International Journal of Phytoremediation, 7:177–197, 2005

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/16226510500214632

CLONAL VARIATION IN SURVIVAL AND GROWTH OF HYBRID POPLAR AND WILLOW IN AN *IN SITU* TRIAL ON SOILS HEAVILY CONTAMINATED WITH PETROLEUM HYDROCARBONS

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Species and hybrids between species belonging to the genera* Populus *(poplar) and* Salix *(willow) have been used successfully for phytoremediation of contaminated soils. Our objectives were to: 1) evaluate the potential for establishing genotypes of poplar and willow on soils heavily contaminated with petroleum hydrocarbons and 2) identify promising genotypes for potential use in future systems. We evaluated height, diameter, and volume after first year budset by testing 20 poplar clones and two willow clones. Unrooted cuttings, 20 cm long, were planted in randomized complete blocks at 0.91- × 0.91-m spacing at Gary, IN, USA (41.5°N, 87.3°W). Four commercial poplar clones (NM6, DN5, DN34, and DN182) were planted as 20- and 60-cm cuttings. Sixty-cm cuttings exhibited greater height and diameter than 20-cm cuttings; however, we recommend continued use and testing of different combinations of genotype and cutting length. We identified promising genotypes for potential use in future systems and we recommend allocating the majority of resources into commercial poplar clones, given their generalist growth performance. However, further utilization and selection of experimental clones is needed. Specific clones rather than genomic groups should be selected based on the geographic location and soil conditions of the site.

KEY WORDS: phytoremediation, plant-enhanced bioremediation, phreatophytes, *Populus*, *Salix*, oil

INTRODUCTION

Global environmental contamination from petroleum hydrocarbons results from industrial petroleum wastes associated with transportation and accidental spills/releases

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(Harris *et al.*, 2001; Maki *et al.*, 2001; Reis, 1996). Problems associated with properly containing such wastes have gained much attention in North America over past decades (LaRiviere *et al.*, 2001; Lombi *et al.*, 2001; Nriagu, 1979). Contaminants from this waste stream have polluted the surface water, groundwater, soil, and sediments much faster than traditional technologies could remediate the problem. Nevertheless, regardless of the source of environmental degradation and duration of impact, we currently have biological technologies to reduce the environmental damage caused by such pollution (Ensley, 2000; Gatliff, 1994; Pardue *et al.*, 2001; Shen *et al.*, 2002). For example, methods that utilize plants alone or plants and their rhizospheric microorganisms to destroy, remove, and stabilize contaminated soils are currently gaining attention in North America because such systems have become increasingly efficient and effective from biological and economical standpoints (Chappell, 1997; Cunningham *et al.*, 1996; McIntyre and Lewis, 1997; Yateem *et al.*, 2000).

Phytoremediation, plant-enhanced bioremediation, consists of using this symbiotic relationship, along with soil amendments and proper management practices, to remediate contaminated soils *in situ* (Cunningham and Ow, 1996; Cunningham *et al.*, 1997; Kulakow *et al.*, 2000). One group of plants currently used for phytoremediation is a limited number of selected cottonwoods and their hybrids (*Populus* species, excluding the aspens and colloquially known as poplars) (Burken and Schnoor, 1998; Campbell *et al.*, 1995; Gilbertson *et al.*, 2001; Isebrands and Karnosky, 2001; McLinn *et al.*, 2001). Poplars have been used to remediate sites with contamination from petroleum hydrocarbons, nitrates, salts, landfill leachates, heavy metals, pesticides, solvents, explosives, and radionuclides (Burken, 2001; Erdman and Christenson, 2000; Gordon *et al.*, 1997; O'Neill and Gordon, 1994; Schnoor, 2000; Thompson *et al.*, 1998). Another group of plants currently used for phytoremediation is species and hybrids between species belonging to the genus *Salix* (hereafter referred to as willows) (Hasselgren, 1998; Landberg and Greger, 1996; Perttu and Kowalik, 1997). Although there is a lack of knowledge of the genetics and genotype × environment interactions of willows in the north central United States, willows have been studied extensively in other regions of North America (Kopp *et al.*, 1993, 2001; Labrecque *et al.*, 1994) and Europe (Christersson, 1986, 1987). In addition, willows (primarily *Salix viminalis* L., *S. dasyclados* Wimm., *S. fragilis* L., *S. kinuyanagi* Kimura, and *S. discolor* Muhl) have been used worldwide for phytoremediation of dairy-farm effluent (Roygard *et al.*, 2001), wastewater sludge (Labrecque *et al.*, 1998), municipal wastes (Perttu, 1993), and cadmium phytoextraction (Eriksson and Ledin, 1999; Greger and Landberg, 1999; Klang-Westin and Eriksson, 2003; Luyssaert *et al.*, 2001; Punshon and Dickinson, 1997).

The complex interaction among the plants, microbes, and soil can be altered to promote dissipation of organic compounds through hydraulic uptake and control driven by evapotranspiration and microbial activity (Cunningham *et al.*, 1997; Hutchinson *et al.*, 2001b; Lappin *et al.*, 1985; Schnoor, 2000). Plants can take up and break down organics (phytodegradation), take up and transpire the contaminant (phytovolatilization), or the microbes of the rhizosphere can degrade the pollutant (rhizodegradation) (Schnoor *et al.*, 1995; Stehmeier *et al.*, 2001), which is most likely the mode of phytoremediation of petroleum hydrocarbons, given their chemical characteristics (Banks *et al.*, 1999; Hutchinson *et al.*, 2001a). Mycorrhizal fungi that are in physical contact with the plant root provide mineral nutrients to the plant while receiving simple sugars from the plant (Schnabel and White, 2000). Root exudates from the trees provide a carbon source for the microbial population.

The objectives of this study were to: 1) evaluate the potential of establishing current genotypes of poplar and willow on soils contaminated with heavy concentrations of petroleum hydrocarbons and 2) identify promising genotypes of poplar and willow for potential use in future phytoremediation systems. To accomplish these objectives, we assume that phytoremediation potential is proportional to survival, height and diameter growth, and volume production, which are our measures of potential benefit, because successful tree establishment is the first requirement for long-term petroleum degradation by the trees and/or their associated rhizospheric microorganisms. We demonstrate that these trees will establish and develop on petroleum-contaminated soils.

MATERIALS AND METHODS

Clone Selection and Plot Establishment

Twenty poplar clones and two willow clones were selected from six and two genomic groups, respectively, during January 2002. The genomic groups were selected based on their current utilization and associated growth potential in the north central United States. Four commercial poplar clones (NM6, DN5, DN34, and DN182) were planted as 20- and 60-cm unrooted cuttings. All other poplar and willow clones were planted as unrooted 20-cm cuttings (Table 1), which were processed from whips grown for one growing season

Table 1 Genomic groups and clones in an experiment evaluating the potential of establishing poplar and willow on soils heavily contaminated with petroleum hydrocarbons

Genomic group	Clone
Populus	
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*	NC14002
〃	NC13649
〃	NC13624
〃	NC13570
〃	NC13377
P. deltoides × *P. deltoides* (F_1 hybrid)	80X00601
P. deltoides × *P. deltoides* (F_2 hybrid)	119.16
P. deltoides × *P. maximowiczii*	NC14107
〃	NC14105
〃	NC14104
P. nigra × *P. maximowiczii*	NM6
P. deltoides × *P. nigra*	DN5
〃	DN34
〃	DN182
P. deltoides	NAT[a]
〃	D125
〃	D124
〃	91.05.02
〃	8000105
〃	7300501
Salix	
S. × *dasyclados*	SV1
S. sachalinensis	SX61

[a]Selection of native *Populus deltoides* growing along the Indiana Harbors Canal. Note: Authorities for the aforementioned species of *Populus* and *Salix* are as follows: *P. deltoides* Bartr. ex Marsh; *P. trichocarpa* Torr. & Gray; *P. maximowiczii* A. Henry; *P. nigra* L.; *S.* × *dasyclados* Wimm.; *S. sachalinensis* F. Schmidt.

in stool beds established at Hugo Sauer Nursery, Rhinelander, WI, USA (45.6°N, 89.4°W). The 60-cm cuttings were acquired from a commercial nursery, which processed the cuttings from whips grown for one growing season in stool beds established in Oregon, USA. The 20- and 60-cm cuttings were processed during the winter of 2002, with cuts made to position at least one primary bud no more than 2.54 cm from the top of each cutting.

During May 2002, the cuttings were soaked in water for 5 d before planting along the shores of the Indiana Harbors Canal and the Lake George Branch of the canal near Gary, IN, USA (41.5°N, 87.3°W) (Figure 1). The Indiana Harbors Canal and surrounding areas are located in the physiographic province known as the Calumet Lacustrine Plain, which is characterized by dunes, beach sands, and lacustrine silts and gravels deposited atop a 15.24–45.72-m thick series of lacustrine clays and glacial till (Schneider, 1966). Bedrock underlies the clay/tills, with unconsolidated deposits hosting a relatively thin but laterally extensive aquifer known as the Calumet Aquifer (Fenlon and Watson, 1993).

The experimental design was randomized complete blocks with five replications (hereafter referred to as reps), five rows per rep, and 20 trees per row. Spacing was 0.91 × 0.91 m between cuttings. Willow cuttings were planted closest to the Indiana Harbors Canal, because of fluctuating water levels (Figure 2) and the potential for periodic flooding. There were 18 ramets per clone for willow, while poplar clones were represented by different numbers of ramets, depending on the status of clone (commercial versus experimental) and the length of cutting. Specifically, there were five and three ramets for commercial poplar clones (NM6, DN5, DN34, and DN182) planted as 20- and 60-cm cuttings, respectively. There were two ramets per genotype for experimental poplar clones. Cuttings of each clone were planted randomly, with ramets of individual clones planted adjacent to one another.

Reps 1–4 were planted along the east shore of the Indiana Harbors Canal, where heavy polycyclic aromatic hydrocarbon (PAH) contamination dominated the upper 0.3 m of the soil column and where shallow groundwater exhibited relatively high dissolved petroleum concentrations. The soils of reps 1–4 along the Indiana Harbors Canal consisted of a clearly visible, impervious, compacted oily layer 15.24 cm thick, underlain by loose, well-sorted sand with relatively high permeability. Specifically, the riparian soils were contaminated with 2–43% (mean = 25%) total petroleum hydrocarbons (TPH), by weight. Soil samples collected in 2001 exhibited a mean total PAH concentration of 4100 $mg \cdot kg^{-1}$ in the riparian sediments, with similar tests of these samples exhibiting a mean TPH concentration of 250 $g \cdot kg^{-1}$ sediment dry weight. Rep 5 was planted along the Lake George Branch of the Indiana Harbors Canal, which served as a control site with little, if any, petroleum contamination. The soils of the control site consisted of 1.52 m of uncontaminated soils underlain by an uncontaminated aquifer. However, lack of adequate moisture, heavy clay content of the soils, and difficult vegetation management resulted in less than ideal planting conditions at the control site.

Based on a controlled-environment treatability study where all plants died due to hydrophobic conditions associated with the contamination of petroleum hydrocarbons (unpublished data), planting consisted of drilling holes with a 7.62-cm diameter auger, filling the holes with sand, and then planting the cuttings in the sand to a maximum depth of 17 and 57 cm for 20- and 60-cm cuttings, respectively. Once initial root systems were established in the sand, roots developed into the contaminated soil (Figure 3). An extensive excavation of two trees following the 2002 growing season revealed the presence of five to seven primary lateral roots, ranging from 0.64 to 0.95 cm in diameter, growing laterally into the upper, heavily oiled, and compacted soil horizon to a distance of 1.2 m and growing down vertically into the ground water. Additional smaller lateral roots were also initiated from

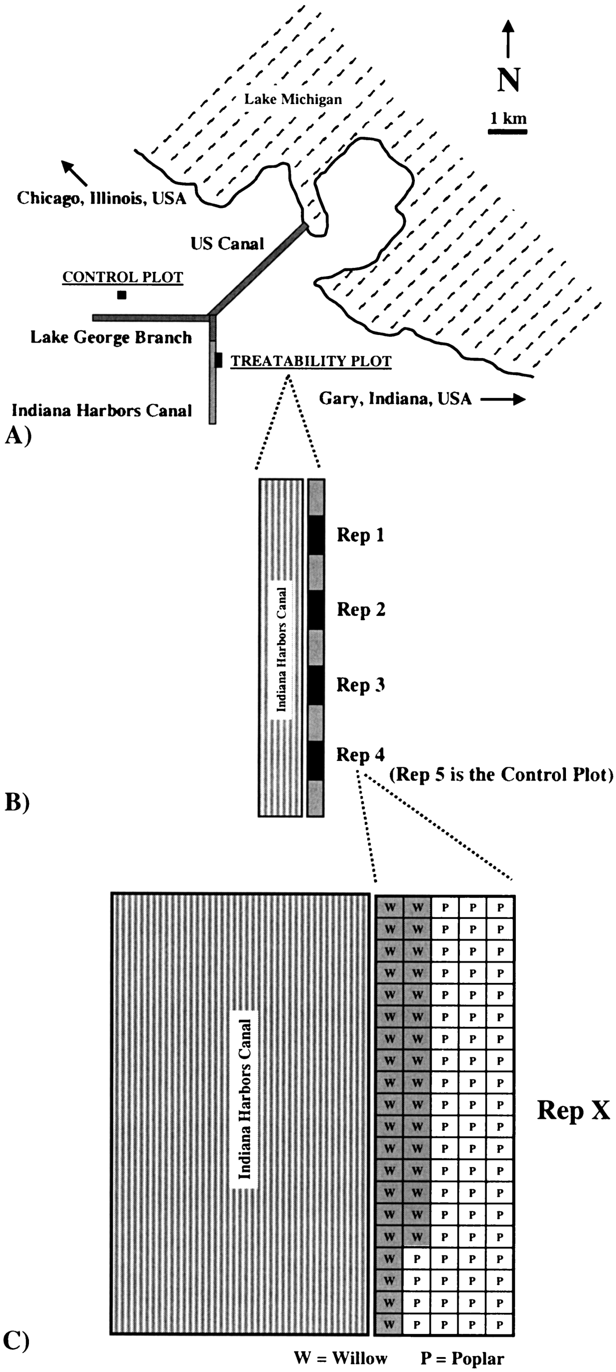

Figure 1 Map depicting the location of the treatability plot and control plot in relation to the Indiana Harbors Canal (A), diagram of the 4 reps along the Indiana Harbors Canal that made up the treatability plot (B), and diagram of a representative rep showing one potential layout of poplar and willow cuttings (C) in a study evaluating the potential of establishing poplar and willow on soils heavily contaminated with petroleum hydrocarbons. Clones were randomly planted with ramets from the same clone planted adjacent to one another.

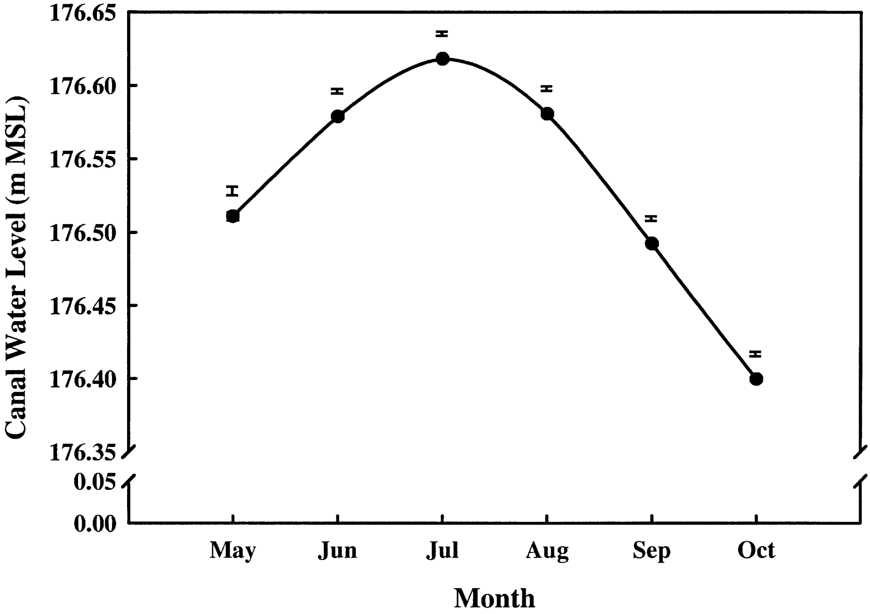

Figure 2 Fluctuating water levels in the Indiana Harbors Canal during the 2002 growing season. Standard errors are listed above the data points. The data were collected by the United States Geological Survey station number 04092750, which was located at Indiana Harbor in East Chicago, IN, USA (41.5°N, 87.3°W).

the cuttings. Secondary and tertiary fine roots were visible along the entire extent of the primary lateral roots.

Data Collection and Analysis

Height to the nearest 1.0 cm and diameter to the nearest 0.01 mm were determined on the terminal shoot of each tree following the first year budset. To reduce experimental error, height was measured from ground level to the base of the apical bud on the terminal shoot and diameter was measured at 10 cm above the soil surface. Survival was also recorded. Volume (cm^3) was estimated using the generalized equation $volume = diameter^2 \times height$, according to Avery and Burkhart (1994).

Height, diameter, and volume data were subjected to analyses of variance according to SAS® (PROC GLM; SAS Institute, 2000) assuming a simple all random effects model, including the main effects of rep and clone, the interaction between rep and clone, and experimental error. Clones were considered random in order to calculate variance components and other genetic parameters. A random model was justified because the clones were not selected based on any knowledge of phytoremediation capabilities, but rather on an arbitrary basis on suitability for the local climate and their general growth rate. Cutting length was not used as an independent variable in the analysis because of the unbalanced nature of clones nested within cutting length. Genus was not used as an independent variable in the analysis because of lack of adequate representation of willow clones. However, like clones representing different lengths of poplar planting stock were coded differently and included in the analysis. In addition, a separate analysis was conducted to compare sites. Fisher's protected least significant difference (LSD) was used to separate means of main effects when no higher-order interactions were present (Carmer and Walker, 1982; Chew, 1976).

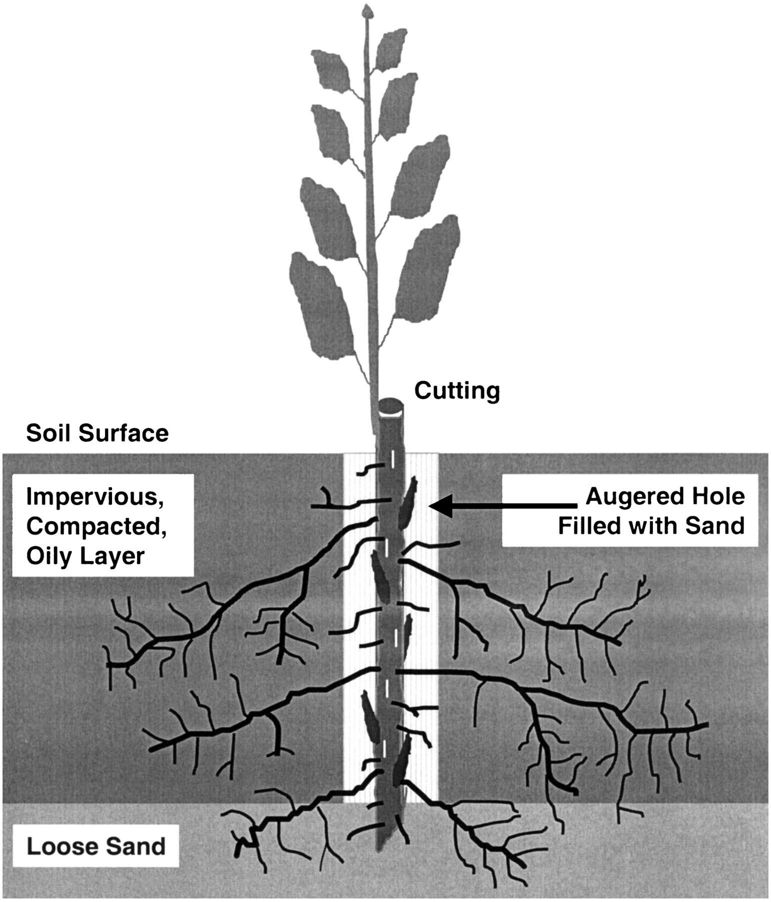

Figure 3 Longitudinal view of the planting design. Given an impervious, compacted, oily layer of soil 15.24 cm thick, cuttings were planted into a 7.62-cm diameter augered hole filled with sand. Once initial root systems were established in the sand, roots developed into the contaminated soil. A 20-cm cutting is shown.

Variance components were estimated by using restricted maximum likelihood (REML) models according to SAS® (PROC VARCOMP; SAS Institute, 2000). Variance components were used to estimate broad-sense heritability, the percent of phenotypic variation among clones due to combined genetic effects, on an individual-tree basis. Coefficients of variation, ratios of the standard deviation to the mean, also were estimated for each variance component.

RESULTS AND DISCUSSION

Survival

The experiment-wide survival rate was 67% (Table 2). The percent survival of trees across reps, genera, and cutting lengths ranged from 26% (rep 1, willow, 20-cm cuttings) to 100% (reps 1, 3, and 4, poplar, 60-cm cuttings). Sixty-cm poplar cuttings had the greatest survival rate (97%), followed by 20-cm poplar (62%) and willow (56%) cuttings. The

Table 2 Percent survival by rep, genus, cutting length, and clone following the 2002 growing season in soils heavily contaminated with petroleum hydrocarbons

		Rep[a]					
Genus—Cutting length[b]	Clone	1	2	3	4	5	Across reps
Poplar—20	NM6	40	100	100	100	100	88
″	NC14107	100	100	50	50	0	60
″	NC14105	100	0	50	50	33	47
″	NC14104	50	100	50	100	50	70
″	NC14002	100	50	100	50	25	65
″	NC13649	100	100	100	50	50	80
″	NC13624	100	100	0	100	50	70
″	NC13570	100	0	100	100	0	60
″	NC13377	100	0	50	100	25	55
″	NAT	100	100	0	na[c]	8	52
″	DN5	67	100	100	80	na	87
″	DN34	100	67	100	88	na	89
″	DN182	75	80	80	86	80	80
″	D125	0	0	100	50	0	30
″	D124	50	0	100	0	25	35
″	91.05.02	100	100	100	50	25	75
″	80X00601	100	50	50	50	75	65
″	8000105	100	100	50	0	0	50
″	7300501	100	0	100	50	0	50
″	119.16	50	50	100	0	0	40
	Across 20-cm poplar cuttings	82	60	74	61	30	62y[d]
Poplar—60	NM6	100	100	100	100	100	100
″	DN5	100	100	100	100	na	100
″	DN34	100	100	100	100	na	100
″	DN182	100	67	100	100	83	90
	Across 60-cm poplar cuttings	100	92	100	100	92	97z
Willow—20	SX61	29	77	71	92	45	63
″	SV1	22	87	35	43	60	49
	Across 20-cm willow cuttings	26	82	53	67	53	56y
Across genera, cutting lengths, and clones[e]		80a	66a	76a	68a	38b	67

[a]Reps 1 to 4 planted along the east shore of the Indiana Harbors Canal, rep 5 planted along the Lake George Branch of the canal (control site). [b]Cutting length in centimeters. [c]No trees planted. [d]Means followed by the same letter for each combination of genus and cutting length are not different; $LSD_{0.05} = 26$, n = 5. [e]Means followed by the same letter within the row are not different; $LSD_{0.05} = 18$, n = 25. Note: Twenty-cm and 60-cm poplar cuttings also were different ($P = 0.0002$) using a paired t-test according to Snedecor and Cochran (1989).

percent survival for reps ranged from 38% (rep 5) to 80% (rep 1). Moreover, the percent survival of clones within any given rep ranged from 0% to 100% and the survival rate of clones across reps ranged from 30% to 100%.

The overall survival rate of 67% in the current study was impressive given report of stunted plant growth with total petroleum hydrocarbons above 1% by weight (Reis, 1996), along with fluctuating water levels in the canal throughout the growing season and uncertainties as to which combinations of genotype and cutting length would perform well given the adverse growing conditions. In addition, we collected a composite sample of leaves from 20 randomly sampled trees and conducted tissue analysis to test for unusual micro- and macro-nutrient concentrations (unpublished data). A notable value was an anomalously heavy zinc concentration of 455.63 $mg \cdot L^{-1}$, which is extremely high for poplars. For example, Schnoor (2000) reported survival rates of 2.5% and >50% in South Dakota, USA, and Kansas, USA, respectively, for poplars grown under conditions of heavy zinc and cadmium concentrations. Furthermore, we believe major contributing factors to the low survival rate of rep 5 (38%) were the extensive clay texture of the soil, along with poor site preparation and the lack of adequate weed control throughout the growing season, which are requirements for successful plot establishment regardless of soil conditions (Hansen *et al.*, 1983, 1993).

The survival rate of 97% for 60-cm poplar cuttings was greater than an anticipated survival of $\leq$ 90%. All four commercial clones (NM6, DN5, DN34, and DN182) planted as 60-cm cuttings surpassed their 20-cm counterparts, but not by dramatic amounts (overall, 97% and 86%, respectively). Nevertheless, greater costs associated with purchasing, handling, and planting the 60-cm cuttings compared with the 20-cm cuttings may negate the benefit from the 11% increase in survival. For example, the stocking level at the current spacing of 0.91 $\times$ 0.91 m is 12,076 trees per hectare. Thus, the aforementioned survival rates of 86% (20-cm cuttings) and 97% (60-cm cuttings) require 14,042 and 12,450 trees per hectare to be planted for 20- and 60-cm cuttings, respectively. The average cost for 20- and 60-cm cuttings is $0.14 US and $0.36 US, respectively. Therefore, the savings in costs of cuttings alone, irrespective of handling and planting costs, would be $2516.12 US per hectare to achieve the desired stocking of 12,076 trees per hectare. Nevertheless, from an academic standpoint and where resources are available, we also recommend limited testing and utilization of cuttings of length 60 cm and above, with upper thresholds for cutting length to be determined.

Relatively poor willow survival rates will support the need for careful consideration of willow deployment in future plantings. However, given the potential random nature of locally contaminated microsites across years, we suggest a relatively lower overall percentage of willow be planted in lieu of discontinuing willow use all together. Another consideration with willow is that productivity often increases following the establishment year (Christersson, 1986; Labrecque *et al.*, 1993). Overall, poplar and willow survival rates were acceptable given impacts from high water levels such as hypoxia and contamination from a petroleum sheen left on the leaves after brief periods of flooding. Overall, depth of water inundation never exceeded the tops of the cuttings for more than seven continuous days.

Commercial clones (NM6, DN5, DN34, and DN182), irrespective of cutting length, exhibited greater survival rates than experimental clones. Nevertheless, broad genetic variation present in current experimental populations warrants further testing of such clones, because identification of superior genotypes may greatly increase establishment potential relative to current commercial standards. Future protocol should address the level of resources to invest in each of these groups. Nevertheless, trends in survival rate for the genomic groups defined in Table 1 appeared to be unstable across clones, which suggested identification of specific clones rather than genomic groups may result in greater establishment success.

Variance Components

Clones accounted for 13.07% and 16.77% of total variation in height and diameter, respectively (Table 3). Broad-sense heritability, the percent of phenotypic variation among clones due to combined genetic effects, was 0.13 and 0.17 for height and diameter, respectively. Within plot (ramet within clone) error constituted 69.99% and 72.12% of the variation in height and diameter, respectively. Evaluation of the coefficients of variation

Table 3 Variance components and derived statistics from analyses of variance of height and diameter

Source of variation	Variance component[a]	Percent of total variation	Genotypic variance[b]	Phenotypic variance[c]	Heritability[d]
Height					
Rep	5.71578	0.47			
Clone	160.78431	13.07	160.78431	160.78431	
Rep × Clone	202.70695	16.48		202.70695	
Within plot	860.96960	69.99		860.96960	
Total	1230.17664	100.01	160.78431	1224.46086	0.13
Diameter					
Rep	0.0149	2.43			
Clone	0.1029	16.77	0.1029	0.1029	
Rep × Clone	0.0533	8.68		0.0533	
Within plot	0.4425	72.12		0.4425	
Total	0.6136	100.00	0.1029	0.5987	0.17

[a]Restricted maximum likelihood (REML) estimates of variance components. [b]Variance attributed to additive, dominant, and epistatic genetic effects (combined genetic effects). [c]Variance attributed to phenotypic effects, where phenotype = genotype + environment + (genotype % environment). [d]Broad-sense heritability estimated on an individual-tree basis.

corroborated these estimates (Table 4). We attributed this substantial variation to the variable effects of the apparent petroleum hydrocarbons in the soil and the fluctuating water levels in the canal. In contrast, the effect of reps was very small relative to other sources of variation, with 0.47% and 2.43% of total variation in height and diameter attributed to reps, respectively (Table 3), which suggested that blocks were not different in their effect on height and diameter and that the variation among blocks was negligible.

Site Effects

Reps did not differ for height ($P = 0.7236$), diameter ($P = 0.1812$), or volume ($P = 0.6351$) (Table 5). Separate analyses of variance were conducted using site and experimental error in the linear additive model. Site differences were negligible for 20-cm poplar cuttings for height ($P = 0.7736$), diameter ($P = 0.5398$), and volume ($P = 0.5958$). Likewise, site differences were negligible for 60-cm poplar cuttings for height ($P = 0.9144$), diameter ($P = 0.5113$), and volume ($P = 0.5358$). In contrast, sites differed for willow cuttings

Table 4 Coefficients of variation from analyses of variance of height and diameter

	Coefficient of variation[a]	
Source of variation	Height	Diameter
Rep	4.99	8.00
Clone[b]	26.46	21.02
Rep × Clone	29.71	15.13
Within plot	61.24	43.60
Phenotypic[c]	73.03	50.71

[a]Coefficient of variation = ratio of standard deviation to the mean, with a mean height of 48 ± 2 cm and a mean diameter of 4.86 ± 1.27 mm. [b]Genotypic variation. [c]Phenotypic variation, where phenotype = genotype + environment + (genotype × environment).

Table 5 Analyses of variance of height, diameter, and volume

Source of variation	df	Mean square	F-variance ratio	*P*-value	Expected mean squares
Height					
Rep	4	642.09	0.52	0.7236	$\sigma^2 + 1.8932\sigma^2\text{RC} + 38.473\sigma^2\text{R}$
Clone	25	3222.70	2.41	0.0012	$\sigma^2 + 2.5082\sigma^2\text{RC} + 10.859\sigma^2\text{C}$
Rep % Clone	77	1406.27	1.47	0.0150	$\sigma^2 + 2.9631\sigma^2\text{RC}$
Within plot	227	953.91			σ^2
Total	333				
Diameter					
Rep	4	0.78	1.58	0.1812	$\sigma^2 + 1.8932\sigma^2\text{RC} + 38.473\sigma^2\text{R}$
Clone	25	1.50	3.01	<0.0001	$\sigma^2 + 2.5082\sigma^2\text{RC} + 10.859\sigma^2\text{C}$
Rep % Clone	77	0.50	1.04	0.4117	$\sigma^2 + 2.9631\sigma^2\text{RC}$
Within plot	227	0.48			σ^2
Total	333				
Volume					
Rep	4	2176.94	0.64	0.6351	$\sigma^2 + 1.8932\sigma^2\text{RC} + 38.473\sigma^2\text{R}$
Clone	25	3191.17	0.98	0.5036	$\sigma^2 + 2.5082\sigma^2\text{RC} + 10.859\sigma^2\text{C}$
Rep % Clone	77	3166.53	0.83	0.8334	$\sigma^2 + 2.9631\sigma^2\text{RC}$
Within plot	227	3827.34			σ^2
Total	333				

for height ($P = 0.0078$) and diameter ($P = 0.0015$), but were negligible for volume ($P = 0.1021$).

Confounding effects relating to site and cultural differences between the treatability plot along the Indiana Harbors Canal and the control plot near the Lake George Branch likely contributed to the negligible differences in height, diameter, and volume of poplar cuttings, regardless of cutting length. In addition to the obvious differences in contamination of the soils at the two locations, poplar trees at the Lake George Branch plot likely failed to outperform their treatability plot counterparts because of a combination of inadequate site preparation, challenging weed control, and heavy clay soils that resulted in less water availability to the trees at the control plot. In essence, the results of the current study showed that poplar performed equally well on petroleum-contaminated soils as on heavy soil conditions with poor vegetation management. The growth advantage of willow at the treatability plot versus the control plot further highlighted the importance of considering these confounding effects relating to soil differences and vegetation management. Mean height of willow along the Indiana Harbors Canal was 68 ! 6 cm and mean height at the Lake George control site was 37 ! 3 cm, while mean diameter along the canal was 5.33 ! 0.33 mm and mean diameter at the control site was 3.33 ! 0.17 mm. Therefore, we cannot conclude that there was an advantage or disadvantage for establishment of the poplar and/or willow cuttings at either site. However, the most important practical implication of these results is that survival and growth of poplar and willow is possible when planted on petroleum-contaminated soils, which indicates the potential for establishing such genotypes on similarly contaminated sites.

Genotypic Effects

Clones differed for height ($P = 0.0012$) and diameter ($P < 0.0001$), but did not differ for volume ($P = 0.5036$) (Table 5). Mean height across clones ranged from 14 ± 2 cm (NC13570) to 73 ! 9 cm (SX61) (Figure 4). Mean height of poplar clones planted as 20-cm

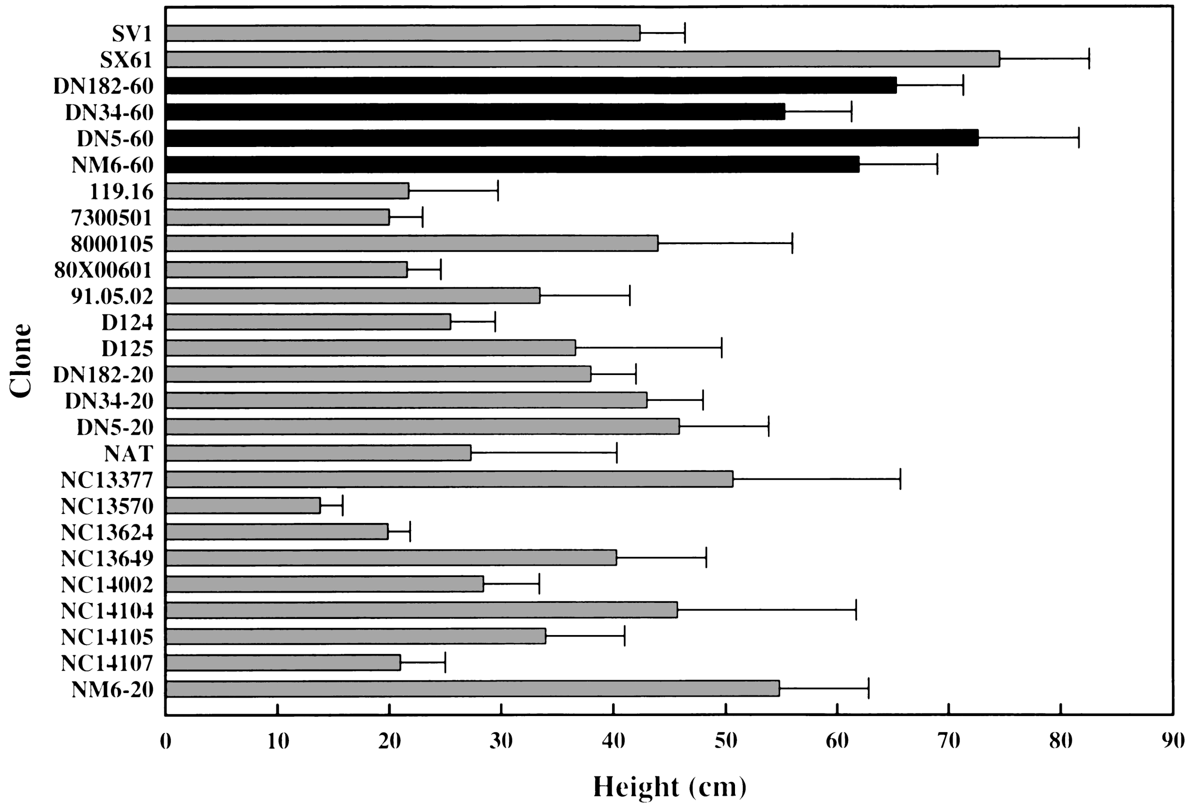

Figure 4 Height of poplar and willow trees in a study evaluating the potential of establishing the clones on soils heavily contaminated with petroleum hydrocarbons. Clones NM6, DN5, DN34, and DN182 were planted as 20- and 60-cm cuttings, which are designated Clone-20 and Clone-60, respectively. Clone means are different at $\alpha = 0.05$ with a LSD critical value = 31.9 cm.

cuttings ranged from 14 ! 2 cm (NC13570) to 55 ! 8 cm (NM6), while mean height of poplar clones planted as 60-cm cuttings ranged from 55 ! 6 cm (DN34) to 73 ! 9 cm (DN5) and mean height of willow clones was 42 ! 4 cm (SV1) and 45 ! 8 cm (SX61). Exhibiting similar trends, mean diameter across clones ranged from 2.00 ! 0.17 mm (NC13570) to 6.86 ! 0.56 mm (DN182) and 6.86 ! 0.68 mm (DN5) (Figure 5). Mean diameter of poplar clones planted as 20-cm cuttings ranged from 2.00 ! 0.17 mm (NC13570) to 5.62 ! 1.15 mm (NC13377), while mean diameter of poplar clones planted as 60-cm cuttings ranged from 5.77 ! 0.73 mm (DN34) to 6.86 ! 0.56 mm (DN182) and 6.86 ! 0.68 mm (DN5) and mean diameter of willow clones was 3.92 ! 0.21 mm (SV1) and 5.59 ! 0.43 mm (SX61).

Three general trends existed for height and diameter. First, commercial poplar clones (NM6, DN5, DN34, and DN182) planted as 60-cm cuttings performed better than all other combinations of genotype and cutting length. Second, broad genetic variation across poplar clones planted as 20-cm cuttings supported the potential for selection of well-performing clones. That is, of all poplar genotypes represented by 20-cm cuttings, selected experimental clones (i.e., NC13377 and NC14104) expressed as much or more potential than most commercial clones, with NM6 being the only exception. Therefore, although commercial poplar clones planted as 60-cm cuttings exhibited the greatest survival and growth in this study, the establishment potential of future studies may be increased with the addition of 20-cm cuttings of new genotypes not currently utilized or identified. Similar results of selected genotypic performance have been reported for other phytoremediation systems (Glass, 2000). For example, of two poplar genotypes resulting from crosses between *P. trichocarpa* and *P. deltoides* (H11-11 and 50–189), both clones exhibited detectable trichloroethylene (TCE) concentrations in their leaves and stems, while TCE was also detected in the roots of H11-11 (Gordon *et al.*, 1997). Greger and Landberg (2001) and Landberg and Greger (1994) tested the ability of *S. viminalis* for phytoextraction of cadmium and reported broad genetic variation among clones. In addition, the variety *angustifolia* of four-wing saltbush [*Atriplex canescens* (Pursh) Nutt.] exhibited the best establishment and growth on shifting dune soils contaminated with uranium mill tailings (Glenn *et al.*, 2001). Other researchers using agricultural crops reported similar results of successful genotype-dependent remediation (Ayoub, 1974; Glenn *et al.*, 2001; Lessani and Marschner, 1978; Wiltse *et al.*, 1998; Yang and Blanchar, 1993), while some showed a lack of varietal differences resulting in less opportunity for the selection of superior genotypes (Russelle *et al.*, 2001). Third, growth of willow clones justified their incorporation into future remediation plantings. We suggest that willow clones should be used in future designs, especially along the Indiana Harbors Canal and similar sites with fluctuating water levels and periods of water inundation, where poplars most likely would not survive (Dickmann *et al.*, 2001; Isebrands and Karnosky, 2001).

Genotype × Environment Interactions

There was an interaction between rep and clone for height ($P = 0.0150$); however, this interaction was negligible for diameter ($P = 0.4117$) and volume ($P = 0.8334$) (Table 5). The variability for height among reps and clones expressed an instability of overall genotypic performance (Table 6). That is, few clones performed consistently across reps, regardless of whether height growth was above average or below average. We attributed most of this clonal instability to specific microsite differences that promoted or failed to promote height growth.

Significant positive correlations between height growth and rooting traits such as root length and root dry weight of poplar have been reported (Riemenschneider and Bauer,

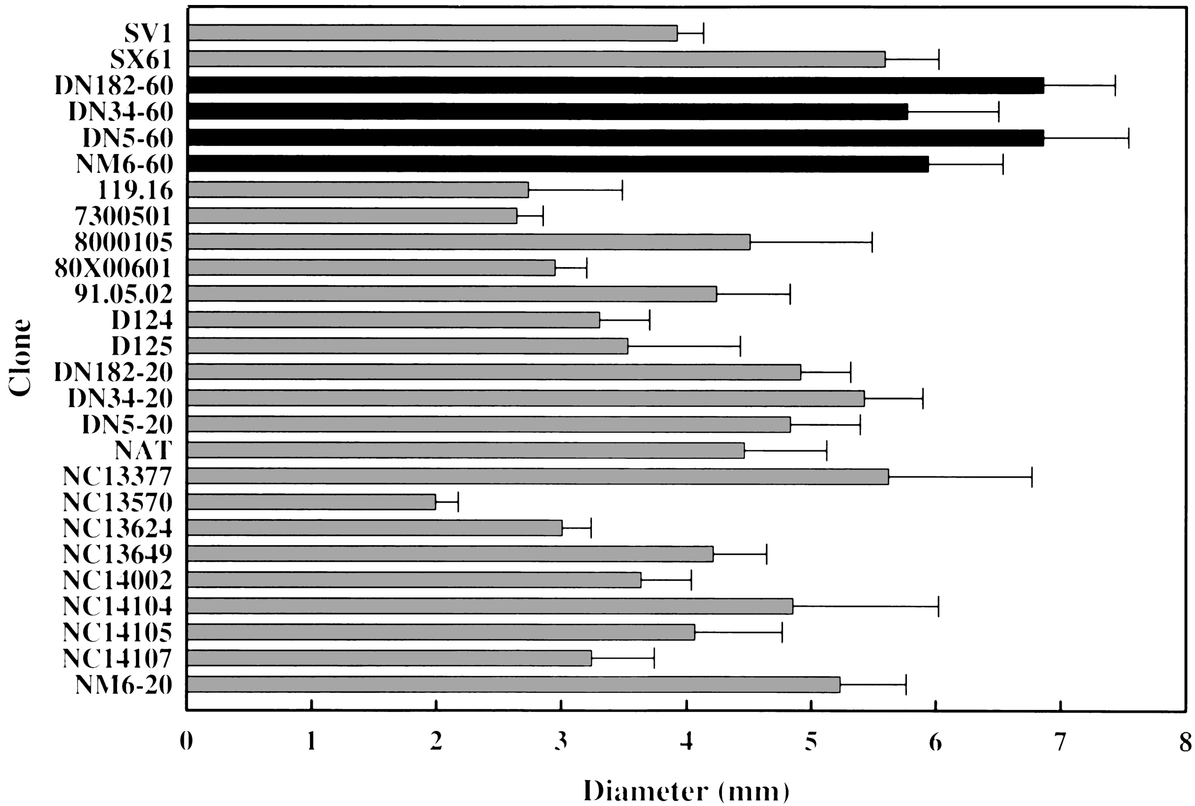

Figure 5 Diameter of poplar and willow trees in a study evaluating the potential of establishing the clones on soils heavily contaminated with petroleum hydrocarbons. Clones NM6, DN5, DN34, and DN182 were planted as 20- and 60-cm cuttings, which are designated Clone-20 and Clone-60, respectively. Clone means are different at $\alpha = 0.05$ with a LSD critical value = 2.28 mm.

Table 6 Rank and mean height (cm) by rep for each clone; standard errors are in parentheses

Clone	Rank	Rep 1		Rank	Rep 2		Rank	Rep 3		Rank	Rep 4		Rank	Rep 5		Rank	Overall[a]	
DN5-60[b]	4	54	(6)	2	92	(43)	4	63	(10)	2	88	(19)	na[c]	na	na	1	73	(9)
DN182-60	6	51	(3)	5	67	(17)	1	93	(15)	3	56	(13)	2	60	(10)	2	65	(6)
NM6-60	8	45	(4)	3	89	(29)	5	61	(7)	6	47	(3)	1	65	(12)	3	62	(7)
DN34-60	10	43	(7)	8	55	(6)	3	70	(20)	4	53	(8)	na	na	na	4	55	(6)
NM6-20	5	54	(19)	1	97	(30)	8	60	(15)	8	36	(2)	5	41	(12)	4	55	(8)
NC13377	1	90	(35)	x[d]	x	x	19	22	()	9	36	(3)	10	32	()	5	51	(15)
DN5-20	7	50	(24)	10	47	(14)	15	35	(8)	7	47	(10)	na	na	na	6	46	(8)
NC14104	14	27	()	4	77	(58)	13	46	()	12	34	(17)	14	25	()	6	46	(16)
SX61	11	39	(4)	7	63	(9)	2	74	(14)	1	138	(21)	9	35	(7)	7	45	(8)
8000105	13	31	(7)	6	66	(27)	17	28	()	x	x	x	x	x	x	8	44	(12)
DN34-20	16	25	(3)	11	43	(10)	12	50	(8)	5	48	(11)	na	na	na	9	43	(5)
SV1	15	26	(8)	9	50	(5)	6	61	(15)	15	22	(5)	8	39	(3)	10	42	(4)
NC13649	2	68	()	14	27	(9)	10	51	(23)	11	34	()	13	25	()	11	40	(8)
DN182-20	18	23	(6)	13	28	(6)	7	61	(9)	13	32	(6)	4	42	(6)	12	38	(4)
D125	x	x	x	x	x	x	11	50	(2)	21	11	()	x	x	x	13	37	(13)
91.05.02	3	63	(16)	15	25	(11)	22	15	(2)	16	22	()	6	41	()	14	34	(8)
NC14105	12	32	(14)	x	x	x	9	56	()	17	20	()	11	30	()	14	34	(7)
NC14002	21	20	(5)	16	23	()	14	36	(16)	14	26	()	7	39	()	15	28	(5)
NAT	25	4	()	12	30	()	x	x	x	na	na	na	3	48	()	16	27	(13)
D124	22	19	()	x	x	x	16	32	(6)	x	x	x	16	19	()	17	26	(5)
199.16	9	44	()	17	18	()	23	13	(1)	x	x	x	x	x	x	18	22	(8)
80X00601	20	21	(1)	19	15	()	20	20	()	22	9	()	12	29	(6)	18	22	(3)
NC14107	19	22	(1)	18	17	(7)	24	13	()	10	36	()	x	x	x	19	21	(4)
7300501	23	18	(5)	x	x	x	18	26	()	19	13	()	x	x	x	20	20	(3)
NC13624	17	25	(7)	20	14	(3)	x	x	x	18	18	(6)	15	23	(3)	20	20	(2)
NC13570	24	14	(2)	x	x	x	21	15	(6)	20	13	(1)	x	x	x	21	14	(2)
Overall[e]		37	(3)		53	(4)		53	(4)		55	(6)		41	(3)		48	(2)

[a]Mean estimated across all reps. [b]Clone-60 = 60-cm cuttings for designated clone; Clone-20 = 20-cm cuttings for designated clone. [c]No trees planted. [d]Lack of experimental trees due to mortality. [e]Mean estimated across all clones. Note: Overall means for clone and rep are not estimable from individual rep × clone interaction means shown in the table due to variable numbers of experimental units resulting from mortality.

1997; Zalesny, 2003; Zalesny *et al.*, 2005). Thus, we believe height growth can be used as a surrogate measure of rooting, which may have increased associated microbial activity in the rhizosphere. Therefore, our better-rooting clones may exhibit greater remedial potential than our recalcitrant clones. The interaction of such clones with specific microsites contributed greatly to the success of our system. Root growth and subsequent plant productivity depend on the availability and uptake of available nutrients (Kelly *et al.*, 2001; Xu and Obbard, 2003) and soil moisture and temperature levels (Hutchinson *et al.*, 2001b; Reilley *et al.*, 1996; Reynolds *et al.*, 2001). Thus, based on results from earlier studies (Churchill *et al.*, 1995; Hutchinson *et al.*, 2001a; Rasiah *et al.*, 1992), we recommend testing our top-performing clones along with additions of nitrogen fertilizer and adequate irrigation. Hutchinson *et al.* (2001a) reported significantly greater reductions of total petroleum hydrocarbons when tall fescue (*Festuca arundinacea* Schreb.) and bermuda grass (*Cynodon dactylon* (L.) Pers.) were fertilized compared with no fertilization. In addition, Hutchinson *et al.* (2001b) found daily irrigation at 30 cm below the soil surface supported the greatest root growth and degradation of petroleum hydrocarbons. Micropores and macropores created by the growth of roots in the soil may also have contributed to the remediation, because of the subsequent oxygen availability in the soil for hydrocarbon degradation (Hutchinson *et al.*, 2001b; Lee *et al.*, 2001; Sepic *et al.*, 1995), along with rooting-induced alteration of soil characteristics in the rhizosphere such as pH, redox conditions, organic matter content, nutrient levels, and hydrophilicity of the soils creating favorable microenvironments for hydrocarbon degradation (Anderson *et al.*, 1993; Andreotti *et al.*, 2001; Crowley *et al.*, 1997; Reilley *et al.*, 1996; Reynolds *et al.*, 2001). In addition, rhizodeposition of root exudates may have contributed to increased microbial population sizes, microbial activity, and subsequent hydrocarbon degradataion in the rhizosphere (Andreotti *et al.*, 2001; Banks *et al.*, 1999; Reilley *et al.*, 1996; Reynolds *et al.*, 2001), which has also been shown to be true for other phytoremediation applications (Crowley *et al.*, 1997; Gaskin and Fletcher, 1997; Gordon *et al.*, 1997; Salt *et al.*, 1998).

The aforementioned clonal instability and negligible genotypic effects for volume led us to conclude that, in order to maximize the potential of establishing poplar and willow on petroleum-contaminated soils, the majority of utilization should be focused on top-performing clones that assume a generalist growth pattern. We recommend concentrating efforts on clones that performed well across reps, even though within any given rep, other clones were identified that had greater height than the generalist clones. However, we make these recommendations identifying that our results are based only on one growing season at two sites in relatively close proximity to one another. Thus, further testing across years and sites is needed before more reliable recommendations can be made. In addition to survival and growth, future testing should be physiological in nature and should include screening of clones for specific phytoremediation effectiveness. Nevertheless, we believe that, for the immediate future at the Indiana Harbors Canal and similar sites, efforts should be placed into planting and tending those clones that expressed the best survival and growth following the 2002 growing season, in addition to continued testing of willow clones and experimental poplar clones (i.e., NC13377 and NC14104). Testing of new genotypes is necessary and, given adequate resources, such genotypes may provide added benefit exceeding those clones we are currently recommending for use in such projects.

CONCLUSIONS

Our results verified the potential of establishing genotypes of poplar and willow on soils that were heavily contaminated with petroleum hydrocarbons. Survival and growth of the trees indicated such establishment was successful. Sixty-cm cuttings exhibited greater height and diameter than 20-cm cuttings; however, we recommend continued use and testing of different combinations of genotype and cutting length. We identified promising genotypes for potential use in future systems of this nature. We recommend allocating the majority of resources into commercial poplar clones because of their generalist growth performance. However, further utilization and selection of experimental clones is needed for increased remedial potential following establishment, as such benefits are proportional to gains from selection. Specific clones rather than genomic groups should be selected based on the geographic location and soil conditions of the site. Providing adequate site preparation and vegetation management is also crucial to ensure successful establishment and subsequent stand development, which are the necessary prerequisites for long-term phytoremediation success.

In addition, from a planting design standpoint, we recommend:

1) Testing more clones with less ramets per clone because of the success of blocking in reducing experimental variation in the field

2) Continuing the use of randomized complete blocks or using designs with greater precision, to capture experimental variation associated with soil heterogeneity
3) Planting monoclonal blocks of the best clones in steps 1 and 2 to reduce long-term intergenotypic competition, along with measuring of interior trees to reduce border effects.

Measuring and using cutting diameter at the time of planting as a concomitant variable in an analysis of covariance to reduce variation associated with the size of the cuttings also would be beneficial in steps 1 and 2.

For selecting clones upon which to perform the aforementioned *in situ* trials, we recommend short-term ($<$70 d) *ex situ* (greenhouse) treatability studies that test the capability of genotypes to grow and survive when continuously irrigated with the contaminant (if applicable) and/or using soil from the contaminated site, if either or both are available. Practical clonal selection indices that are commonly used in plant breeding such as multiplicative, weighted summation, and rank-summation indices are efficient and easy to develop, and should be used with phenotypic traits such as number of leaves, leaf area, shoot height, number of roots, root length, and dry weights of aboveground and belowground plant components, along with survival.

ACKNOWLEDGMENTS

In addition to the affiliations of the authors, this research was the result of cooperation from the following organizations: United States Environmental Protection Agency Office of Research and Development, Purdue University, United States Environmental Protection Agency Region V, United States Fish and Wildlife Service, Indiana Department of Environmental Monitoring, Roy F. Weston, Inc., and British Petroleum. We thank Steve Rock for conducting the controlled-environment treatability study that led to our planting methodology. We are grateful to the following people for review of earlier versions of this manuscript: Deahn DonnerWright, Rob Doudrick, Bill Headlee, Assibi Mahama, and Adam Wiese.

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