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

Jill A. Zalesny
Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, USA

Ronald S. Zalesny Jr. and Adam H. Wiese
USDA Forest Service, Northern Research Station, Institute for Applied Ecosystem Studies, Rhinelander, Wisconsin, USA

Richard B. Hall
Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, USA

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

INTRODUCTION

Landfills produce leachate from the infiltration of precipitation and internal biological processes (Duggan, 2005). The leachate composition of organic compounds, inorganic ions, and heavy metals changes due to the chemical and biological processes that occur during natural degradation of the waste products (Gettinby, Sarsby, and Nedwell, 1996). Although

Address correspondence to Jill A. Zalesny, USDA Forest Service, Northern Research Station, Institute for Applied Ecosystem Studies, 5985 Highway K, Rhinelander, WI, 54501, USA. E-mail: jzalesny@fs.fed.us

contaminant levels generally decrease with landfill age, leachate treatment is necessary to avoid ground-and surface-water contamination (Wong and Leung, 1989). Economically-sound and environmentally-sustainable options are available to land managers for on-site leachate remediation (Glass, 1999; Schnoor *et al.*, 1995). Such options reduce the costs associated with transportation and treatment. These treatment costs can last for years after landfill closure, and the resulting reduction in revenue can complicate the ability of resource managers to afford traditional leachate processing methods (Duggan, 2005). An alternative treatment that decreases expenditures is to utilize the leachate as a fertilization and irrigation source for species and interspecific hybrids of the genus *Populus* (Erdman and Christenson, 2000).

Hybrid poplars have been utilized in a variety of phytoremediation projects (Bañuelos *et al.*, 1999; Burken, 2001). Selected poplar genotypes are ideal for remediation due to their ability to establish quickly after planting and produce large plant biomass, produce extensive root systems, transpire large volumes of water, be propagated easily and inexpensively from hardwood cuttings, and grow on marginal lands (Isebrands and Karnosky, 2001). Although a variety of clonal material has been utilized, research efforts have focused on a few commercially-available clones, which may not offer maximum remedial benefits to researchers and project managers. Breeders across the North Central United States have spent decades developing more than 100,000 new poplar genotypes for multiple uses such as fiber, bioenergy, riparian stabilization, wood products, cordwood, and now phytoremediation (Heilman, 1999; Riemenschneider *et al.*, 2001; Zalesny *et al.*, 2005a). This untapped supply of different genotypes offers a unique opportunity to study and identify clones that either perform well across most sites or perform well in sites with specific contamination problems (Zalesny, Riemenschneider, and Hall, 2005b), such as elevated salt concentrations in the leachate and/or soil.

Phyto-recurrent selection involves the adoption of crop and tree improvement strategies to identify and select superior-performing clones for specific remediation efforts (Zalesny and Bauer, 2007). Specifically, this method involves the evaluation, identification, and selection of favorable clones using multiple testing cycles. The length of each cycle increases concurrently with the precision of the data that are acquired. Consequently, as the complexity of the data increases, the number of clones tested in each cycle decreases. The identification of such clones is accomplished with experimental procedures such as those outlined below, with adjustments to allow for site-specific features such as soil type and leachate characteristics.

The primary objective was to evaluate the early growth and productivity of different poplar genotypes when irrigated with landfill leachate or water. Crop and tree improvement concepts were utilized to develop a phyto-recurrent selection model that would help identify superior genotypes tailored to specific objectives. Three selection cycles used to choose eight poplar genotypes for an *in situ* landfill study (cycle 4) are described. Twenty-five clones were tested in cycle 1, while the best 12 genotypes were evaluated in cycles 2 and 3. Fifteen different below- and above-ground traits were tested. The null hypotheses for each cycle were that clones would not respond differently to leachate and water irrigation, and that clones would not vary for all traits. This information enhances the body of research that was already conducted using poplars for landfill remediation, because there is a general lack of knowledge about clonal comparisons for establishment success, growth, and productivity of poplar genotypes when irrigated with leachate. In addition, the use of crop and tree improvement methodologies and phyto-recurrent selection offers project managers a tool for the identification and selection

Table 1 Genomic groups and clones of *Populus* irrigated with landfill leachate and municipal water

Genomic group	Clone
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*	NC13451, NC13460, NC13475, NC13608, NC13652,
”	NC13661, NC13668, NC13670, NC13672, NC13680,
”	NC13807, NC13850, NC13857, NC14018
P. deltoides × *P. deltoides* (F_1 hybrid)	80X00601
P. deltoides	7300501, 8000105, 91.05.02
P. deltoides × *P. maximowiczii*	DM115, NC14104, NC14106
P. deltoides × *P. nigra*	DN5, DN182
P. nigra × *P. maximowiczii*	NM2, NM6

Note: Authorities for the aforementioned species are: *P. deltoides* Bartr. ex Marsh; *P. trichocarpa* Torr. and Gray; *P. nigra* L., *P. maximowiczii* A. Henry.

of superior clones that may help to increase the success of future projects of this nature.

MATERIALS AND METHODS

Initial Clone Selection and Cutting Preparation

Twenty-five poplar (*Populus* spp.) clones (Table 1) were selected from six genomic groups during January 2005 for phyto-recurrent selection cycle 1, an *ex situ* study testing early root, stem, and leaf growth in an effort to select the best 12 clones for selection cycles 2 and 3. The genomic groups and clones were selected based on current growth in the North Central United States, past clonal screening tests that demonstrated regional growth success (biomass data), representation of hybrids from multiple species (specifically *P. deltoides, P. nigra, P. maximowiczii,* and *P. trichocarpa*), and clonal availability.

Dormant, unrooted cuttings, 25.4 cm long, were processed from whips collected during December 2005. The whips were grown for one growing season in stool beds established at Hugo Sauer Nursery in Rhinelander, WI, USA (45.6 °N, 89.4 °W). During processing, cuts were made to position at least one primary bud not more than 2.54 cm from the top of each cutting. Cuttings were stored in polyethylene bags at 5 °C, then soaked in water to a height of 15 cm for 3 d before planting. The trees were grown in a greenhouse at the Institute for Applied Ecosystem Studies in Rhinelander with a 16-h photoperiod and a daytime and nighttime temperature of 24 and 20 °C, respectively.

Leachate Description

Leachate was collected from the Oneida County Landfill on 14 January 2005. The landfill was located 6 km west of Hugo Sauer Nursery. Leachate was collected and sent to Northern Lake Service, Inc. (Crandon, WI, USA) on 25 January and 23 February 2005 for chemical analysis using approved United States Environmental Protection Agency methods. The leachate was brownish-green in color, with a putrid odor, an electrical conductivity of 10.2 ± 0.02 mS cm^{-1} at 25 °C, and a pH of 8.4 ± 0.39. The concentration of nitrogen (N), phosphorus (P), and potassium (K) was 745 ± 15 mg N L^{-1} (191 kg N ha^{-1}), 2.1 ± 0.1 mg P L^{-1} (0.5 kg P ha^{-1}), and 450 ± 30 mg K L^{-1} (115 kg K ha^{-1}). The primary

Table 2 Leachate composition over time of parameters relevant to the current study compared with those in the published literature

Sampling date	pH	Electrical conductivity (mS cm^{-1})	Biological oxygen demand (mg L^{-1})	Chemical oxygen demand (mg L^{-1})	Cl^-(mg L^{-1})
19 April 2001	8.0	8.7	1600	2800	1000
9 April 2002	7.9	8.7	270	1300	980
10 October 2002	7.7	10.0	1600	2600	1100
30 April 2003	8.1	6.8	380	1500	1300
28 October 2003	8.6	13.0	690	2300	1600
6 April 2004	8.1	7.0	69	880	790
15 October 2004	8.9	3.4	210	1100	1200
25 January 2005[a]	8.0	10.2	14	1100	1400
23 February 2005[a]	8.8	10.2	48	1000	1400
28 April 2005	8.8	5.7	16	670	820
19 October 2005	8.8	6.6	26	650	750
Other leachate[b]	4.5–9.0	2.5–35.0	20–57000	140–152000	150–4500

[a]Leachate used in the current study.
[b]Ranges based on 14 studies cited in Kjeldsen *et al*. (2002).

toxicity concern was the relatively high chloride (Cl^-) concentration of 1400 ± 0 mg L^{-1} (359 kg Cl^- ha^{-1}).

Final closure and capping of the Oneida County Landfill occurred in 2002. Since that time, the concentrations of inorganics, organics, and metals have declined annually. Heavy metals and volatile organic compounds were not detectable in the leachate analysis and, therefore, not a concern with respect to plant establishment. Leachate composition varies by local environmental conditions and deposition of waste from residential, commercial, or industrial sources (Kjeldsen *et al*., 2002), which was exhibited by variable pH, salinity, biological oxygen demand, chemical oxygen demand, and Cl^- concentration since the Oneida County Landfill closure (Table 2).

Experimental Design

The trees of each selection cycle were arranged in a split-plot design, with blocks (random), treatments (fixed whole plots), and clones (fixed sub plots). Clones were arranged in randomized complete blocks to minimize effects of any potential environmental gradients in the greenhouse. Treatments and clones were considered as fixed in the analysis and, therefore, we evaluated means rather than variances.

Selection Cycle 1

Tree establishment and irrigation regime. Four blocks, two treatments, and 25 clones (200 experimental units) were tested. The trees were established in folding book planters (four cells per planter, 10 planters per rack) containing a standard greenhouse potting mix consisting of equal parts of sand, peat, and vermiculite (v:v:v). The irrigation regime was 100-mL treatments of landfill leachate or water (the control) on Monday, Wednesday, and Friday, from 19 January to 2 February 2005.

Data collection. After 14 d, the trees were harvested, washed, and dissected into roots, stems, leaves, and the cutting. In addition, the number of roots and leaves were recorded and the leaf area was determined (Li Cor Model 3100 Area Meter). All plant components were oven dried at 70 °C for 72 h and dry mass was obtained for roots, stems, leaves, and cuttings.

The number of leaves was determined according to the leaf plastochron index (LPI), which is an index of morphological time scale that supports plant comparisons under large environmental and/or developmental variance (Larson and Isebrands, 1971). Specifically, we used a leaf lamina width of 2 cm as a unified reference for plastochron index development. Lamina width was chosen as a reference because it is an arbitrary non-destructive developmental measure. Therefore, LPI 0 was the index leaf of 2 cm; LPI −1, −2, and so on were the leaves above LPI 0 that were not yet 2 cm; and LPI 1, 2, and so on were the leaves below LPI 0 that were greater than 2 cm. Leaves of LPI 0 and greater were used for the analysis.

Data analysis. The number of roots and leaves, leaf area, and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split-plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The non-significant ($P > 0.25$) block × clone interaction for all variables was pooled with the three-way interaction into a common error term to increase precision of F-tests (Zalesny *et al.*, 2005b).

Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 14 DAP (1.05 to 7.73 g). Cutting dry mass was a significant covariate for root and top dry mass ($P = 0.0080$, $P < 0.0001$, respectively), along with number of leaves ($P = 0.0032$); however, cutting dry mass did not have a significant effect on number of roots or leaf area ($P = 0.0567$, $P = 0.1631$, respectively). Therefore, all means except for number of roots and leaf area were adjusted for the variation in cutting dry mass. Fisher's protected least significant difference (LSD) was used to compare adjusted and unadjusted means (Chew, 1976).

Selection Cycle 2

Tree establishment and irrigation regime. Four blocks, two treatments, and 12 clones (96 experimental units) were tested. The trees were established in specially-designed rhizotrons that supported two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots until the final harvest. Wiese, Riemenschneider, and Zalesny (2005) provided a description of the rhizotrons and types of data that can be collected with them. Each rhizotron had a capacity of 6,675 cm^3 of soil. The growing medium was sand (rather than the potting mix in cycle 1) to support easier identification of roots during digital root analysis and to supply an inert growing environment. The irrigation regime was 30-mL treatments of leachate or water on Monday, Wednesday, and Friday, from March to June 2005. In addition, drip irrigation with water only was applied for 15-s intervals twice daily. The supplemental irrigation was applied to simulate natural rainfall, along with helping to meet the water demands of the trees.

Data collection. Digital photographs of the root systems were taken each Monday and Thursday of the experimental period beginning 21 DAP, when roots were present on all clones. The photographs were subjected to digital analysis using WinRHIZO Tron software (Regent Instruments, Inc., Quebec, Canada) to determine total root length at 21, 24, 28, 31, 35, 38, 42, and 45 DAP. Number of leaves (as described above) and tree height was recorded on all photograph dates. Tree height was measured at the point of attachment between the stem and the original cutting in order to reduce measurement error.

After 45 d the trees were harvested, washed, and dissected into roots, stems, leaves, and the cutting. Leaf area was determined (Li Cor Model 3100 Area Meter) and all plant components were oven dried at 70 °C for 72 h to obtain dry mass for roots, stems, leaves, and cuttings.

Data analysis. Number of leaves, height, and root length data were subjected to repeated measures analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming a split-plot, repeated measure design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The repeated measure was time (*i.e.*, DAP). Given correlated errors associated with DAP, the results from multivariate analyses of variance (MANOVA) were interpreted to provide correct F-variance ratios and to reduce the probability of incorrectly claiming significant differences when, in fact, there were none (*i.e.*, Type I Errors). A pooled error term was used for all traits as in selection cycle 1. Leaf area and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split-plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot) using a pooled error term.

Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 45 DAP (1.41 to 9.04 g). Cutting dry mass was a significant covariate for leaf area, leaf dry mass, stem dry mass, and aboveground dry mass, along with number of leaves (except 24, 42, and 45 DAP) and height throughout the study ($P < 0.05$). However, cutting dry mass did not have a significant effect on root dry mass and root length ($P > 0.05$). Therefore, all means except for number of leaves at 24, 42, and 45 DAP, along with rooting traits, were adjusted for the variation in cutting dry mass.

Selection Cycle 3

Tree establishment and irrigation regime. Six blocks, two treatments, and 12 clones (144 experimental units) were tested. The trees were established in specially-designed planters constructed of an aluminum framework with plexiglass walls and base. Each planter consisted of four individual tree chambers, each with a capacity of 22,052 cm^3 of soil (25.0 cm high × 29.7 cm wide × 29.7 cm deep) (Figure 1). Three concentric rings of hardware cloth (0.635 cm × 0.635 cm heavy metal screen) were attached to one another, divided into three layers, and placed in every cell of the planters. Hardware cloth was used to hold roots in place during development and data collection. Cuttings were planted in the middle of the concentric rings. The growing medium was sand to reduce experimental error associated with loss of roots during excavation and to supply an inert growing environment. The irrigation regime was 300-mL treatments of water for a 10-d establishment period, followed by irrigating with 300 mL of leachate or water on Monday, Wednesday, and Friday for the remaining 20 d of the experiment.

Data collection. Number of leaves and tree height was recorded on all treatment irrigation dates. These traits were determined as described for selection cycle 2. After 30 d the trees were harvested, washed, and dissected into root, stem, leaf, and cutting components. The number of roots was recorded for each layer described above. In addition, leaf area was determined (Li Cor Model 3100 Area Meter) followed by all plant components

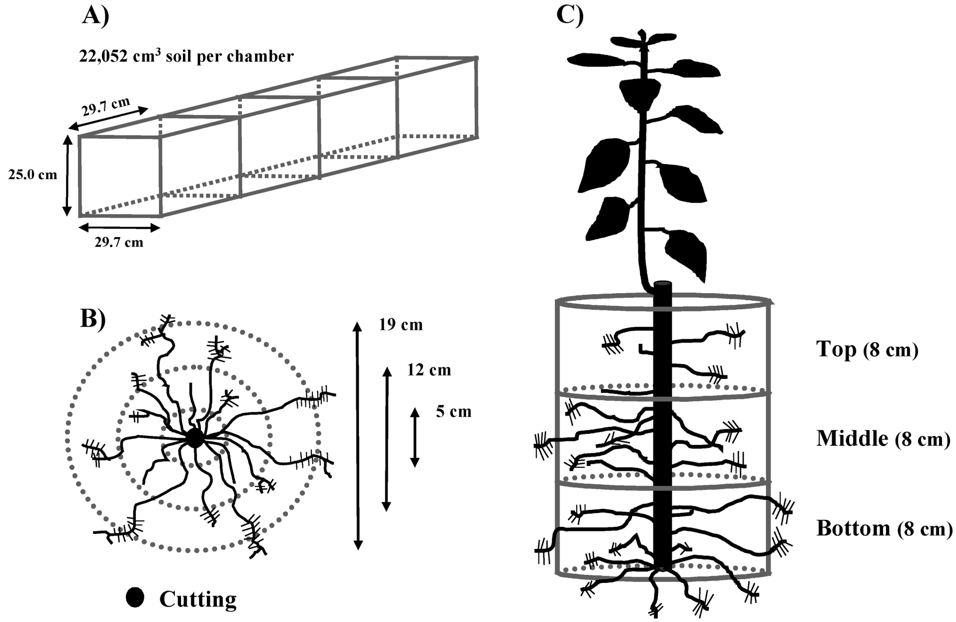

Figure 1 Specially-designed planters used to quantify root initiation and development along the length of the cuttings, in addition to aboveground traits. Within each chamber (A), one cutting was planted in the middle of three concentric rings of hardware cloth (B) that were attached to one another and divided into three layers (C).

being oven dried at 70 °C for 72 h. Dry mass was obtained for roots (within each layer), stems, leaves, and the cutting.

Data analysis. Number of leaves and height data were subjected to repeated measures analyses of variance according to SAS® as described for selection cycle 2. Leaf area, number of roots, and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split-plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The block × clone interaction was negligible ($P > 0.25$) for leaf area, number of roots in the first and third layer, total number of roots, and root dry mass in layers 1 and 2. Thus, for these variables, a pooled error term was used.

Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 30 DAP (1.08 to 7.51 g). Cutting dry mass was a significant covariate for leaf area ($P < 0.0001$), leaf dry mass ($P = 0.0070$), stem dry mass ($P = 0.0012$), and aboveground dry mass ($P = 0.0050$), along with root dry mass in the first ($P = 0.0045$) and second layers ($P = 0.0398$). However, cutting dry mass did not have a significant effect on number of leaves, height, or the remaining rooting traits ($P > 0.05$). Therefore, all means except for number of leaves, height, and the remaining rooting traits were adjusted for the variation in cutting dry mass. Fisher's protected LSD was used to compare adjusted and unadjusted means (Chew, 1976).

Weighted Summation Indices

For the phyto-recurrent selection indices used in cycles 1 to 3, weighted allometric traits (sum of weights = 1) were used based on their relative importance for early establishment and perceived contribution to subsequent phytoremediation. The weights were multiplied by the adjusted or unadjusted means for the traits of interest, followed by summation of values. In general, roots and leaves each had 40% weight, while stems contributed to 20% of each index value. Favorable genotypes were those that exhibited greater relative index values.

Selection cycle 1. The traits of interest were root number (RN), root dry mass (RDM), leaf number (LN), leaf area (LA), and combined leaf and stem dry mass (LSDM). The following phyto-recurrent selection model was used in the analysis:

$$\text{Index Value (IV)} = 0.15^*\text{RN} + 0.25^*\text{RDM} + 0.1^*\text{LN} + 0.15^*\text{LA} + 0.35^*\text{LSDM}.$$

Selection cycle 2. The traits of interest were root length (RL), RDM, height (HT), LN, LA, stem dry mass (SDM), and leaf dry mass (LDM). The following model was used:

$$\text{IV} = 0.1^*\text{RL} + 0.3^*\text{RDM} + 0.15^*\text{HT} + 0.05^*\text{LN} + 0.2^*\text{LA} + 0.05^*\text{SDM} + 0.15^*\text{LDM}.$$

Selection cycle 3. The traits of interest were root number in layers 1 to 3 (RN_{L1}, RN_{L2}, RN_{L3}), root dry mass in layers 1 to 3 (RDM_{L1}, RDM_{L2}, RDM_{L3}), HT, LN, LA, SDM, and LDM. The following model was used:

$$\text{IV} = 0.025^*\text{RN}_{L1} + 0.075^*\text{RN}_{L2} + 0.1^*\text{RN}_{L3} + 0.025^*\text{RDM}_{L1} + 0.075^*\text{RDM}_{L2} + 0.1^*\text{RDM}_{L3} + 0.15^*\text{HT} + 0.05^*\text{LN} + 0.2^*\text{LA} + 0.05^*\text{SDM} + 0.15^*\text{LDM}.$$

RESULTS

Selection Cycle 1

Clones responded differently to leachate and water irrigation. Treatment main effects were significant for number of roots ($P = 0.0005$), root dry mass ($P = 0.0050$), number of leaves ($P = 0.0003$), leaf area ($P < 0.0001$), and combined dry mass of leaves and stems ($P = 0.0001$). Likewise, clone main effects were significant for these traits ($P = 0.0016$ for root dry mass; $P < 0.0001$ for all others). Nevertheless, the treatment × clone interaction governed these traits ($P = 0.0035$ for root dry mass; $P = 0.0022$ for number of leaves; $P < 0.0001$ for all others). Overall, the number of roots across treatments and clones ranged from 0.3 ± 0.3 to 37.5 ± 5.0, with a mean of 12.6 ± 2.4, while root dry mass ranged from 0.0 ± 0.0 to 197.7 ± 17.7 mg, with a mean of 29.4 ± 15.0 mg. Similarly, number of leaves across treatments and clones ranged from 0.0 ± 0.0 to 8.6 ± 0.8, with a mean of 3.0 ± 0.8, while leaf area ranged from 0.0 ± 0.0 to 72.0 ± 4.9 cm^2, with a mean of 15.3 ± 3.5 cm^2. The combined dry mass of leaves and stems across treatments and clones ranged from 7.3 ± 7.3 to 274.0 ± 18.0 mg, with a mean of 96.7 ± 17.9 mg (Figure 2). Leachate and water treatments differed for seven of the 14 backcross clones [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*], one pure *P. deltoides* clone, and all clones belonging to the remaining genomic groups (*P. deltoides* × *P. maximowiczii; P. deltoides* × *P. nigra; P. nigra* × *P. maximowiczii*). Similar interaction trends existed for all other traits.

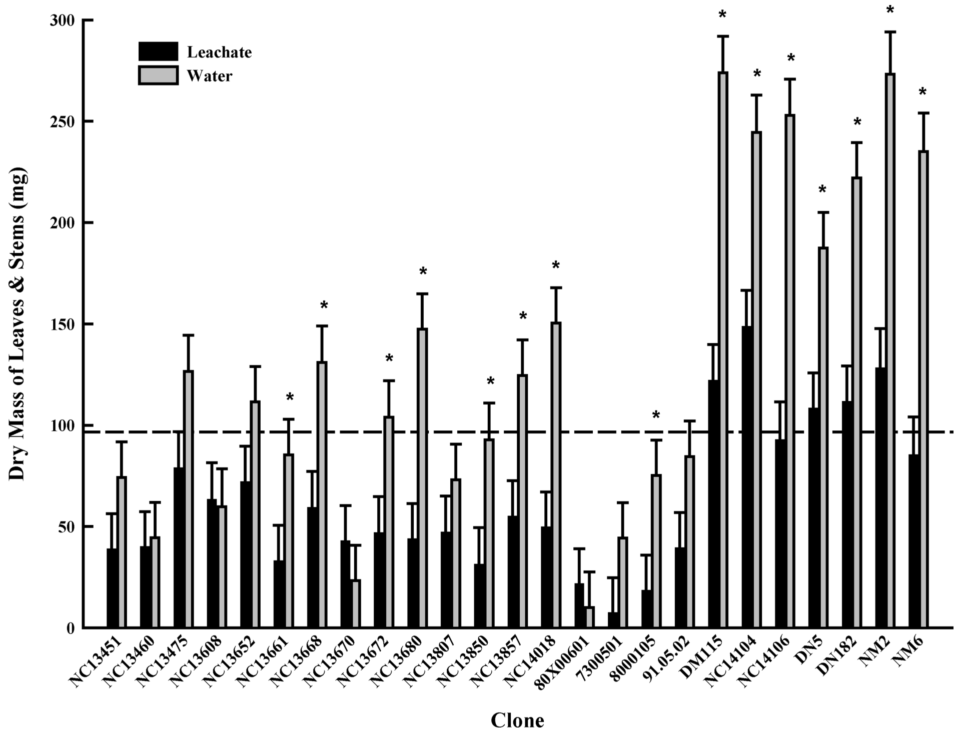

Figure 2 Combined dry mass of the leaves and stems for each combination of treatment (leachate, water) and clone during phyto-recurrent selection cycle 1. Each bar represents the mean adjusted for cutting dry mass with one standard error. Asterisks above bars indicate differences between treatments, according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, $n = 4$, LSD = 51.3 mg). The dashed line represents the overall mean.

Selection Cycle 2

Clones responded similarly to leachate and water irrigation. The number of days after planting affected treatment main effects for height ($P_{MANOVA} = 0.0117$) and number of leaves ($P_{MANOVA} < 0.0001$). From a univariate standpoint, treatment main effects were significant for height at 21 and 24 DAP ($P = 0.0276$, $P = 0.0310$, respectively) and for number of leaves at 21, 24, and 28 DAP ($P = 0.0362$, $P = 0.0329$, $P = 0.0487$, respectively). The difference between treatment means at each measurement date decreased over time for these traits (Figure 3). Furthermore, clone main effects were significant for height and number of leaves, regardless of DAP ($P < 0.05$), along with leaf area ($P < 0.0001$), stem dry mass ($P = 0.0010$), and leaf dry mass ($P < 0.0001$). In contrast, clone main effects only affected root length at 21 and 24 DAP ($P = 0.0128$, $P = 0.0382$, respectively). Overall, leaf area across treatments and clones ranged from 39.1 ± 37.6 to 302.4 ± 44.7 cm^2, with a mean of 172.0 ± 38.3 cm^2. Similar clonal variation existed for all other significant traits.

Selection Cycle 3

Clones responded differently to leachate and water irrigation for rooting traits, but responded similarly for aboveground traits. The number of days after planting affected clone main effects for height ($P_{MANOVA} < 0.0001$) and number of leaves ($P_{MANOVA} = 0.0114$).

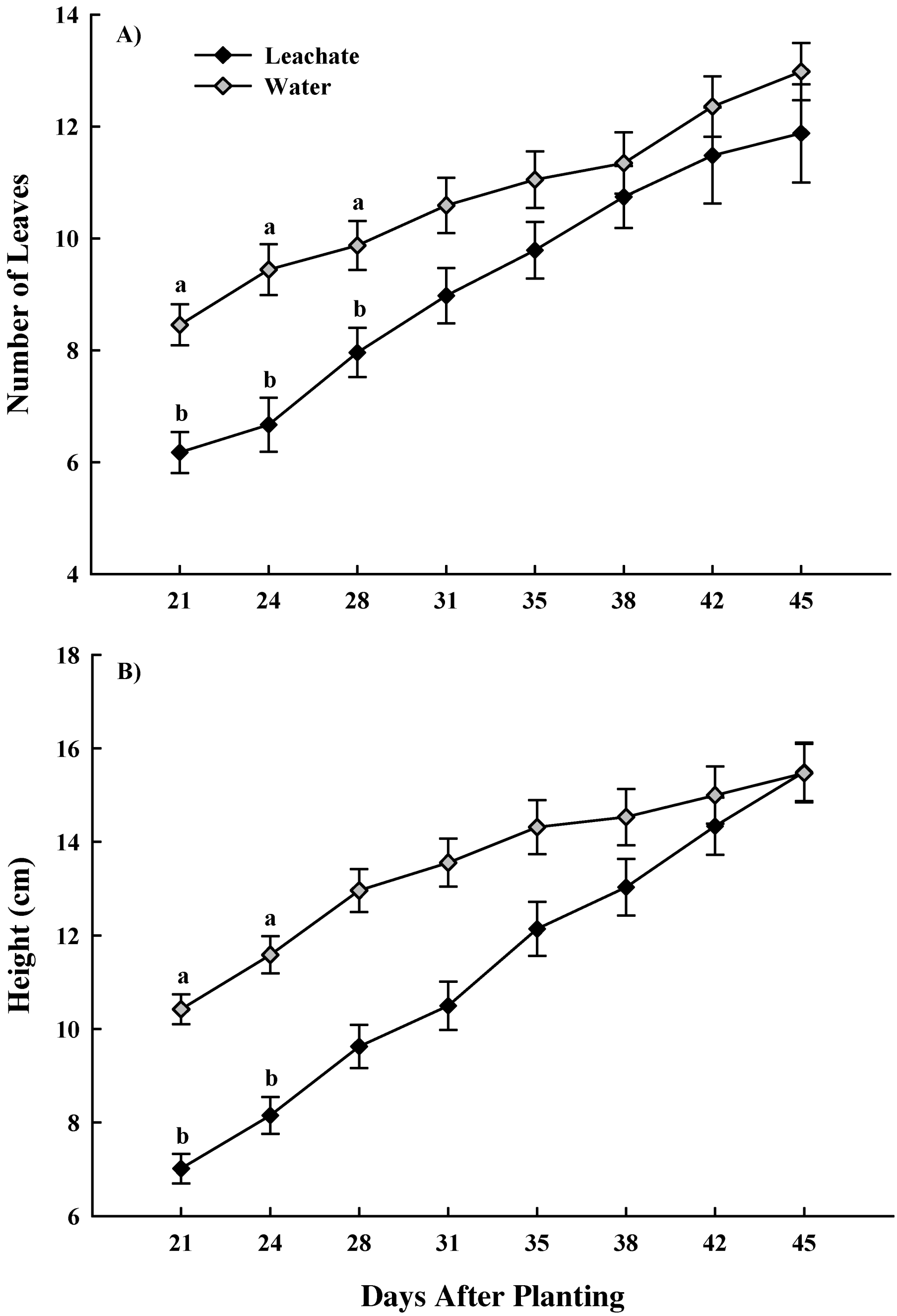

Figure 3 Number of leaves (A) and height (B) across clones vs. days after planting (DAP) for each treatment (leachate, water) during phyto-recurrent selection cycle 2. Each diamond represents the mean ($n = 48$) adjusted for cutting dry mass (except number of leaves at 24, 42, and 45 DAP that were unadjusted), with one standard error. Treatments with different letters within a day were different, according to a split-plot, repeated measures ANOVA ($P < 0.05$).

From a univariate standpoint, clone main effects were significant for all traits ($P < 0.0001$). There was broad variation in clonal responses to treatments for rooting traits at different layers. The treatment × clone interaction was significant for number of roots in the top layer ($P = 0.0064$), along with root dry mass in top and middle layers ($P = 0.0045$, $P = 0.0398$, respectively). Overall, the number of roots in the top layer across treatments and clones ranged from 0.0 ± 0.0 to 8.8 ± 0.6, with a mean of 4.0 ± 1.1. Although mean root dry mass across treatments and clones in the top, middle, and bottom layer was 17.2 ± 6.3, 79.0 ± 22.6, and 223.4 ± 46.5 mg, respectively, significant differences in clonal responses to treatments were present only in the top and middle layers (Figure 4).

Weighted Summation Indices

A summary of index values and outcomes of the weighted summation indices for each selection cycle is presented in Figure 5. The favorable clones of selection cycle 1 belonged to every genomic group except that of pure *P. deltoides* and the F_1 hybrids of *P. deltoides*. Nevertheless, we chose *P. deltoides* clone 91.05.02 for selection cycles 2 and 3 because cuttings of the clone ranked eighth (NC13608) were of poor quality and because we had an academic interest in advancing 91.05.02, which ultimately was not selected for cycle 4. In contrast, the favorable clones of selection cycles 2 and 3 excluded pure *P. deltoides* genotypes. Clone NC13460 was selected over DN182 and NC13475 because there was a discrepancy between the indices for these clones and because cuttings of NC13475 were of poor quality. Overall, selection cycle 1 took 1 mo to complete (beginning January 2005), while cycles 2 and 3 lasted an additional 5 mo. Selection cycle 4, an *in situ* trial at the Oneida County Landfill in northern Wisconsin, was completed during August 2006, 20 mo after the initial planting of cuttings in cycle 1.

DISCUSSION

The main goals of this study were to test poplar genotypes for differences in below- and above-ground growth when irrigated with landfill leachate or water, and to develop and evaluate a phyto-recurrent selection model to help researchers and resource managers choose superior clones for phytoremediation field applications. Clones responded differently to treatments for all traits in selection cycle 1. This supports the postulate of testing clonal compatibility for leachate composition and concentration. Early root and shoot development is necessary for plant survival, with increased below- and above-ground growth indicating genotype-specific tolerance and/or the capability for exclusion of leachate-imposed stresses (Larcher, 1995). Sensitivity to elevated concentrations of salts in landfill leachate, such as Cl^- in our samples, elicited strong environmental pressure to eliminate weaker and less-tolerant genotypes. The elimination of unsuccessful genotypes from the experiment at the earliest selection cycle is imperative to the final goal of field deployment of well-suited clones. The number of clones depends on the size of the planting and relatedness of the selected genotypes.

Clones responded similarly to treatments during selection cycle 2. Repeated measurements for the number of leaves and height across clones were different early on. However, treatment differences became more negligible over time. Half of the experimental genotypes were removed after selection cycle 1, with the remaining clones performing uniformly during selection cycle 2. The leachate appeared to be more detrimental during the root initiation stage, when young tissues were responsive to osmotic stress. Once established,

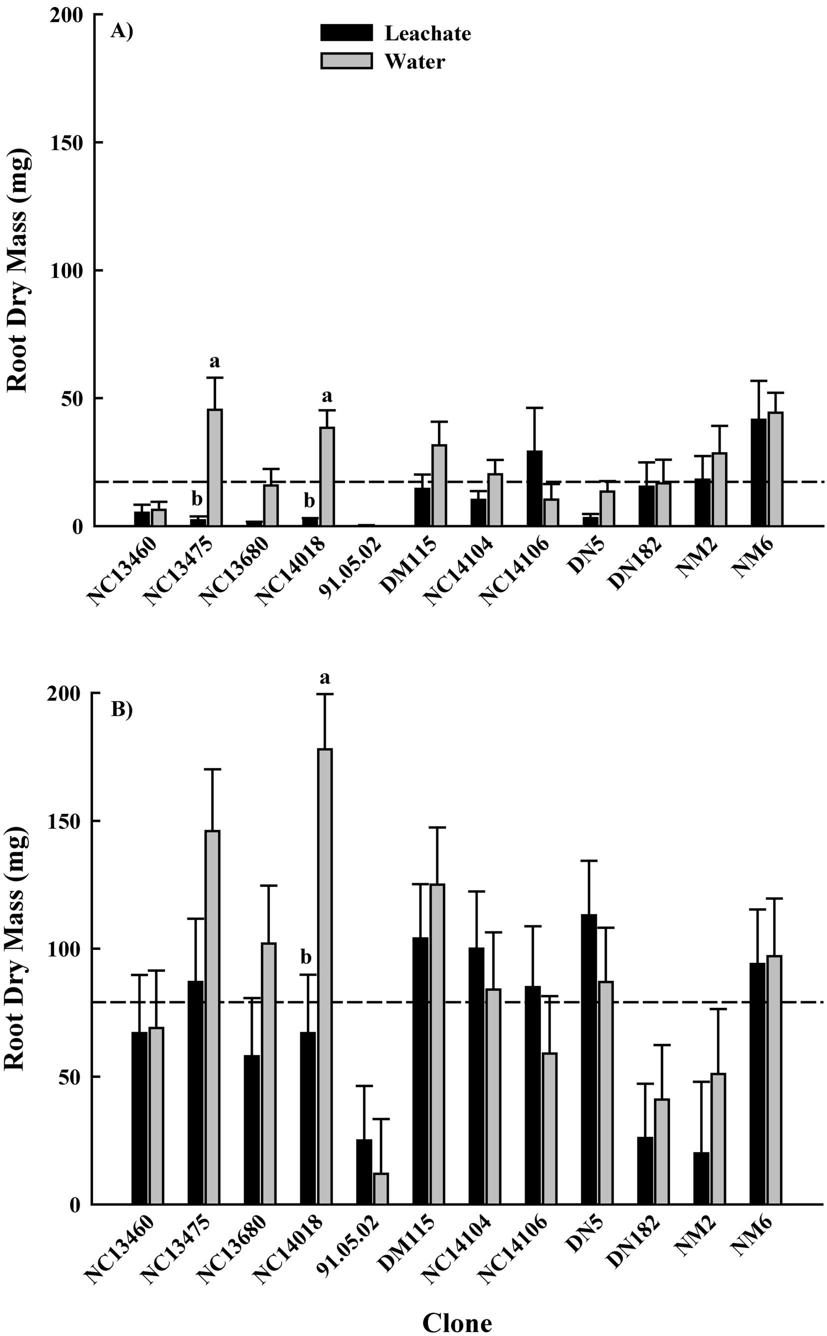

Figure 4 Root dry mass in the top (A) and middle (B) layers of specially-designed planters for each combination of treatment (leachate, water) and clone during phyto-recurrent selection cycle 3. Root dry mass in the bottom layer was negligible ($P = 0.1197$). Each bar represents the mean unadjusted (A) and adjusted (B) for cutting dry mass, with one standard error. Treatments with different letters within a clone were different, according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, $n = 6$, $LSD_{top} = 20.0$ mg, $LSD_{middle} = 60.0$ mg). The dashed line represents the overall mean.

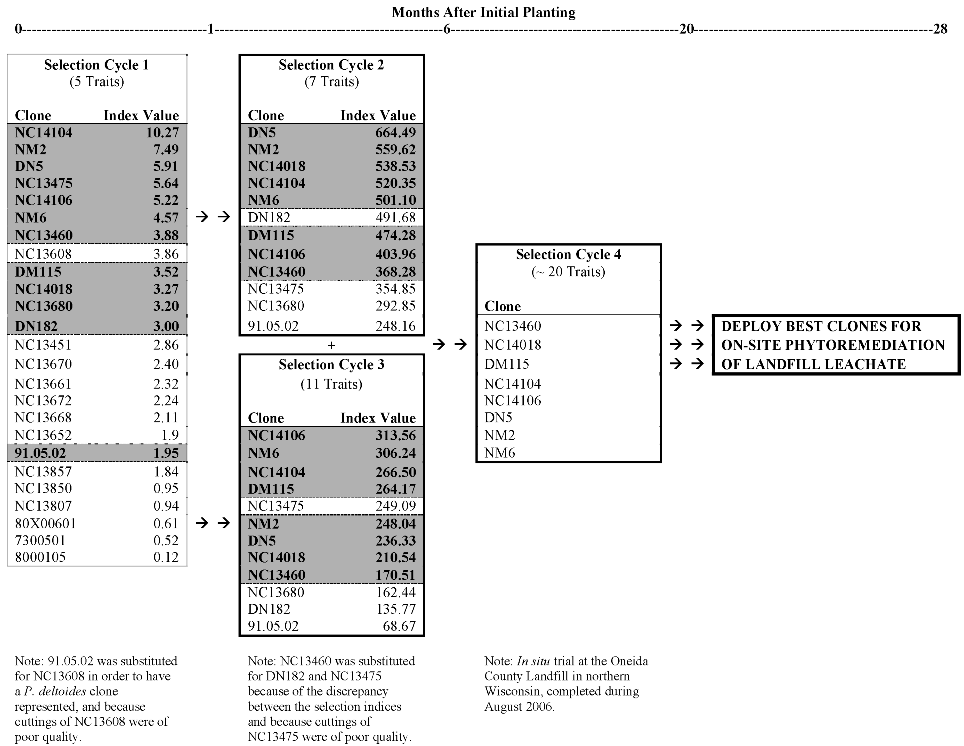

Figure 5 Phyto-recurrent selection cycles using weighted summation indices to choose eight *Populus* clones for selection cycle 4, an *in situ* study testing phytoremediation effectiveness following irrigation with landfill leachate or water. See the Materials and Methods section for quantitative descriptions of the indices.

the trees were able to tolerate high Cl^- concentrations. Overall, the apparent stress had diminished before the end of the experiment. The trends in Figure 3 indicated the potential for trees receiving leachate to exhibit greater number of leaves and height after 45 DAP. Thus, it may be beneficial in future studies to test the trees for an additional week, or until the roots are no longer discernible from one another in the photographs (this depends upon the exact clones studied).

Generally, treatments did not affect clones for aboveground growth in selection cycle 3. However, clones responded differently to treatments for rooting in the upper two-thirds of the cutting length (*i.e.*, top and middle planter layers). Similar variation in tissue-specific responses across five willow clones belonging to four genomic groups was reported (Dimitriou, Aronsson, and Weih, 2006). Amplified root growth is most likely due to increased nutrients available for exploitation (Rytter and Hansson, 1996). These results are useful to phytoremediation applications because an extensive rhizosphere and its associated microorganisms may offer improved remedial benefits (Anderson, Guthrie, and Walton, 1993). Likewise, root biomass should be positively correlated with phytoremediation capability (Zalesny et al., 2005a).

The usefulness of phyto-recurrent selection lies with balancing the acquisition of meaningful data with successfully identifying and selecting favorable genotypes that can be used for field applications. Although it is next to impossible to simulate field conditions in a greenhouse or growth chamber, our method is a useful technique that includes multiple selection cycles within a short time period and that can be done with limited resources. In the current study, selection cycle 1 lasted 1 mo and five traits were evaluated (Figure 5). Selection cycles 2 and 3 were conducted simultaneously for 5 mo, with an additional seven and 11 traits tested, respectively. Although actual phytoremediation effectiveness was not evaluated in these cycles, a longer (15-mo) *in situ* study (cycle 4) was conducted that involved evaluating approximately 20 traits. The tissue concentration of contaminants in the roots, stems, and leaves, along with soil and leachate concentrations, were tested (Zalesny et al., 2007a; 2007b; 2007c). Ultimately, superior clones will be selected for the resource manager as early as 21 mo from the planting of selection cycle 1. The number of clones decreased from 25 in cycle 1 to eight in cycle 4 that will be used for on-site treatment of landfill leachate. As stated above, the number of clones for the phytoremediation system is dependent upon the size of the planting and the parentage of the clones. From a genetics standpoint, it is important to have enough diversity among genotypes to guard against insect/disease outbreaks, changes in soil conditions, and unfavorable genotype × environment interactions (Zalesny and Bauer, 2007).

Short rotation intensive forestry systems using poplars, along with willows (*Salix* spp.), have provided resource managers with multiple uses and associated products (Heilman, 1999). In addition to phytoremediation, short rotation woody crops provide secondary benefits such as aesthetic improvement and value-added products during short rotation harvests. However, such high-intensity cropping requires optimal plant nutrition for biomass production and for resistance to disease and insect outbreaks (Coyle and Coleman, 2005). Leachate is an excellent source of irrigation and nitrogen fertilization for poplar and willow (Hasselgren, 1992); despite this elevated levels of heavy metals and salts may reduce growth (Stephens, Tyrrel, and Tiberghien, 2000).

The nitrogen application rate of 191 kg N ha^{-1} in the leachate was within the range of optimal nutrient estimates reported for pure *P. deltoides* and hybrid genotypes (105 to 276 kg N ha^{-1}) (Heilman and Stettler, 1986; Nelson, Switzer, and Lockaby, 1987). Coleman, Friend, and Kern (2004) reported that 2-yr-old *P. deltoides* 'D105' grown in northern Wisconsin, USA, acquired at most 120 kg N ha^{-1} yr^{-1} from synthetic and soil N sources, with trees receiving 50 and 100 kg N ha^{-1} yr^{-1} exhibiting near-optimal growth. In contrast, elevated Cl^- concentrations impose osmotic stress and associated negative effects on tree growth such as leaf chlorosis, early leaf abscission, growth inhibition, and increased mortality (Cureton, Groenevelt, and McBride, 1991; Menser, Winant, and Bennett, 1983). In the current study, the elevated leachate Cl^- concentration (1400 mg CL^- L^{-1}) likely diminished the overall positive effect on growth and productivity associated with N fertilization. Stephens *et al.* (2000) demonstrated an inverse relationship between leachate Cl^- concentrations greater than 2500 mg L^{-1} and growth/productivity of *Salix viminalis* L. 'Q683'.

Excessive chloride and elevated electrical conductivity (EC) can be related to increased plant stress and associated reductions in productivity (Neuman *et al.*, 1996; Shannon *et al.*, 1999). The variability in chloride tolerance among the clones used in this study was indicative of the broad genetic variation among *Populus* genotypes at the section, species, and clone level (Rajora and Zsuffa, 1990). Mechanisms of chloride tolerance for poplars are not well understood; however, optimal growth was reported at an EC ranging from 1 to 5 mS cm^{-1} (Neuman *et al.*, 1996). It has been shown that salt tolerance existed for *P. deltoides* × *P. nigra* (DN) clones. Aw and Wagner (1993) found that DN clones ranged in sensitivity when irrigated with wastewater with an EC of 3.21 mS cm^{-1}, while Shannon *et al.* (1999) reported growth reduction at 3.3 mS cm $^{-1}$. Overall, this corroborated the advancement of clone DN5 to selection cycle 4, while DN182 was removed from the experiment at the end of selection cycle 3, given an EC of the leachate of 10.2 mS cm^{-1}.

Overall, based on our results, there is potential for on-site treatment of landfill leachate using poplars. However, there are concerns with leachate application associated with optimizing plant growth while minimizing environmental impact. In some cases, where levels of contaminants are toxic, the leachate should be diluted before application to alleviate the potential phytotoxic effects to the trees. For example, Wong and Leung (1989) reported the need to dilute leachate in order for *Brassica* (cabbage) and *Acacia* (acacia) plants to sustain the nutritional advantages of the fertilization without exhibiting phytotoxic impacts. In general, *Brassica* species had greater yields when irrigated with leachate, while species of both genera experienced inhibited root growth from leachate application. Nevertheless, the need for dilution depends upon the specific leachate composition and the genotypes used for remediation. Cheng and Chu (2007) irrigated 12 tree species for 90 d with leachate exhibiting broad differences in chemical properties. Overall, none of the leachate sources caused toxic effects, growth inhibition, or decreased biomass accumulation, but leachate irrigation did increase leaf N concentration. Furthermore, pioneer tree species that shared silvicultural traits with *Populus* and *Salix* withstood harsh conditions imposed by elevated contaminant levels in the leachate. Toxic effects to plants are easier to see and measure than toxic effects to the environment. Application rates need to be carefully considered and monitored to minimize or eliminate contamination to the soil or groundwater.

These methods have important academic and practical implications, assuming that there are adequate levels of money and time. However, it is realized that the phyto-recurrent selection techniques used in the current study may be more complicated and time consuming than researchers and resource managers can allocate for genotypic testing. An easier and more-efficient selection effort could involve the use of only one or two of the selection cycles. For example, methods described for selection cycle 1 could be used with larger containers and greater numbers of clones for an extended length of time. In addition, less complex observations and data collection may be sufficient for selection of the best performing clones tailored to specific project objectives. A second consideration not addressed in the current study is the field deployment of rooted planting stock. Root initiation in this experiment was sensitive to osmotic stress, which might be overcome with selection cycles and field deployment of rooted cuttings. In addition, the use of rooted material would allow land managers access to hundreds of genotypes that otherwise will not survive in the field due to erratic rooting capability. There is a potential for improvement of remedial success because root initiation is essential to cutting survival, but the ability of a cutting to root in the greenhouse compared with the field has no bearing on remediation effectiveness. Overall, the most useful recommendation for researchers and resource managers is to test genotypic material prior to field deployment to make superior clonal selections for enhancement of remedial efforts.

In summary, 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. Future landfill leachate remediation research in regions similar to the North Central United States should evaluate rooted cuttings as well as unrooted cuttings, especially for *P. deltoides* genotypes that do not establish well from unrooted cuttings because of erratic rooting. Additionally, there is a need to assess the effect of landfill leachate on macro- and micro-organisms in the rhizosphere.

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