Case Study: Obtaining Regulatory Approval and Implementing Field Trials of Transgenic Plants for Mercury Phytoremediation

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Transgenic Plants to Improve Phytoremediation

Why Use Genetic Engineering?

- ***For metals:*** Improve bioavailability, uptake, storage capacity.
- ***For organics:*** Improve biodegradative capability, create new pathways.
- General growth enhancements, agronomic improvements (e.g., pest resistance).

What's Been Holding it Back?

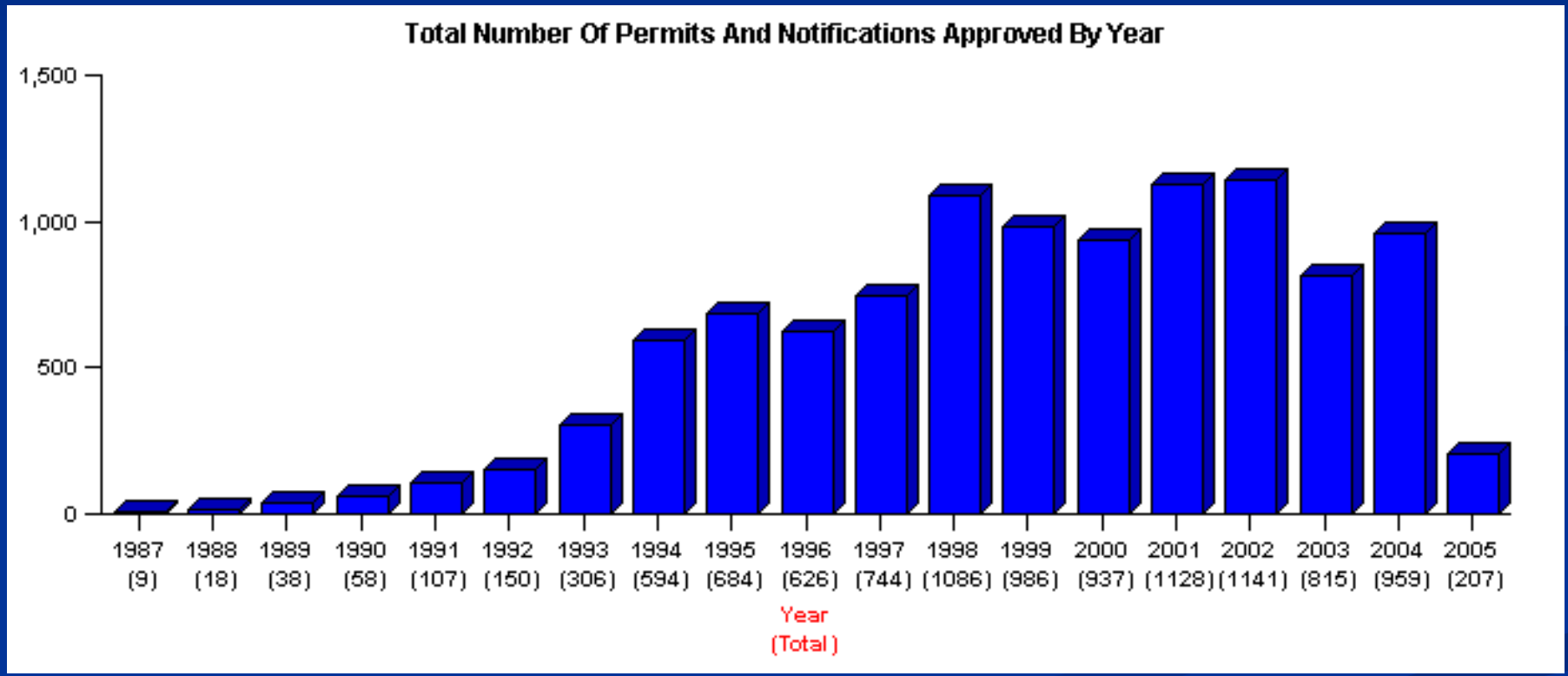
- Lack of a clear technical need for transgenics.
- Long time frames and cost of genetic engineering research.
- Economic realities of remediation market.
- Regulatory uncertainty??

History and Status of Regulation of Transgenic Plants

Transgenic Plants are Now Widely Used in Agriculture

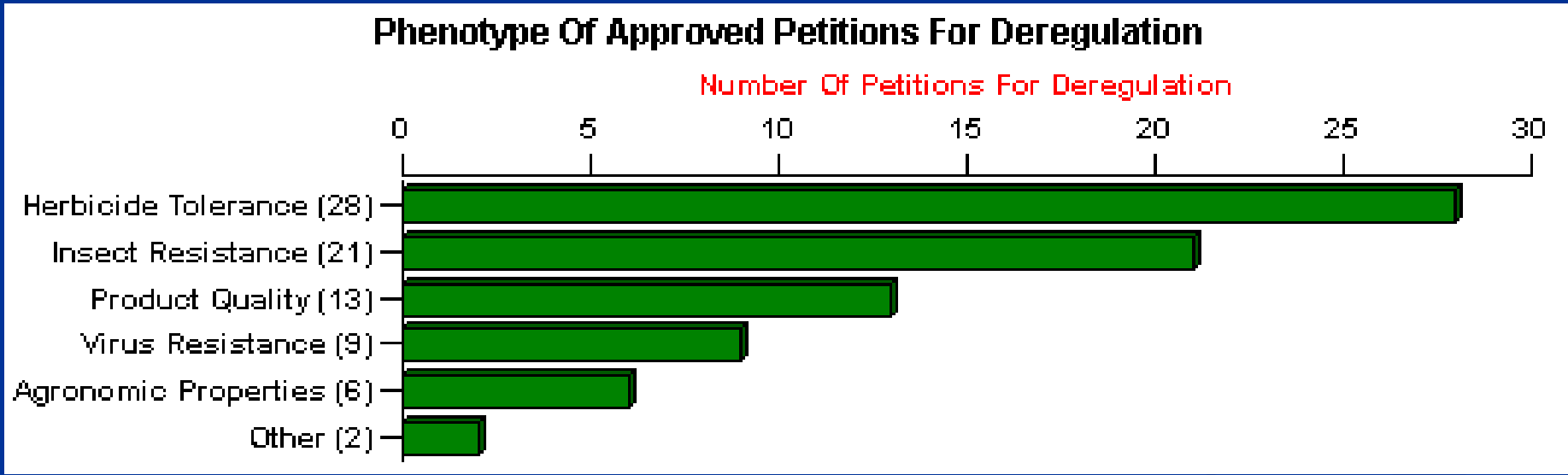
- First plants transformed: 22 years ago (1983).
- First U.S. field tests of transgenic plants: 18 years ago (1987).
- Tens of thousands of field tests have taken place all over the world since then.

U.S. Approvals for Field Releases of Transgenic Plants, 1987-2005



Through 3/18/05. Total= 10,593. Not all approvals represent tests actually conducted, but many approvals cover multiple sites. Source = www.isb.vt.edu.

Transgenic Plants Approved (Delisted) for Commercial Sale in U.S.



Through 3/18/05. Total= 97, but some petitions cover multiple phenotypes so the actual number of approved petitions is lower. Source = www.isb.vt.edu.

U.S. Regulation of the Environmental Uses of Biotechnology

Environmental Protection Agency

- Microbial pesticides.
- Plant pesticides (e.g. transgenic plants).
- Microbes used for nitrogen fixation and bioremediation.

U.S. Department of Agriculture

- Transgenic plants.
- Transgenic animals.
- Plant pests.

USDA Biotechnology Regulation *Summary*

- USDA Regulations administered by Animal and Plant Health Inspection Service (APHIS).
  - ***Notifications***: 30 day advance notice before field use.
  - ***Permits***: 120 day advance notice before field use.
  - ***Delisting***: petitions to exempt specific varieties from the regulations, to allow commercial use and sale.
- Regulations substantially relaxed over time.
- USDA has decided that all phytoremediation field uses require permits rather than notifications.

USDA Biotechnology Regulation
Applications for Permits

- Submit permit application 120 days before field test.
- Describe the host plant, the genetic modifications, the proposed conditions of the field test, assess environmental impacts of field test.

Early Approvals for Field Tests of Transgenic Plants for Phytoremediation

Year of Submission	Institution	Organism/Gene(s)
1989	U. of Kentucky	***Tobacco***/Mouse Metallothionein
1990	U. of Kentucky	***Tobacco***/Mouse Metallothionein
2000	U. of Georgia	***Tobacco***/E. coli Mercuric ion reductase (*mer*A gene)
2001	U. of Georgia	***Poplar***/E. coli Mercuric ion reductase (*mer*A gene)

Recent Approvals for Field Tests of Transgenic Plants for Phytoremediation

Year of Submission	Institution	Organism/Gene(s)
2003	Agricultural Research Service	***Brassica juncea***/APS, ECS, GS genes (*Banuelos et al., ES&T ASAP Article, February 1, 2005*)
2003	Applied PhytoGenetics, Inc.	***Cottonwood (Populus deltoides)***/E. coli *mer*A, *mer*B genes (2 permits covering five potential sites)
2003	Applied PhytoGenetics, Inc.	***Rice***/E. coli *mer*A, *mer*B genes (one potential site)

Recent Approvals for Field Tests of Transgenic Plants for Phytoremediation

Year of Submission	Institution	Organism/Gene(s)
2004	Applied PhytoGenetics, Inc.	***Cottonwood (Populus deltoides)***/ECS gene
2004	Applied PhytoGenetics, Inc.	***Cottonwood (Populus deltoides)***. Renewals of two permits issued in 2003
2004	Edenspace Corporation	***Tobacco/***Citrate synthase gene

Applied PhytoGenetics, Inc. Mercury Phytoremediation Technology

Applied PhytoGenetics, Inc.

- Applied PhytoGenetics (APGEN) founded in 1999 by Laura Carreira, Richard Meagher, Michael Coia.
- Located in Athens, Georgia.
- Expertise in the use of select native plant species for phytoremediation of organics, other contaminants.
- Using genetic engineering to develop technologies for phytoremediation of elemental contaminants, including technology licensed from the University of Georgia.

APGEN's Phytoremediation Strategy for Mercury

- *Detoxify mercury* with genes from bacteria – *mer*A, *mer*B encode enzymes which change oxidation state, convert mercury into less toxic form.

Methylmercury Can be Degraded to Less Toxic Non-Biomagnified Products

10^6-10^8 fold biomagnifiable		Not biomagnifiable		Not biomagnifiable
CH_3Hg^+	MerB →	Hg(II)	MerA →	Hg(0)↑
methyl-mercury		ionic mercury		metallic mercury

APGEN's Phytoremediation Strategy for Mercury

- *Detoxify mercury* with genes from bacteria – *mer*A, *mer*B encode enzymes which change oxidation state, convert mercury into less toxic form.
- *Sequester mercury* inside plants, through engineered over-expression of metal-binding proteins – e.g. ECS gene to enhance synthesis of phytochelatins.
- Cottonwood chosen as initial platform for transgenic technology.

Applied PhytoGenetics, Inc. Field Tests of Transgenic Plants for Mercury Phytoremediation

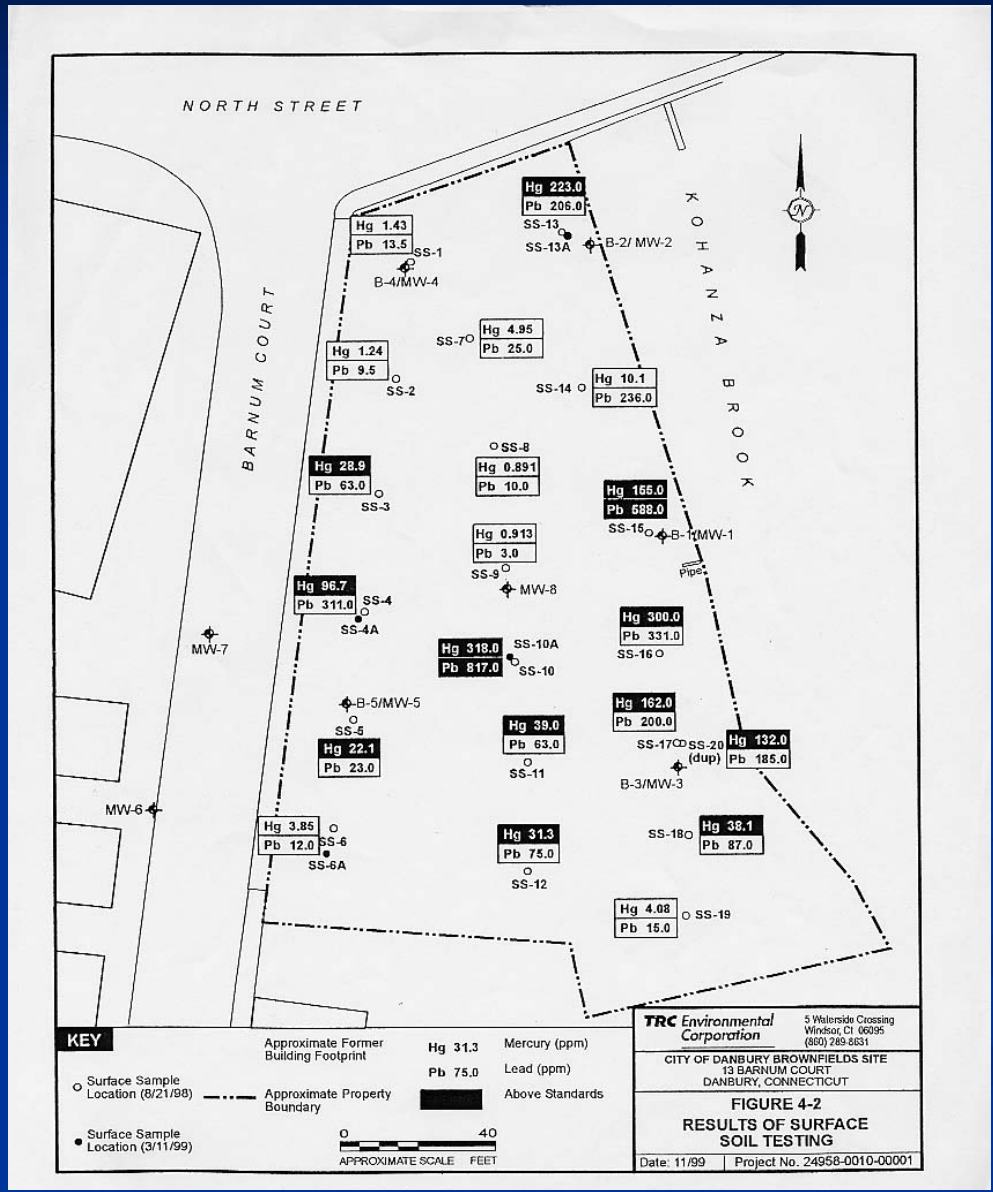
APGEN Field Trials: *Overview*

- Two multi-year field trials of cottonwood expressing *mer*A were begun in 2003.
- Brownfields site in Danbury, Connecticut: field test began July 2003.
- Private industrial site in Colbert County, Alabama: pilot field demonstration began November 2003.

Danbury Field Site

- Danbury had more than 35 hat factories until the early 1900s; mercury used in felt production; many sites are now contaminated.
- The City of Danbury owns an abandoned former hat factory, on 0.33 acres of land in a mixed use area of the city.
- The site is contaminated with lead, mercury and some petroleum hydrocarbons.
- City wants the site cleaned for redevelopment.

Danbury Field Site



Danbury Field Site: *Regulatory Chronology*

- ***December 2002:*** Initial meeting with USDA.
- ***February 2003:*** Permit application submitted.
- ***June 2003:*** Permit granted by USDA.
- ***July 2003:*** Field test begins.
- ***August 2003:*** One APHIS site visit/inspection.
- ***June 2004:*** Permit renewed, 3 site visits in 2004.
- ***January 2005:*** Second renewal submitted.

Danbury Field Site: *Field Test Chronology*

- Planting date: July 16, 2003.
- 120 trees (60 *mer*A, 60 wild type) planted.
- First thinning of trees: August 23, 2003.
- Fall soil sampling August 23, 2003.
- Trees survive first winter well, vigorous growth seen in spring, summer 2004.
- 2004 activities include additional thinning of plots, fall soil and leaf sampling.


July 16, 2003


July 16, 2003


July 16, 2003


July 16, 2003


August 12, 2003


August 12, 2003

June 21, 2004

Danbury Field Site: *Initial Results*

- No significant changes have yet been seen in soil concentrations (sampling at 15 months).
- Leaves of *mer*A plants show extremely low levels of Hg compared to wild type and native trees, showing that *mer*A is expressed and is processing Hg.
- Winter survival of trees in northeastern U.S. is encouraging: suggests that technology will be applicable throughout U.S.?

Alabama Field Site

- Industrial site in northern Alabama.
- Site is approximately 200 feet by 300 feet in area.
- Mercury contamination from former industrial use.
- Site owner has contracted with APGEN for pilot demonstration of mercury phytoremediation technology.

Alabama Field Site: *Field Test Chronology*

- Planting date: November 3, 2003 (plants taken to site to harden off in October).
- 55 *mer*A cottonwood, 55 wild type, 35 holes with no trees, planted in a 48 ft. x 48 ft. grid.
- First soil sampling at T=0.
- Trees survive first winter well, vigorous growth seen in spring, summer 2004.
- 2004 activities include site inspections, installation of irrigation system, fall soil and leaf sampling.

Conclusions

- Biotechnology regulatory issues are not prohibitive, and can easily be managed by companies and academic institutions.
- APGEN's field tests are among the first of commercially-relevant transgenic plants for phytoremediation.
- Transgenic plants can be expected to play a role in commercial phytoremediation.