Phyto-Recurrent Selection:
A Method for Selecting *Populus* & *Salix* Genotypes for Environmental Applications

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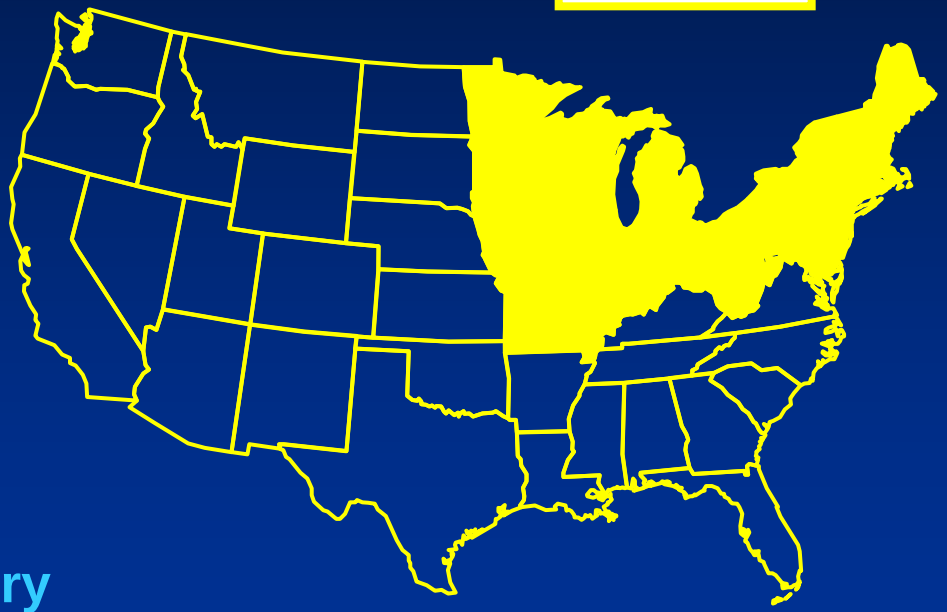
U.S. Forest Service Research & Development

Research Stations

- Northern
- Pacific Northwest
- Pacific Southwest
- Rocky Mountain
- Southern
- Forest Products Laboratory
- International Institute of Tropical Forestry



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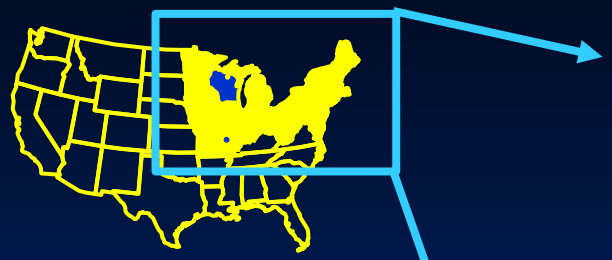
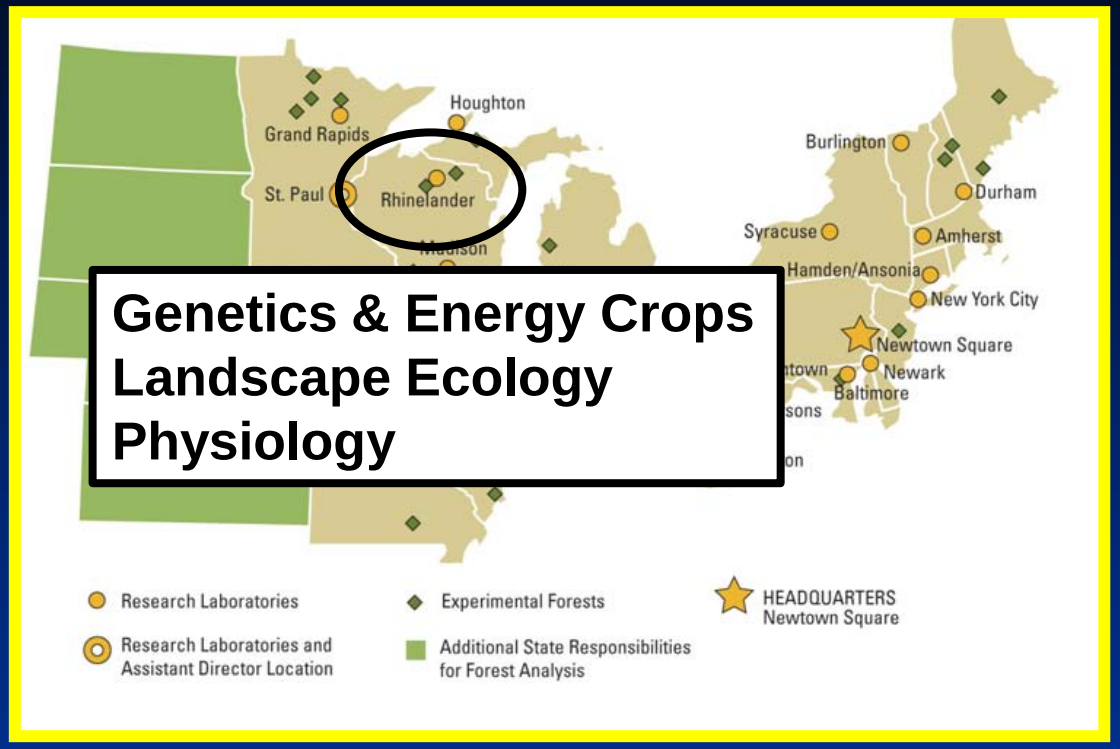




Genetics & Energy Crops
Landscape Ecology
Physiology

<u>Research Themes:</u>

1) Forest Disturbance Processes

2) Providing Clean Air & Water

3) Sustaining Forests

4) Urban Natural Resource Stewardship

5) Natural Resources Inventory & Monitoring

Genetics & Energy Crop Production Unit

Our objective is to use the link between energy, climate, & tree genetics to:

1) develop fast-growing tree crops as energy feedstocks;
2) develop sustainable forest biomass removal strategies;
3) understand climate change effects on natural & plantation forests;
4) fill critical knowledge gaps in 1), 2), & 3).

- **Short rotation woody crops for energy, fiber, &**

<u>PHYTOTECHNOLOGIES</u>

- **Ecological sustainability of using forest residues for energy**
- **Carbon sequestration & climate change adaptation of conifers**

Poplar Genetics Research

- **Northeastern - 1920's**

  1924 to 1939: 13,000 hybrids

- **Lake States**

  1950's (IL), 1960's (MN), 1980's (IA & WI)

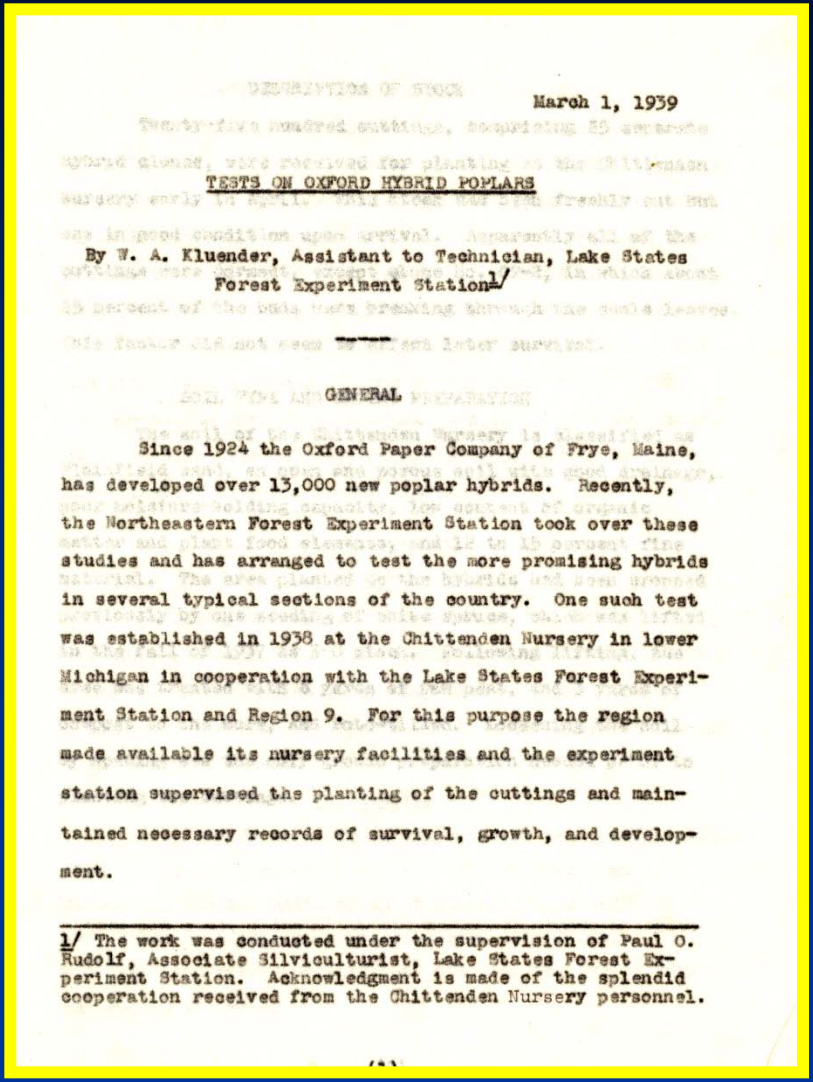
- **Pacific Northwest - 1960's**

- **USFS**

  1937 - 1940: 25 Oxford Paper Company varieties planted in lower Michigan

  1950: LSFES rejected Schreiner's idea for collaborative study

  1983: Poplar genetics research began



March 1, 1939

TESTS ON OXFORD HYBRID POPLARS

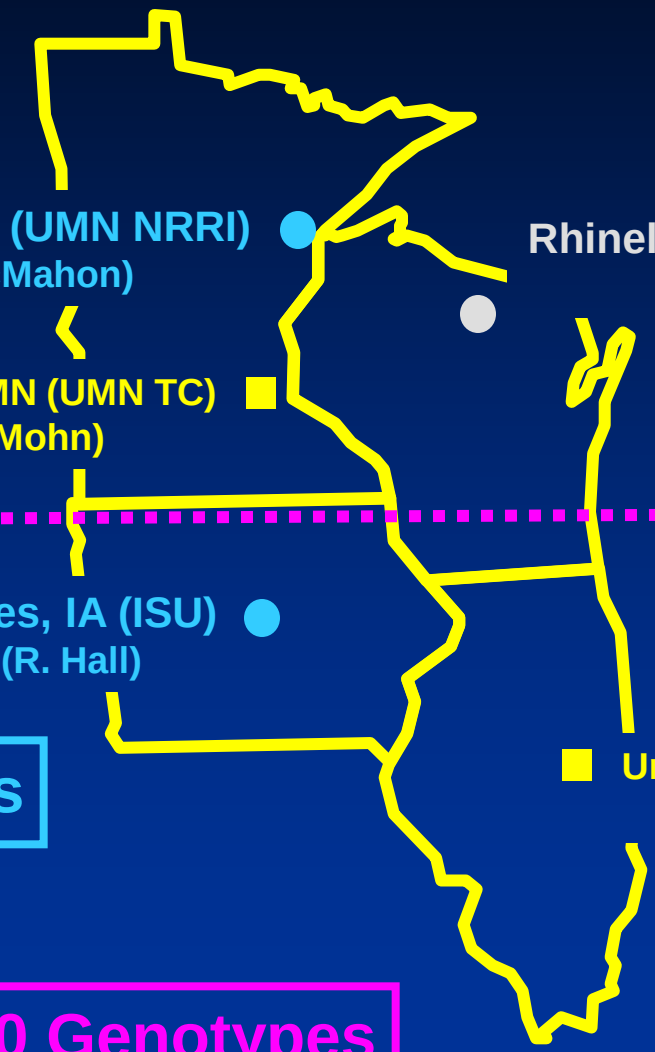
By W. A. Kluender, Assistant to Technician, Lake States Forest Experiment Station[1/]

GENERAL

Since 1924 the Oxford Paper Company of Frye, Maine, has developed over 13,000 new poplar hybrids. Recently, the Northeastern Forest Experiment Station took over these studies and has arranged to test the more promising hybrids in several typical sections of the country. One such test was established in 1938 at the Chittenden Nursery in lower Michigan in cooperation with the Lake States Forest Experiment Station and Region 9. For this purpose the region made available its nursery facilities and the experiment station supervised the planting of the cuttings and maintained necessary records of survival, growth, and development.

1/ The work was conducted under the supervision of Paul O. Rudolf, Associate Silviculturist, Lake States Forest Experiment Station. Acknowledgment is made of the splendid cooperation received from the Chittenden Nursery personnel.

North Central Poplar Breeding



~40,000
+
~48,000

~88,000 Genotypes

+ Previous

>100,000 Genotypes

Commonly Used Species

- ***P. deltoides*** **Bartr. ex Marsh**

  Eastern cottonwood

- ***P. nigra*** **L.**

  European black poplar

- ***P. trichocarpa*** **Torr. & Gray**

  Western black cottonwood

- ***P. suaveolens*** **Fischer subsp.** ***maximowiczii*** **A. Henry**

  Japanese poplar

Schreiner's trials (12 species): ***angulata, berolinensis, candina, charkowiensis, laurifolia, maximowiczii, nigra, petrowskyana, plantierensis, rossica, sargentii, trichocarpa***

Genotypes

(10 genomic groups, 187 clones)

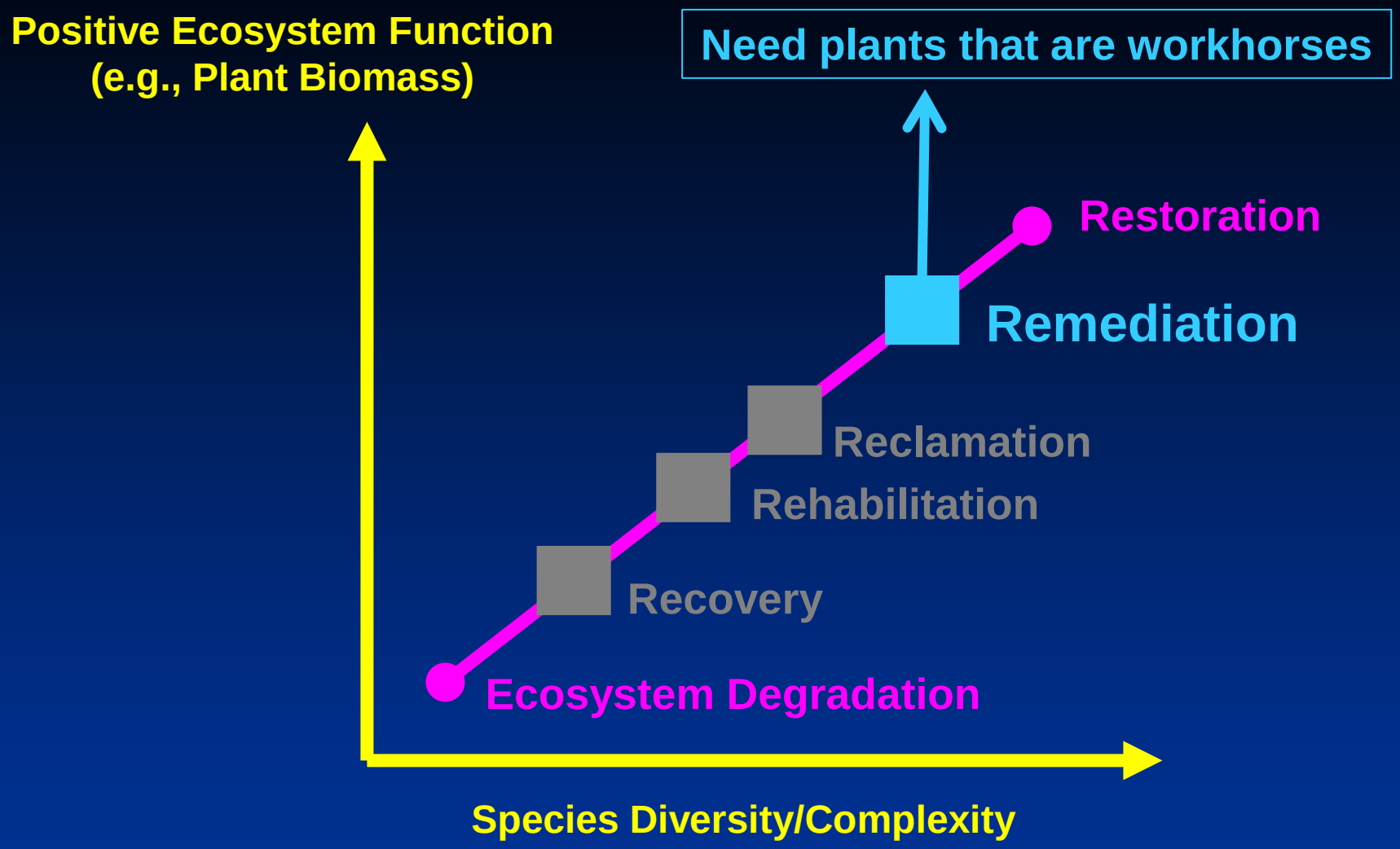
Genomic group	Number of clones 1995	1997	2000
P. deltoides	40	46	12
P. nigra	0	1	1
P. deltoides *P. deltoides*	4	17	5
P. deltoides *P. nigra*	2	1	1
P. deltoides *P. suaveolens* subsp. *maximowiczii*	8	13	0
P. nigra *P. suaveolens* subsp. *maximowiczii*	1	1	2
P. alba *P. alba*	2	0	0
P. alba *P. grandidentata*	1	0	0
P. alba (*P. alba* *P. grandidentata*)	1	0	0
(*P. trichocarpa* *P. deltoides*) *P. deltoides*	0	0	32
Total	59	79	53

Zalesny, R.S. Jr., et al. 2009. Biomass and genotype × environment interactions of *Populus* energy crops in the Midwestern United States. BioEnergy Research 2:106-122.

Environmental Applications

- **Incorporating intensive forestry with waste management for the application of phytotechnologies**

Utilizing sustainable recycling of waste waters as irrigation & fertilization for alternative biomass feedstock production systems



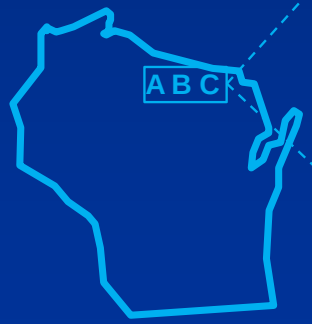
Continuum between complete ecosystem degradation & pre-disturbance condition (restoration) for positive ecosystem function & species diversity/complexity.

Phytotechnologies Research
(Rhinelander)

- **Began in mid-1990's**
- **Emphasis on *Populus* (i.e., poplars) & *Salix* (i.e., willows) as biological filters atop or adjacent to closed landfills**
  - **Recycle & reuse municipal solid waste (MSW) landfill leachate on-site to reduce economic & ecological costs associated with treating the waste waters**
  - **Maintain regional environmental integrity of groundwater aquifers & nearby water bodies**

Phytoremediation Projects

- Municipal wastewater
- Ammonia
- Petroleum hydrocarbons
- Polychlorinated biphenyls (PCB's)
- Nitrates / fertilizer residues
- Heavy metals
- Salts
- Landfill leachate



A: Oneida County Landfill
Zalesny et al. (2007a, 2008a, 2008b, 2009a)

B: Institute for Applied Ecosystem Studies
Zalesny and Bauer (2007a)
Zalesny and Zalesny (2009)
Zalesny et al. (2007b, 2009c)

C: Former Rhinelander City Landfill
Zalesny and Bauer (2007b, 2007c)
Zalesny et al. (2006)

Environmental Applications

- A common protocol has been to utilize a limited number of readily-available genotypes with decades of deployment in other applications (e.g., fiber, windbreaks)
- It is possible to increase the success of phytotechnologies with proper genotypic screening & selection, followed by field establishment of favorable clones

Phyto-Recurrent Selection

Crop Development Strategy

Phytotechnologies

	Rooting	Pest & Disease	Yield	Other
P. deltoides	E	G	G	?
P. trichocarpa	VG	VB	G	?
P. nigra	G	B	G	?
*P. suaveolens**	VG	B	G	?
Hybrids	G	E	VG	?
Adv. Generation	G?	G?	G?	?

P. maximowiczii is currently classified as a subspecies of *P. suaveolens*

Phyto-Recurrent Selection

- Consists of revising & combining crop & tree improvement protocols to utilize superior *Populus* & *Salix* clones for phytotechnologies.

- Such information is lacking for environmental clean-up technologies, but centuries of plant selection success in agronomy, horticulture, & forestry validate the need for similar approaches for environmental applications.

Project Development

↓

Clone Selection

↓

Tree Establishment

↓

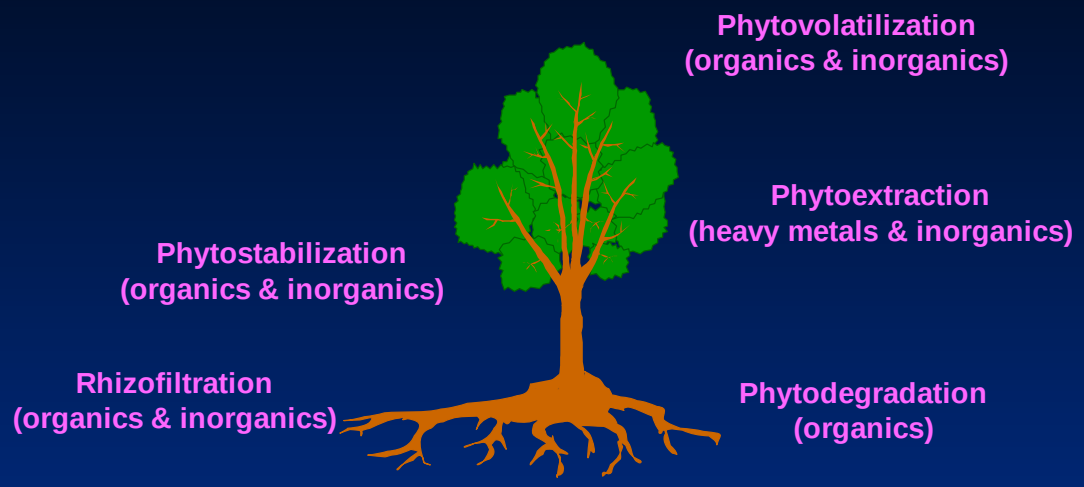
Evaluation of Success Metrics

Zalesny, R.S. Jr., & Bauer, E.O. 2007. Selecting & utilizing *Populus* & *Salix* for landfill covers: implications for leachate irrigation. Intl. J. Phyto. 9:497-511.

Project Development

Identify:

Objectives
Biological Processes
Plant Traits

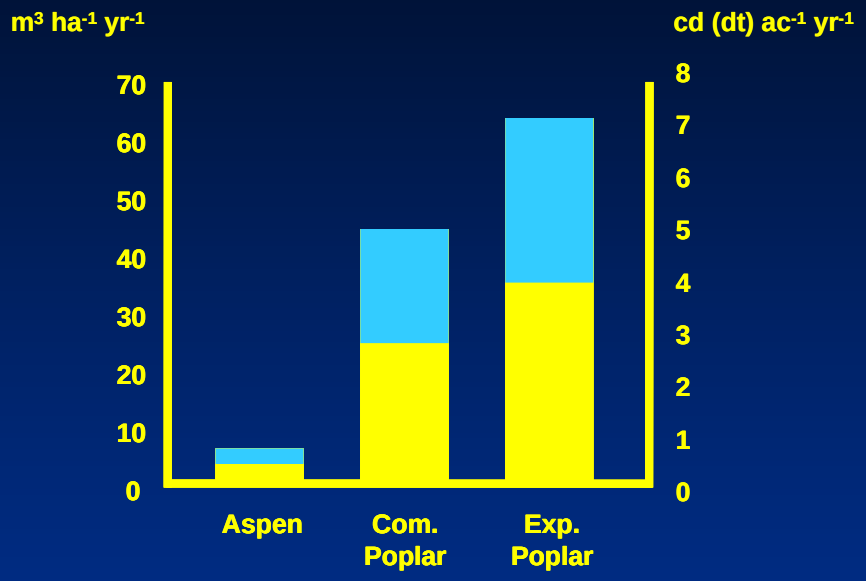


- **Objectives vary with site characteristics, contaminant properties, & choice of plant species.**
- **Biological processes dictate plant traits that should be evaluated.**
- **Parallel consideration of processes & traits fosters sound, realistic management goals.**

Traits of Interest

Rooting Ability

Biomass



Pest / Disease Resistance

Clone Selection

Identify:

Favorable Clones from Phyto-Recurrent Selection Cycles

- **Assess traits of interest during each selection cycle, with precision & complexity increasing as the number of clones tested decreases.**
- **Early testing of genotypes conducted in controlled environments such as growth chambers & greenhouses, then moved to nurseries & field sites with successive selection cycles.**

Recurrent Selection in Plant Improvement

Primary Objectives

1. Increase frequency of favorable alleles & improve mean of new population
2. Retain genetic variation of original population

Continue next cycle with favorables

1. Intermate Individuals to Develop Progenies

2. Evaluate Progenies for Traits of Interest

3. Select Favorable Genotypes

Discard Undesirables

Zalesny, R.S. Jr., & Bauer, E.O. 2007. Selecting & utilizing *Populus* & *Salix* for landfill covers: implications for leachate irrigation. Intl. J. Phyto. 9:497-511.

Phyto-Recurrent Selection in Phytotechnologies

Primary Objectives

Choose clones for field deployment that have:

1. Improved phytoremediation potential over original set of clones

2. Adequate genetic variation to guard against insect/disease outbreaks, changes in soil conditions (e.g., flood/drought), & unfavorable genotype environment interactions

Select Initial Clones for Testing

↓

1. Evaluate Clones when Irrigated with Leachate

↓

2. Select Favorable Clones Based on Phenotypic, Physiological, & Anatomical Traits

Continue next cycle with favorables → 1. Evaluate Clones when Irrigated with Leachate

Discard Undesirables ← 2. Select Favorable Clones Based on Phenotypic, Physiological, & Anatomical Traits

Zalesny, R.S. Jr., & Bauer, E.O. 2007. Selecting & utilizing *Populus* & *Salix* for landfill covers: implications for leachate irrigation. Intl. J. Phyto. 9:497-511.

Tree Establishment

Consider:

Silvicultural Guidelines as with Other Applications

- **Site requirements (soil depth, fertility, moisture, aeration, etc.)**
- **Cutting collection & processing (cutting type, time of shoot harvest in dormant season, etc.)**
- **Site preparation (mechanical, chemical or both)**
- **Planting (spacing, soil temperature, etc.)**
- **Site maintenance (vegetation management, irrigation, insect/disease surveys, etc.)**

Success Metrics

Non-Plant Related Variables:

- Soil testing before & after treatment
  - Concentrations of inorganics & organics, along with pH, texture, cation exchange capacity, & presence/absence of soil fauna
- Contaminant levels in irrigation leachate & water

Success Metrics

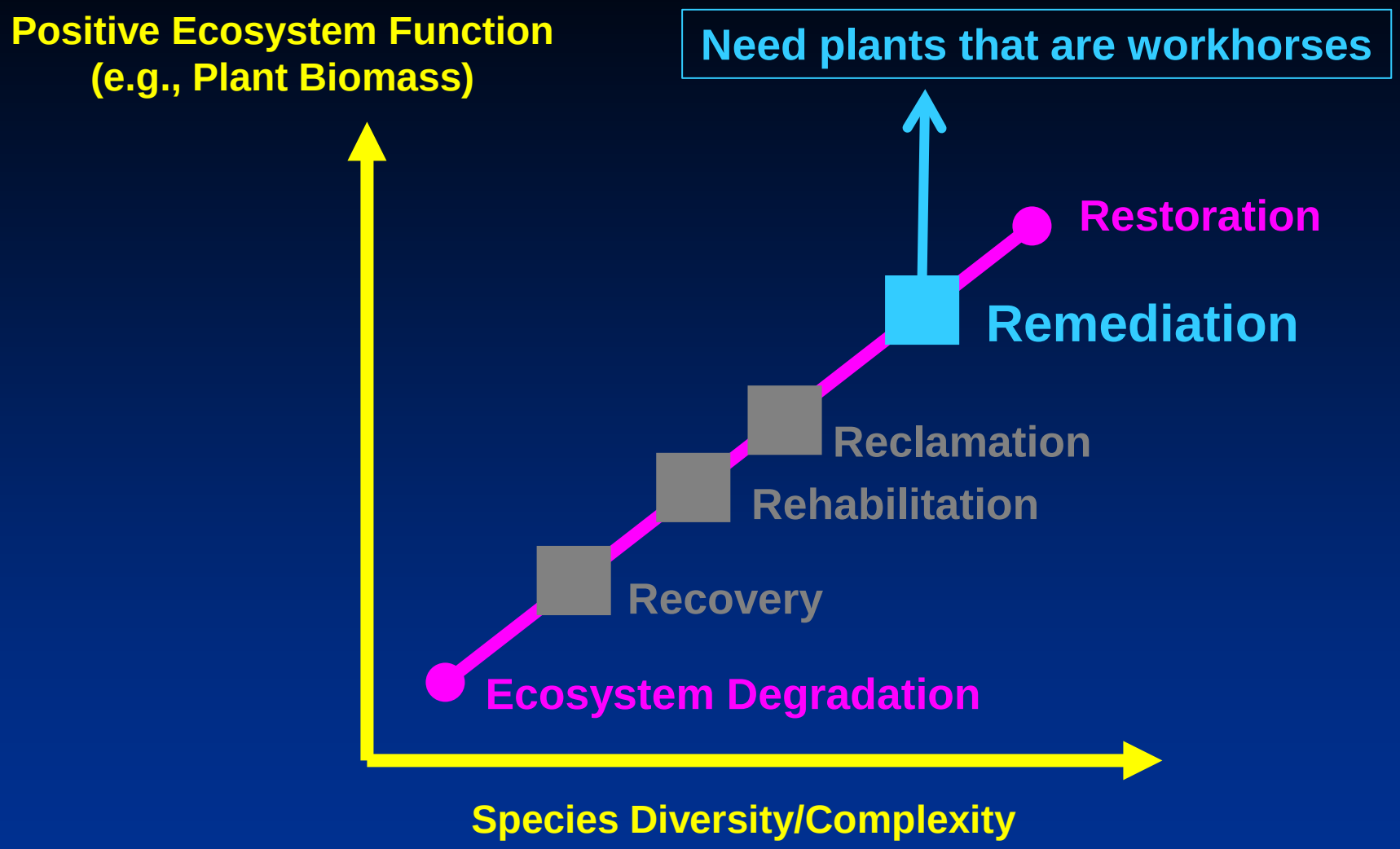
Allometric Traits:

- **Useful for evaluating tree establishment & health, along with becoming *in situ* components of each phyto-recurrent selection cycle.**
- **Drawback of evaluating such traits is that they do not provide information about specific remediation success.**
- **Common allometric traits include: height, diameter, biomass, leaf area, number of leaves, root area, root length, number of roots, & root architecture.**

Success Metrics

Physiological & Anatomical Traits:

- Useful for evaluating specific remediation success.
- Drawback of evaluating such traits is that testing is laborious & expensive.
- Common physiological & anatomical traits include: contaminant concentrations in roots, stems, & leaves, sapflow/water usage, stomatal conductance, & presence of root exudates.



Continuum between complete ecosystem degradation & pre-disturbance condition (restoration) for positive ecosystem function & species diversity/complexity.

Phyto-Recurrent Selection

Months After Initial Planting

0----------1----------6----------20----------28

Selection Cycle 1
(5 Traits)

Clone	Index Value
NC14104	10.27
NM2	7.49
DN5	5.91
NC13475	5.64
NC14106	5.22
NM6	4.57
NC13460	3.88
NC13608	3.86
DM115	3.52
NC14018	3.27
NC13680	3.20
DN182	3.00
NC13451	2.86
NC13670	2.40
NC13661	2.32
NC13672	2.24
NC13668	2.11
NC13652	1.9
91.05.02	1.95
NC13857	1.84
NC13850	0.95
NC13807	0.94
80X00601	0.61
7300501	0.52
8000105	0.12

→ →

Selection Cycle 2
(7 Traits)

Clone	Index Value
DN5	664.49
NM2	559.62
NC14018	538.53
NC14104	520.35
NM6	501.10
DN182	491.68
DM115	474.28
NC14106	403.96
NC13460	368.28
NC13475	354.85
NC13680	292.85
91.05.02	248.16

→ →

Selection Cycle 3
(11 Traits)

Clone	Index Value
NC14106	313.56
NM6	306.24
NC14104	266.50
DM115	264.17
NC13475	249.09
NM2	248.04
DN5	236.33
NC14018	210.54
NC13460	170.51
NC13680	162.44
DN182	135.77
91.05.02	68.67

→ →

Selection Cycle 4
(~ 20 Traits)

Clone
NC13460
NC14018
DM115
NC14104
NC14106
DN5
NM2
NM6

→ → **DEPLOY BEST CLONES FOR**
→ → **ON-SITE PHYTOREMEDIATION**
→ → **OF LANDFILL LEACHATE**

Zalesny, J.A., et al. 2007. Choosing tree genotypes for phytoremediation of landfill leachate using phyto-recurrent selection. Intl. J. Phyto. 9:513-530.

Practical Implications

- **Well-developed experimental designs & associated precision levels support estimation of quantitative genetic data that lead to recommendations of generalist genotypes that perform well over broad contaminant ranges or specialist genotypes that perform well for specific contaminants.**

- **Phyto-recurrent selection can help researchers & resource managers move closer to obtaining long-term (i.e. rotation age) remediation data from landfill covers & other phytoremediation systems.**

SUSTAINABILITY

International Journal of Phytoremediation, 9:513–530, 2007
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DOI: 10.1080/15226510701709754

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CHOOSING TREE GENOTYPES FOR PHYTOREMEDIATION OF LANDFILL LEACHATE USING PHYTO-RECURRENT SELECTION

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Information about the response of poplar (Populus spp.) genotypes to landfill leachate irrigation is needed, along with efficient methods for choosing genotypes based on leachate composition. Poplar clones were irrigated during three cycles of phyto-recurrent selection to test whether genotypes responded differently to leachate and water, and to test whether the methodology had merit as a tool for plant selection during remediation. Fifteen below- and above-ground traits were evaluated. Twenty-five clones were tested in cycle 1, while the best 12 genotypes were evaluated in cycles 2 and 3. Eight clones were selected and subsequently tested in an in situ landfill study (cycle 4). Results from cycles 1, 2, and 3 are presented here. Overall, clones responded differently to irrigation treatments, with certain genotypes exhibiting better below- and above-ground growth with water than leachate. However, growth was greater with leachate irrigation for some clones. In addition, differences between treatments within clones decreased with days after planting (DAP). There were no treatment differences for number of leaves, height, and root length at the end of cycle 2 (45 DAP) or cycle 3 (30 DAP). These results detail the extensive variation in clonal responses to leachate irrigation, along with the need and efficacy of using phyto-recurrent selection to choose superior genotypes.

KEY WORDS: leachate irrigation, wastewater treatment, chloride stress, clonal selection index, short rotation woody crops, *Populus*, poplar

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SELECTING AND UTILIZING *POPULUS* AND *SALIX* FOR LANDFILL COVERS: IMPLICATIONS FOR LEACHATE IRRIGATION

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The success of using Populus and Salix for phytoremediation has prompted further use of leachate as a combination of irrigation and fertilization for the trees. A common protocol for such efforts has been to utilize a limited number of readily-available genotypes with decades of deployment in other applications, such as fiber or windbreaks. However, it may be possible to increase phytoremediation success with proper genotypic screening and selection, followed by the field establishment of clones that exhibited favorable potential for clean-up of specific contaminants. There is an overwhelming need for testing and subsequent deployment of diverse Populus and Salix genotypes, given current availability of clonal material and the inherent genetic variation among and within these genera. Therefore, we tail phyto-recurrent selection, a method that consists of revising and combining crop and improvement protocols to meet the objective of utilizing superior Populus and Salix s for remediation applications.

h such information is lacking for environmental clean-up technologies, centuries of ction success in agronomy, horticulture, and forestry validate the need for similar in phytoremediation. We bridge the gap between these disciplines by describing pment, clone selection, tree establishment, and evaluation of success metrics f their importance to utilizing trees for phytoremediation.

hyto-recurrent selection, phytoremediation, poplar, willow, short rotation

Poplar Research Database

Constraints

- Only peer-reviewed manuscripts
- Published between 1989 & 2009
- Focused on poplars, cottonwoods, aspens, & their hybrids

  grown as short rotation woody crops
- Focused on research conducted in North America
- Focused on at least one topic area

12 Topic Areas

- Conservation
- Diseases
- Economics & Social Science
- Genetics
- General
- Cell & Tissue Culture
- Growth & Productivity
- Insects & Mites
- Physiology
- Phytotechnologies
- Silviculture
- Harvesting & Wood Products

Thank you!

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