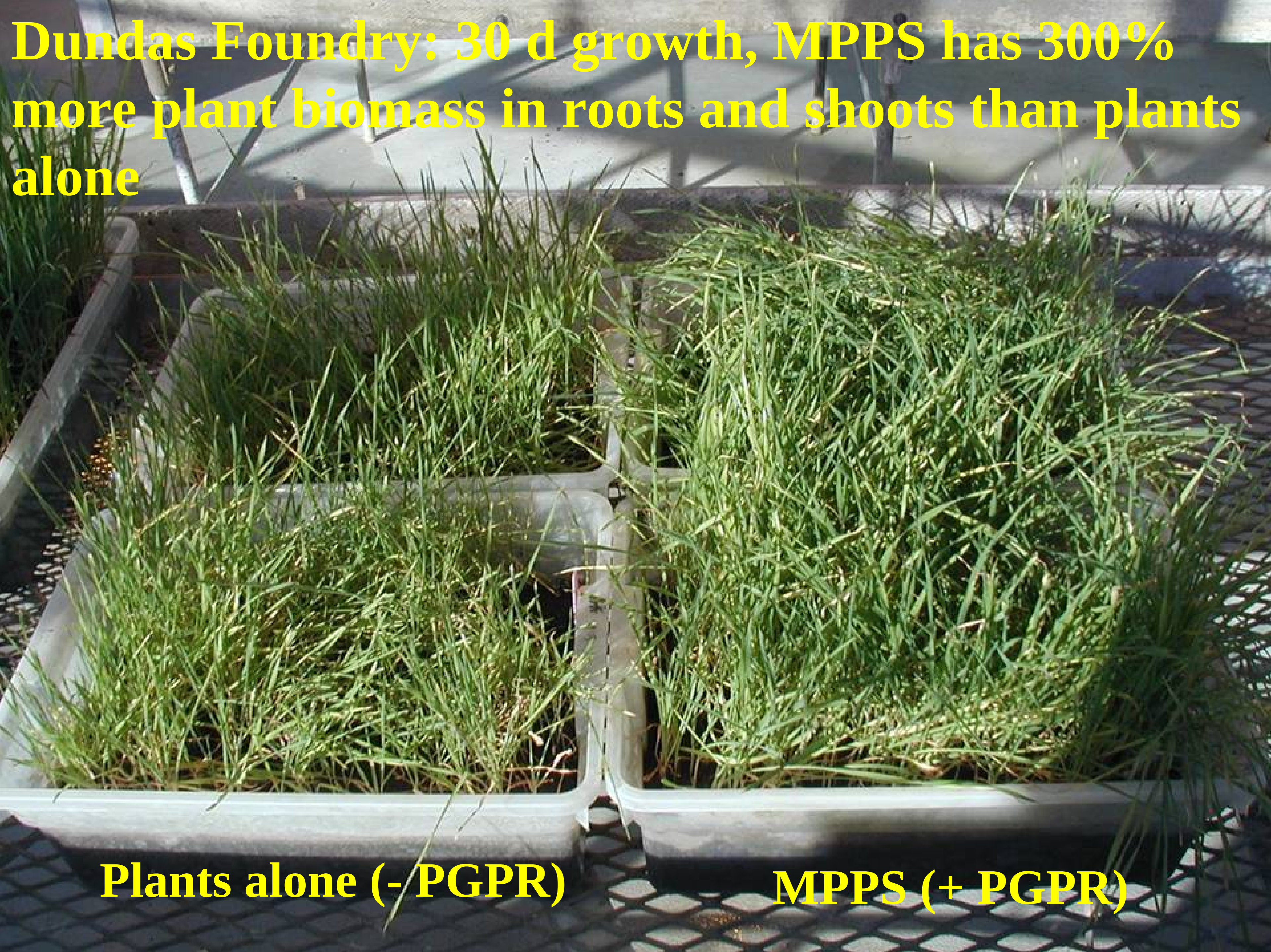


Barley/Rye + PGPR - PGPR

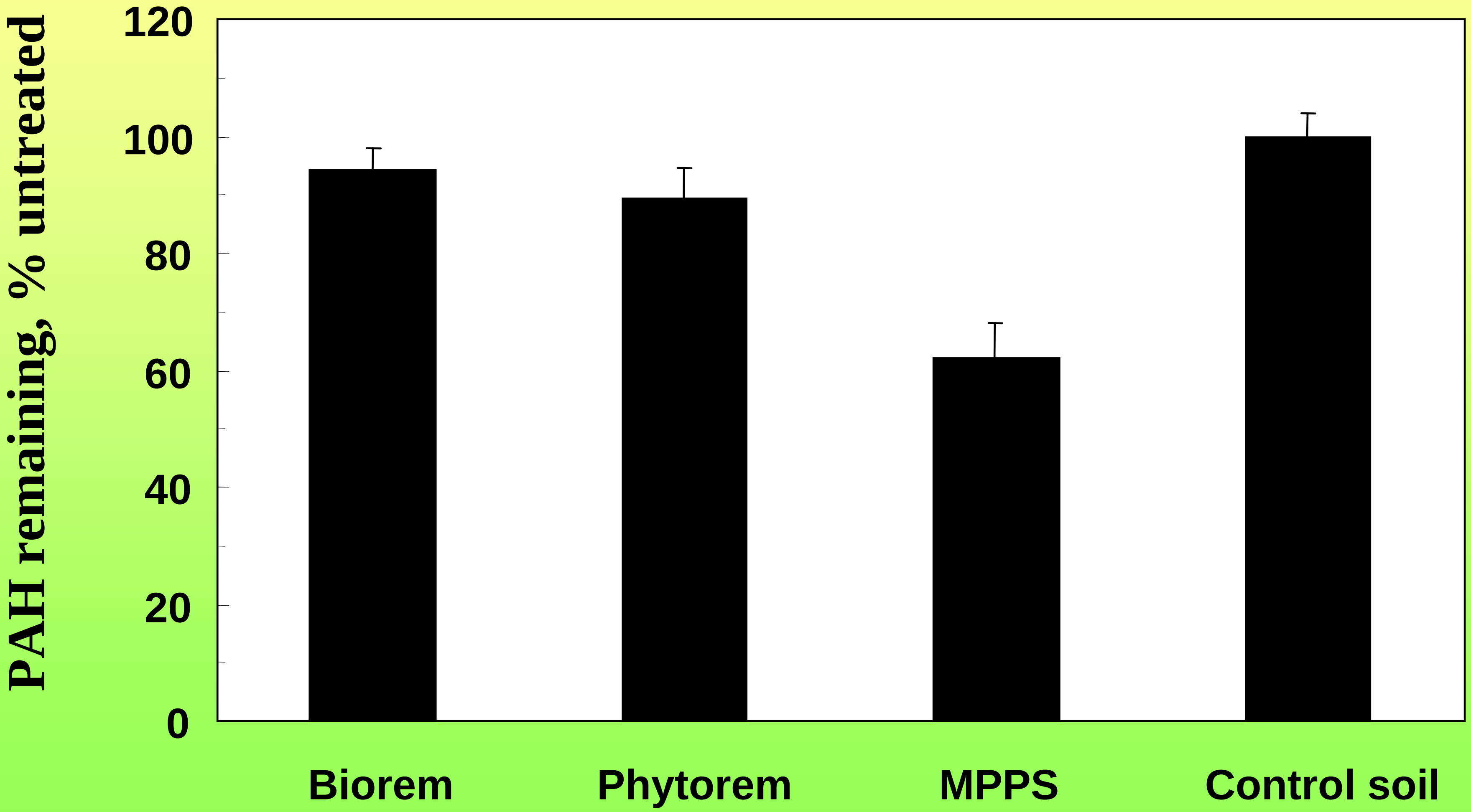

Control
Cu 1.0g/ kg
+ PGPR
Cu 1.0g/ kg
No PGPR
Remediation of Metals

PGPR Effect on Plant Growth with 2% Salt in Irrigation Water

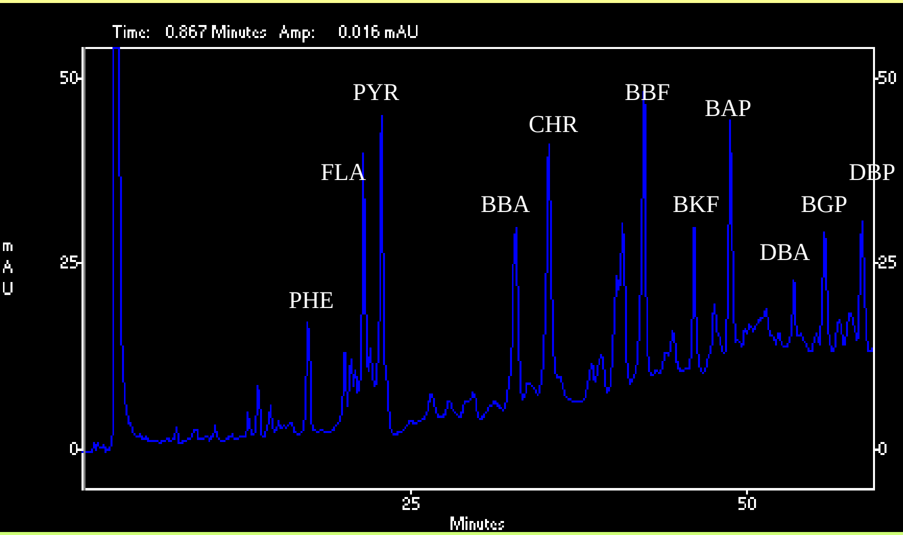

Dundas foundry soil – PAH contamination ~ 500 ppm
MPPS: germinated earlier and grew faster in the contaminated soil
MPPS (+ PGPR)
Plants alone (- PGPR)


Dundas Foundry: 30 d growth, MPPS has 300% more plant biomass in roots and shoots than plants alone
Plants alone (- PGPR)
MPPS (+ PGPR)

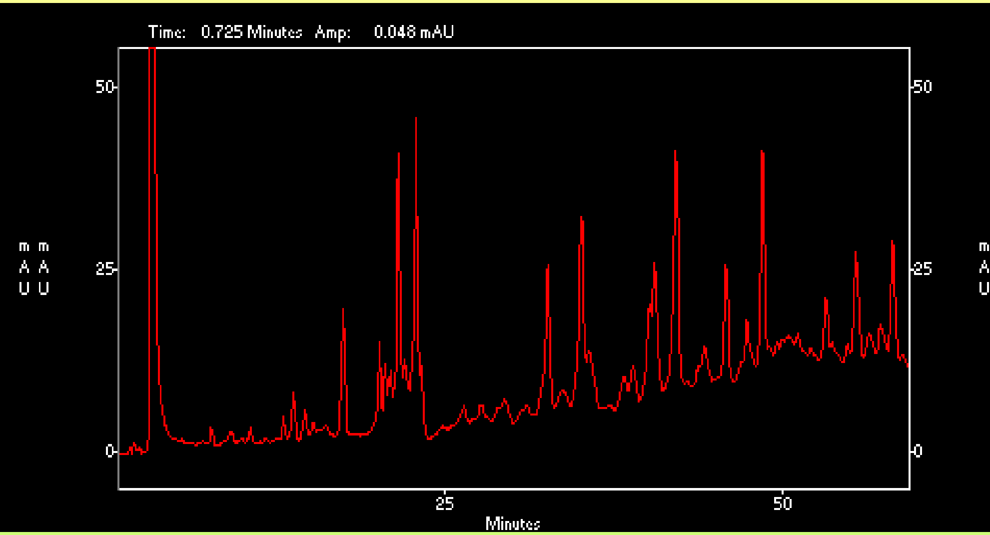
Effectiveness of the MPPS for Remediation From Dundas Foundry Soil: 90 days



Multi-Process System for Remediation of PAH Contaminated Brownfield: 90 d remediation leads to ~ 40 % removal of organics

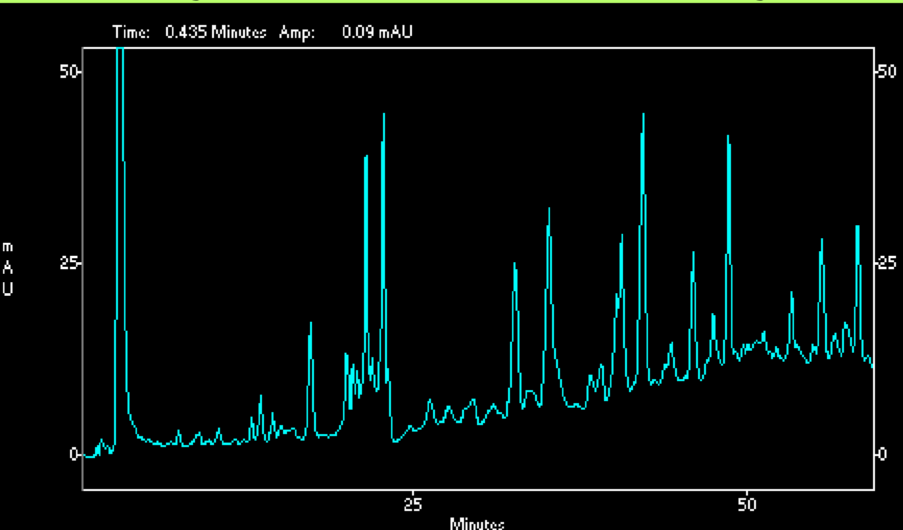


Untreated brownfield soil

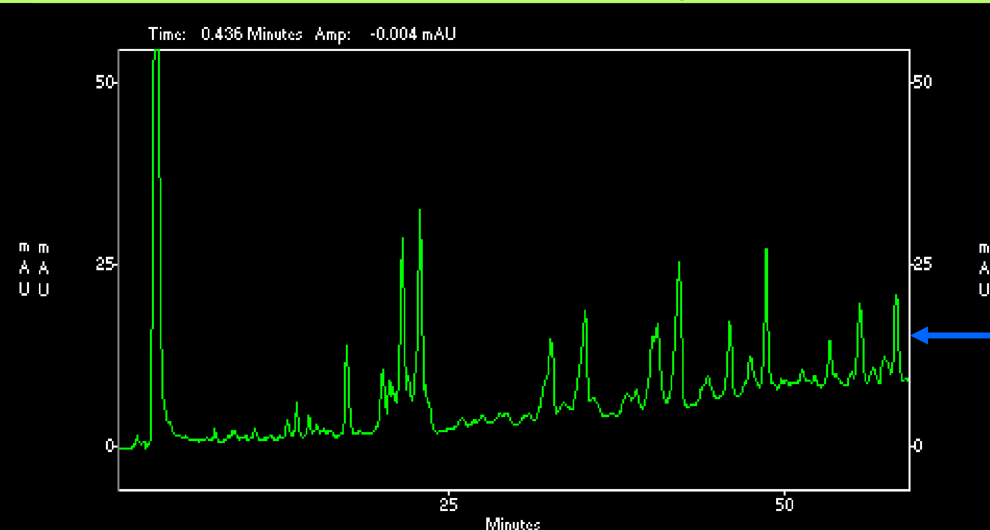


Bioremediation for 90 days

Phytoremediation for 90 days



MPPS for 90 days



DDT Remediation with Millet Summer 2005, Simcoe, ON

70 d growth

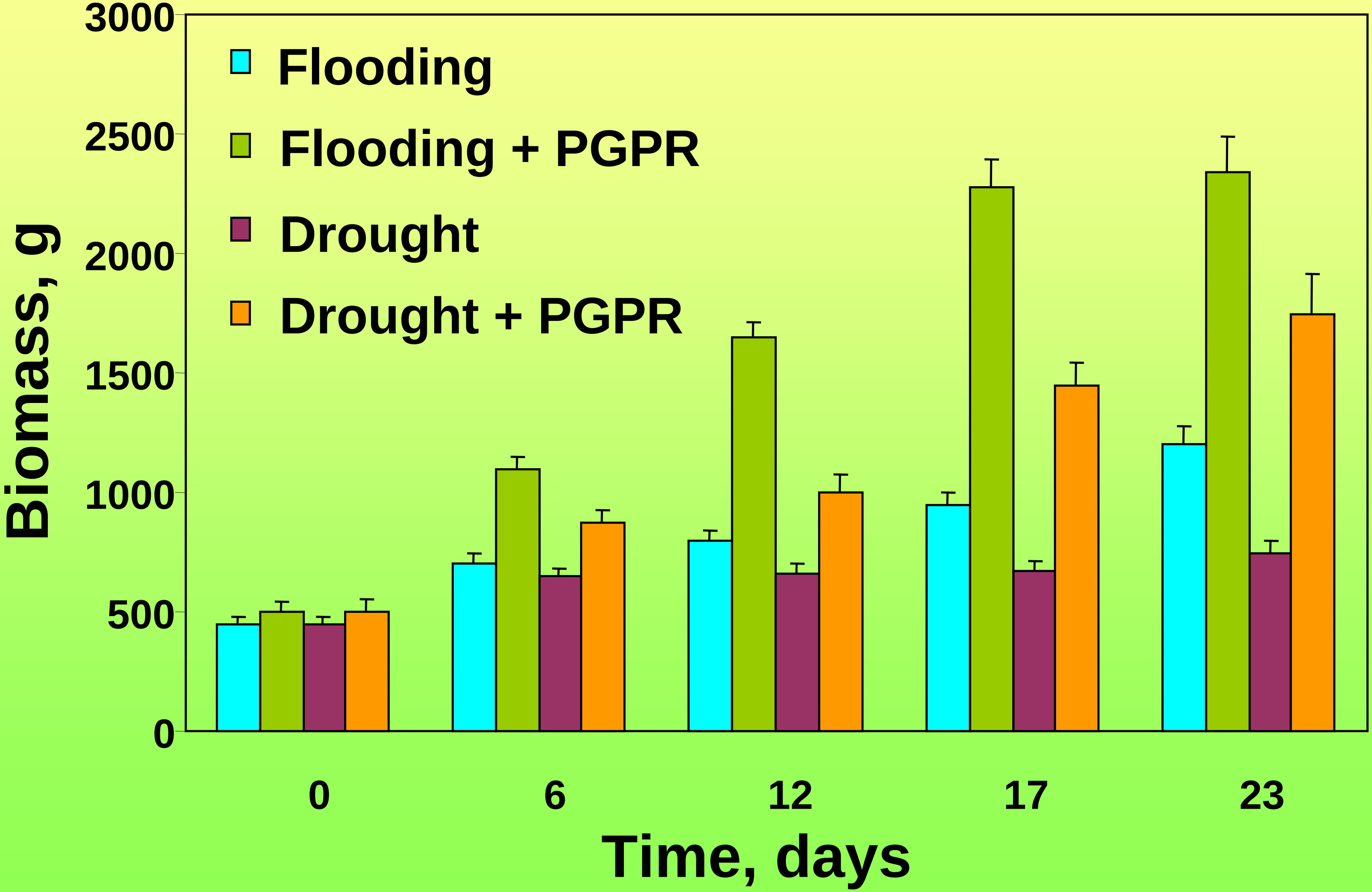
+ PGPR
+ Peat Moss w/PGPR

+ PGPR
- Peat Moss

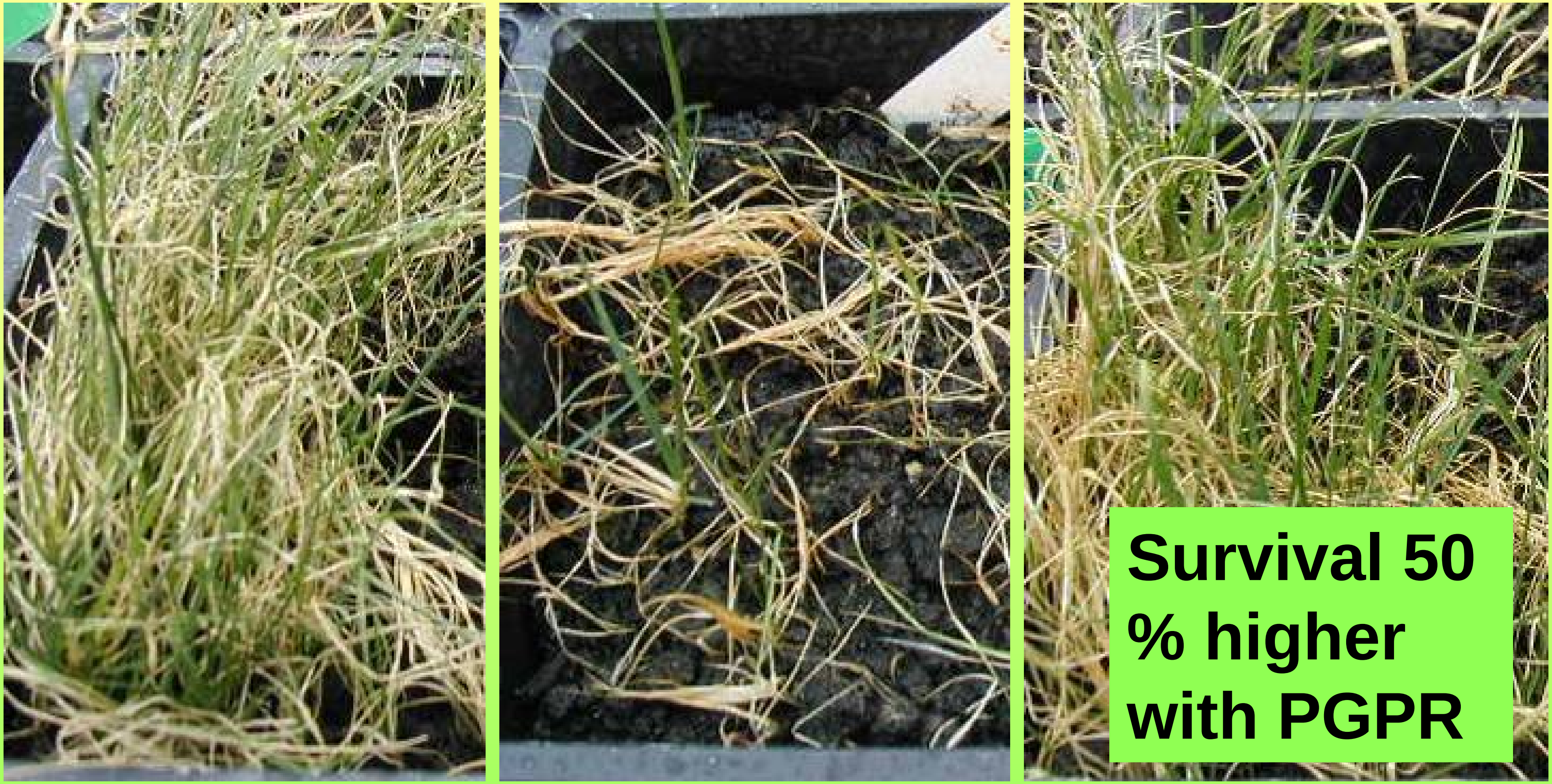
Remediation of DDT

- **Approximately a 0.5 Hectare site**
- **SW Ontario farmland**
- **Homogeneous levels of DDT at ~ 0.8 mg/kg (ppm)**
- **Half planted with Millet and half planted with fall rye**
- **<u>40 % overall DDT remediation</u> after 90 d**
- **DDT breakdown products (DDE or DDD) not found**
- **Chlorinated compounds can be degraded by phytoremediation**

Effect of PGPR on Plant Growth during Flooding or Drought Stress



Growth of rye grass in salt contaminated soil



EP3 (a salt tol. PGPR) **-PGPR** **UW4+EP3 (2 PGPRs)**

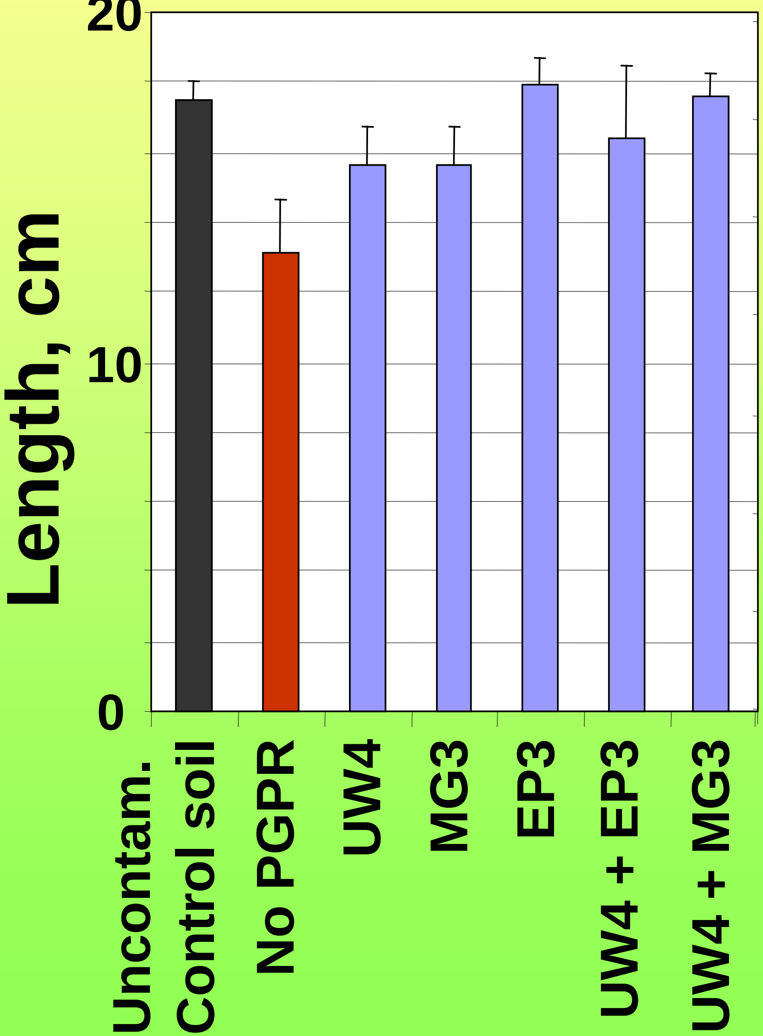
Salt impacted clay-loam soil Fenn-Big Valley.

Preliminary greenhouse experiment.

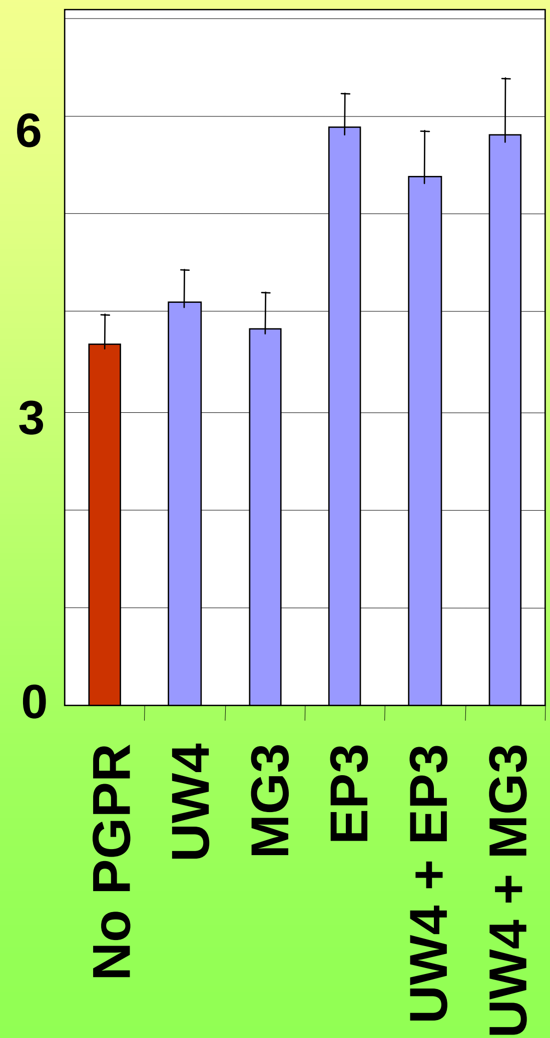
Cl: 1200-2000 mg/ml, SAR: 11-15, ECe: 9-15 dS/m

Growth of fall rye in salt contaminated soil from Fenn-Big Valley

Fall rye shoots



Fall rye roots



MG3/EP3: PGPR from salted soils

50 % more survival + 20 - 30 % more growth ≅ 100% more biomass

Triticale/Barley Mix
Year 1: Summer 2005, North East, AB
~ 1 % TPH on a 7 Hectare site

- PGPR   +PGPR

70 d growth on good soil

Remediation of the NE Alberta Site

- A very large site: > 7 hectares
- Overall ~1 % TPH, with high variability in TPH levels (0.3 to 3 % TPH)
- 6 Hectares planted: Large scale pilot remediation; All plants (barley/fall rye mix, 2000 kg of seeds) were PGPR treated
- 1 Hectare was used as a random block test for PGPR efficacy. Barley, fall rye and triticale were used
- Pilot area: 15% to 30 % remediation was observed in 70 d
- Random block test (70 d), % remediation:

	+ PGPR	- PGPR
F3	2.8 ± 0.5	- 4.2 ± 0.7
F4	8.9 ± 1.3	1.9 ± 0.3
Total	21.9 ± 4.7	16.7 ± 2.3
F4G	32.6 ± 10.9	28.7 ± 5.3

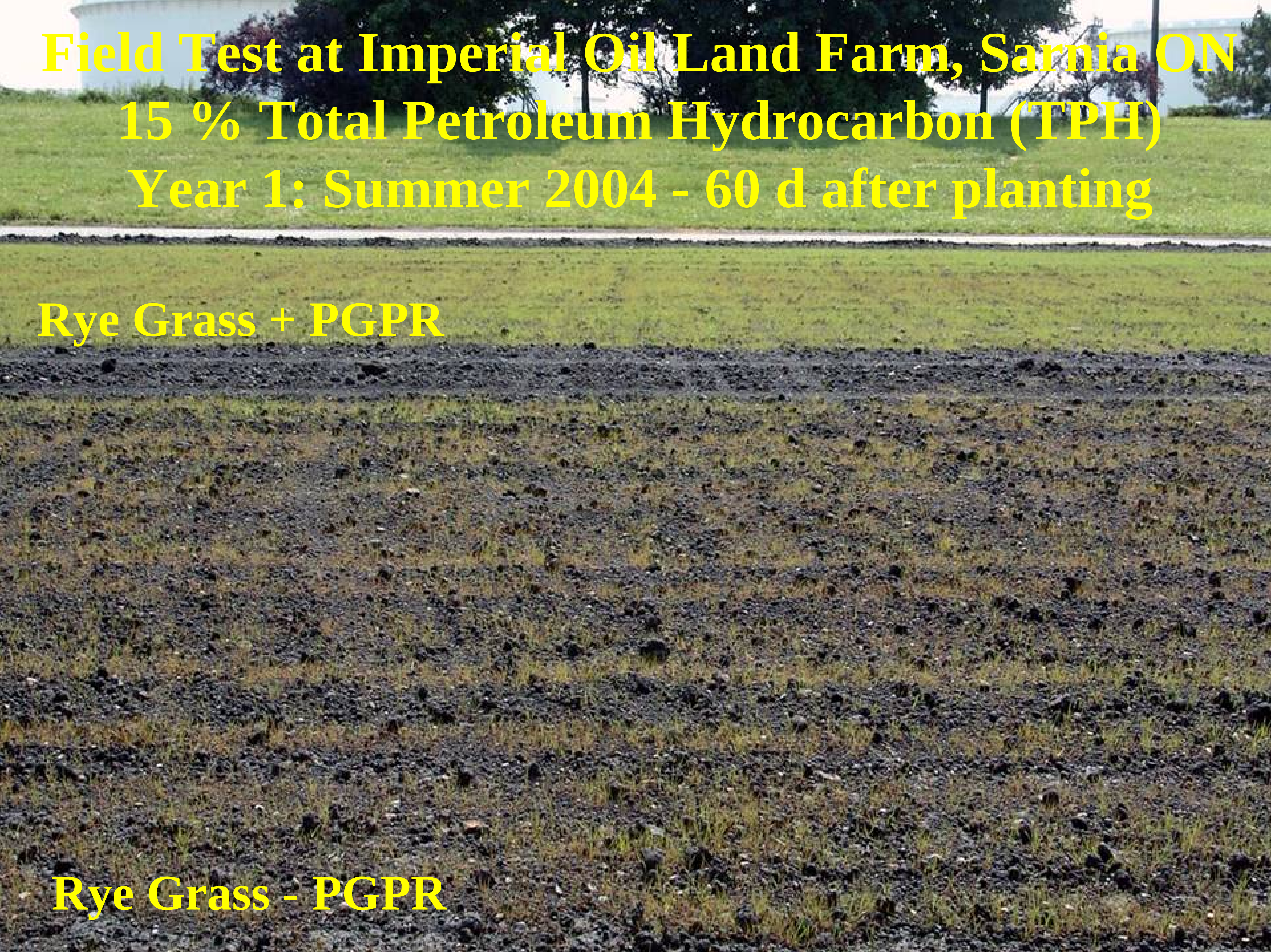
- Consistent rates of remediation observed
- Evidence of PGPR improvement, PGPR impact on par with Turner Valley
- 2006 season: Planted only + PGPR Barly/Rye on June 20.

Multi-Process Phytoremediation System (MPPS)

1. Complicated mixtures of contaminants are present in the environment
2. Many techniques based on an individual process failed or were ineffective
3. Contaminants are too toxic to plants and bacteria for remediation
4. Use and understanding of different remediation mechanisms
5. Multiple remediation kinetics resulting in effective and efficient remediation

Field Test at Talisman Energy Biopile (1 % TPH)
Turner Valley Alberta
Summer 2005 – Rye/Fescue Plant Growth

Average root depth: 21 cm


Field Test at Imperial Oil Land Farm, Sarnia ON
15 % Total Petroleum Hydrocarbon (TPH)
Year 1: Summer 2004 - 60 d after planting
Rye Grass + PGPR
Rye Grass - PGPR

Field Test at Imperial Oil Land Farm, 120 d