Coppice growth responses of two North American willows in acidic clay soils on coal mine overburden

A. Mosseler and J. E. Major

Natural Resources Canada, Canadian Forest Service – Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick, Canada E3B 5P7 (e-mail: amosseler@nrcan.gc.ca).

Received 5 February 2014, accepted 21 May 2014. Published on the web 29 May 2014.

Mosseler, A. and Major, J. E. 2014. **Coppice growth responses of two North American willows in acidic clay soils on coal mine overburden**. Can. J. Plant Sci. **94**: 1269–1279. Acid-generating mine spoils with low pH are a major problem for revegetation and site reclamation. We compared growth responses of 15 genotypes from two widespread willow species, *Salix discolor* Muhl. (DIS) and *S. eriocephala* Michx. (ERI), native to eastern and central North America on two adjacent coal mine spoil sites that differed strongly in both pH (3.6 vs. 6.8) and soil texture. Despite significantly poorer growth responses for several coppice biomass traits on a highly acidic clay deposit compared with adjacent shale overburden, these willow clones demonstrated a surprising tolerance for extremely acidic soil conditions. Analysis of survival and growth uncovered genotype × environment interactions, indicating that both species and genotypic differences within species could be used to select better-adapted genotypes for extreme conditions. Most ERI and DIS clones grew comparatively better on the shale overburden site, but two of eight ERI clones and one of seven DIS clones grew significantly better on the acidic clay site, indicating the possibility for clonal selection for specific site adaptations within a species. Allometric relationships between coppice height and basal stem diameter were constant at both the species and site levels. However, there was a divergence of height and diameter in their relationship with green mass yields on the two different site types.

Key words: Acidic soil, genotype variation, growth responses, mine reclamation, *Salix* spp., willows

Mosseler, A. et Major, J. E. 2014. **Croissance des taillis de deux saules d'Amérique du Nord sur l'argile acide des morts-terrains d'une mine de charbon**. Can. J. Plant Sci. **94**: 1269–1279. Les déblais miniers à faible pH, qui libèrent des acides, entravent sérieusement les efforts de végétalisation et de restauration. Les auteurs ont comparé la croissance de 15 génotypes de deux espèces très communes de saule, *Salix discolor* Muhl. (DIS) et *S. eriocephala* Michx. (ERI), indigènes à l'est et au centre de l'Amérique du Nord sur deux sites adjacents de déblais miniers dont le pH (3,6 c. 6,8) et la texture du sol variaient considérablement. Malgré la réaction significativement plus faible de plusieurs caractères de croissance associés à la biomasse observée sur le dépôt d'argile très acide, comparativement aux résultats obtenus sur le mort-terrain d'ardoise adjacent, les clones se sont avérés étonnamment tolérants aux sols très acides. L'analyse du taux de survie et de la croissance révèle des interactions entre le génotype et l'environnement, signe qu'on pourrait se servir des écarts entre les espèces et les génotypes pour sélectionner des variétés mieux adaptées à des conditions extrêmes. La plupart des clones de ERI et de DIS poussent comparativement mieux sur l'ardoise, mais deux des huit clones de ERI et un des sept clones de DIS poussaient nettement mieux sur l'argile acide, ce qui laisse croire qu'on pourrait sélectionner des clones spécifiquement adaptés à certains sites. Les liens allométriques entre la hauteur des taillis et le diamètre des tiges à la base restent constants pour les deux espèces et les deux sites. On relève toutefois une différence dans la relation entre la hauteur/le diamètre et le rendement en biomasse verte aux deux endroits étudiés.

Mots clés: Sol acide, variation du génotype, croissance, restauration des mines, *Salix* spp., saule

In North America, there has been a longstanding interest in the use of willows (*Salix* spp.) as a source of biomass for energy purposes (Mosseler et al. 1988; Zsuffa 1990; Labrecque and Teodorescu 2005; Volk et al. 2006). More recently, there has been a growing interest in the potential use of native willows for various environmental applications (Kuzovkina and Volk 2009; Mosseler et al. 2014a), and in particular, for use in revegetation and reclamation of highly disturbed mine sites affected by soil acidification and related heavy metal toxicities (Andersson 1988; Punshon and Dickinson 1997, 1999; Landberg and Greger 2002; Vyslouzilova et al. 2003; Kuzovkina et al. 2004; Kuzovkina and Quigley 2005; Shanahan et al. 2007). Of special concern for reclamation purposes is the identification of plants that can tolerate highly acidic mine tailings and rock overburden (Bagatto and Shorthouse 1999; Berti and Cunningham 2000; Green et al. 2003; Kuzovkina, et al. 2004; Shanahan et al. 2007). For instance, soil acidification can induce aluminum (Al) toxicity in plants, and although trees are generally tolerant of high Al concentrations, irreversible damage to tree roots can occur at pH < 4.2, resulting in decreased growth and biomass yields (Andersson 1988; Rout et al. 2001; Klang-Westin and Eriksson 2003).

Abbreviations: DIS, *Salix discolor*; **ERI**, *Salix eriocephala*; **MBG**, Montreal Botanical Garden; **VIM**, *Salix viminalis*

Can. J. Plant Sci. (2014) 94: 1269–1279 doi:10.4141/CJPS-2014-046

With more than 350 species worldwide, willows are widespread across the northern hemisphere. Canada has 76 native willows (Argus 2010), which are widely distributed across every region and are adapted to a large range of site conditions. However, in our field-testing activities with seven different North American willow species (Mosseler et al. 2014a), we observed anecdotally that some species have poorer survival and growth in heavy-textured clay deposits on the shale coal mine overburden where we have been field testing willows for reclamation purposes. Others have also reported poorer growth of willows on heavy-textured clay soils (Niiyama 1990; Ledin 1996), where roots may have difficulty growing through such hard, compacted substrates, especially during periods of drought (Desrochers and Tremblay 2009). However, Gage and Cooper (2004) reported better survival of several montane willow species on finer-textured soils. Periodic flash flooding and ponding of water on the former Salmon Harbour coal mine site near Minto, New Brunswick (NB), Canada (lat. 46.07°N, long. 66.05°W) have created a patchwork of small areas covered by thick clay deposits scattered over the broken shale rock overburden that dominates these coal mine spoils. The highly variable post-mining landscape at the Salmon Harbour mine site provided an opportunity to assess growth performance in a set of 15 genotypes from two willow species, *Salix discolor* (DIS) and *S. eriocephala* (ERI), that were established together on shale overburden with, and without, the heavy clay deposits that result from pooling of water from surface runoff following rainstorms and from the pumping of flood waters draining from former mine shafts.

These two willows are widespread across eastern and central Canada and were being field tested in common-garden studies because both species appeared promising as fast-growing sources of biomass production (Mosseler et al. 1988). Both DIS and ERI are shrub willows and can be found together in natural populations on disturbed wetland sites throughout eastern and central North America, but DIS is also commonly found colonizing drier upland sites throughout its botanical range and is a common, naturally occurring species on the Salmon Harbour coal mine site, where it invades exposed shale overburden along with several other willow species (e.g., *S. bebbiana* Sarg. and *S. lucida* Muhl.), aspen (*Populus tremuloides* Michx.), balsam poplar (*P. balsamifera* L.), birches (*Betula papyrifera* Marsh. and *B. populifolia* Marsh.) and pin cherry (*Prunus pensylvanica* L.), whereas ERI is more commonly associated with stream banks disturbed by fast-flowing water.

Our objective was to assess growth performance (biomass yield) and coppice structure in 2-yr-old coppice growth from selected genotypes of DIS and ERI on two adjacent sites with very different soil substrates, despite having originated from the same shale rock overburden, in order to identify variation in species and genotypic responses to highly acidic, heavy-textured soil conditions. In order to assess the adaptation of willows for mine site reclamation, as well as biomass production for other purposes, it is important to understand the growth impacts of different soil types. There are several studies on the impacts of heavy metal tolerance on willows (Landberg and Greger 1996, 2002; Punshon and Dickinson 1997; Klang-Westin and Eriksson 2003), but very little *in situ* research has been done to define soil impacts on growth and survival in willows, and there is very little information on genetic variation within and among different willow species in relation to site adaptation. Here, we use a set of 15 genotypes from two willow species that were common to two different, adjacent soil types to assess the potential impact of a highly acidic, heavy clay deposit vs. a well-drained, coarse-textured shale overburden.

MATERIALS AND METHODS

A common-garden field test and a clonal gene bank of selected genotypes of DIS and ERI contained 15 genotypes in common (Table 1). Both field tests were established on two adjacent sites separated by approximately

Table 1. Origins of *Salix discolor* and *S. eriocephala* genotypes used for biomass yield and coppice trait measurements taken in two common garden studies at the Salmon Harbour mine site near Minto, NB

Species	Population	Latitude N.	Longitude W.	Selected clones
S. discolor	Levis, QC	46°78′	71°18′	LEV-D3, LEV-D6
	Lower Anfield, NB	46°92′	67°49′	ANF-D1
	Hawkesbury, ON	45°39′	74°75′	HAW-D5
	Montmagny, QC	46°94′	70°60′	MON-D1
	Mud Lake, ON	45°88′	76°78′	MUD-D4
	Richmond Fen, ON	45°13′	75°82′	RIC-D2
S. eriocephala	Ste. Anne de la Perade, QC	46°56′	72°20′	ANN-E6
	Bristol, NB	46°47′	67°58′	BRI-E2
	Fosterville, NB	45°78′	67°76′	FOS-E1
	Fredericton, NB	45°94′	66°62′	FRE-E1
	Green River, NB	47°34′	68°19′	GRE-E1
	Norton, NB	45°67′	65°81′	NOR-E10
	Shepody Creek, NB	45°71′	64°77′	SHE-E3
	Rivière au Saumon, QC	47°21′	70°35′	SAU-E3

300–480 m on the Salmon Harbour coal mine spoils near Minto, NB (lat. 46.07°N, long. 66.05°W), on a property operated by NB Coal Ltd., a subsidiary of NB Power, the local electrical power utility. These adjacent mine spoil sites consisted of recently bulldozed and landscaped shale overburden that resulted from coal strip-mining operations. However, one of these sites had existed for approximately 3 yr as a settling pond that was part of a watercourse draining the mine site. This pond deposited a thick layer of clay covering a rectangular area measuring approximately 70 m × 40 m over broken shale rock overburden. The clay depth on this deposit was determined by digging 44 holes with a soil auger in a grid pattern at 6 m × 6 m intervals across the area of the clone bank and measuring the depth to bedrock. The average depth of the clay layer was 35 cm, and ranged in depth from 11 cm to 48 cm.

A soil analysis based on six soil samples taken from each of these two sites indicated significant differences in soil characteristics (Table 2), with the clay deposit having a much higher proportion of clay (42.3%) than the normal shale overburden, which had a much higher proportion of coarser material such as sand (67.2%) and an average of 56.5% stone content that would not pass through a 2-mm sieve. The clay deposit also had a much higher proportion of organic matter, carbon (C), nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S). The sulfur content in the clay deposit was 10 times higher than the shale rock overburden, which probably explains the extreme acidity of the clay deposit (pH = 3.6 vs. pH = 6.8 for the shale overburden).

The genotypes were established on the clay deposit in May 2009 to maintain 31 of the best-performing genotypes of DIS and ERI that had been selected from a common garden field test established at the Montreal Botanical Gardens (MBG) in Montreal, Quebec, Canada, in 2005. Also included on both the clay deposit and the shale overburden sites was a check clone of *S. viminalis* (clone 5027) (VIM) introduced from the Swedish willow breeding program, and which has been widely used in short-rotation willow biomass trials across Canada (Guidi-Nissim et al. 2013). The study site on the clay deposit was a uniform area measuring 62 m × 21 m. Each genotype was established as a single linear row plot consisting of 21 ramets per genotype spaced at 1 m between ramets within the row plot and 2 m between adjacent rows, giving each plant approximately 2 m^2 of growing space. In order to analyze this test as a blocked (replicated) experiment, we divided each 21-ramet linear row plot into three artificial, or post hoc, blocks each with a single seven-ramet sample plot per clone per block. This results in a restriction on randomization within the experiment on the clay deposit. However, the clay deposit test was a small and very uniform area, and the overriding difference between it and the shale overburden site was the soil substrate: a deep clay deposit vs. broken shale rock overburden, and a pH of 3.6 vs. 6.8, respectively. Survival in the clone bank was determined over the 21-ramet row plot.

The shale overburden site was established in 2008 adjacent to the clone bank and included many of the same genotypes established in the clone bank on the clay deposit described above. The common garden on the shale overburden was located approximately 300 m from the clone bank (clay deposit) and extended for a distance of approximately 180 m along the watercourse that drained the mine site. The common garden test on the shale overburden was established along a gentle slope that had been landscaped to minimize soil erosion and surface runoff into the adjacent watercourse. Each genotype in the common garden test was established as a five-tree linear row plot with the five ramets spaced at 0.5 m within the plot and 2 m between plots. Each clonal plot was replicated three times in a randomized complete block test design.

Both study sites on the clay deposit and shale overburden were established with 20-cm-long unrooted stem cuttings collected during the dormant season from vigorous 1-yr and/or 2-yr-old stem sections (as per Densmore and Zazada 1978) from coppiced plants in a common garden established at the MBG. Therefore, each plant on the shale overburden had 1 m^2 of growing space versus 2 m^2 on the clay deposit. However, growth of the willow clones on both sites was quite poor relative to the much more fertile site of the MBG from where these clones had been selected (Mosseler et al. 2014a), and none of the plants fully occupied their growing space or appeared to be in competition for space. From October 24 to 26, 2011, the above-ground biomass of both the common garden and the clone bank was harvested at the root collar to induce coppicing. After 2 yr of coppice growth, both sites were harvested in the fall of 2013, when plants were still small enough at

Table 2. Soil properties for the two sites, rock and clay, at the Salmon Harbour (SH) mine site. Sites with different letters are significantly different using ANOVA test, $\alpha = 0.05$

Site	Organic matter (%)	Carbon (%)	Nitrogen (%)	Potassium (meq 100 g^{-1})	Calcium (meq 100 g^{-1})	Magnesium (meq 100 g^{-1})	Phosphorus (ppm)
SH-rock	0.79 ± 0.40*b*	0.46 ± 0.23*b*	0.102 ± 0.011*b*	0.233 ± 0.037*a*	7.33 ± 0.58*b*	0.66 ± 0.12*b*	3.98 ± 0.65*b*
SH-clay	9.14 ± 0.40*a*	5.31 ± 0.23*a*	0.207 ± 0.017*a*	0.297 ± 0.037*a*	14.84 ± 0.58*a*	2.90 ± 0.12*a*	10.75 ± 0.65*a*
Site	Sand (%)	Silt (%)	Clay (%)	pH	C:N ratio	Sulfur (%)	
SH-rock	67.2 ± 2.4*a*	23.4 ± 2.5*b*	9.4 ± 1.9*b*	6.8 ± 0.2a	4.6 ± 1.4*a*	0.008 ± 0.012*b*	
SH-clay	12.9 ± 2.4*b*	44.9 ± 2.5*a*	42.3 ± 1.9*a*	3.6 ± 0.2*b*	25.9 ± 1.4*b*	0.079 ± 0.012*a*	

both study sites that differences in spacing between these sites did not affect coppice growth from intra- or interclonal competition.

The first representative plant from each of the three, five-ramet plots was selected for harvest of the 2-yr-old coppice growth. The number of coppice stems per coppice was counted, and the total green mass was measured in the field to the nearest 10 g using an electronic weight scale (Digital Infant Scale, model ACS-20A-YE, Wuxi Weigher Factory Ltd., Wuxi City, Jiangsu, China). Previous measurements of fresh and dry mass taken in the fall at the MBG common garden demonstrated that percent moisture content was approximately 50 ± 2% regardless of species or clones (Mosseler, unpublished data). For each of the three harvested plants per genotype per study site, the length of each coppice stem was measured to the nearest 1 cm using an aluminum meter ruler, and the basal diameter of each coppice stem was measured to the nearest 0.1 mm using an electronic caliper. There was a total of 90 samples in the hierarchal nested experiment. Green mass in tonnes per hectare was calculated by converting the harvested biomass per plant to biomass production per hectare to a standard 1 m^2 by multiplying by 10 (e.g., multiplying by 10 000 plants per ha divided by 1000 kg per tonne). Overall yield was the product of yield and survival.

Statistical Analysis

The data from both study sites were subjected to analyses of variance (ANOVA) in which site, species and clones were considered fixed effects. The ANOVA model used was as follows:

$$Y_{ijklm} = u + B_{i(j)} + T_j + S_k + C_{l(k)} + S_k T_j + C_{l(k)} T_j + e_{ijklm}$$

where Y_{ijklm} is the dependent ramet trait of the *i*th block, of the *j*th site, of the *k*th *species*, of the *l*th clone, of the *m*th ramet, and *u* is the overall mean. B_i is the effect of the *i*th block ($i = 1, \ldots 3$). T_{j} is the effect of the *j*th site ($j = 1, 2$), S_k is the effect of the *k*th species ($k = 1, 2$), $C_{l(k)}$ is the effect of the *l*th clone ($\mathrm{l} = 1, \ldots 8$) nested within the *k*th species, and $S_k T_j$ is the effect of the species × site interaction, $\mathrm{C}_{l(k)} T_j$ is the effect of the clone nested within species x site interaction, and $e_{ijkl\mathrm{m}}$ is the random error component.

Allometric growth relationships were analyzed using analysis of covariance (ANCOVA). In these analyses, three sources of variation were studied: (1) covariate (i.e., diameter), (2) independent effect (site or species), and (3) independent effect × covariate. The analyses were based on the following model:

$$Y_{ij} = B_0 + B_{0i} + B_1 X_{ij} + B_{1i} X_{ij} + e_{ij}$$

where Y_{ij} is the dependent trait of the *j*th tree of the *i*th site or species, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are the site or species-specific coefficients, X_{ij} is the independent variable, and e_{ij} is the error term. Results were considered statistically significant at $\alpha = 0.05$, although individual *P* values are provided for all traits so that readers can make their

Table 3. Willow productivity trait ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), *P* values, and coefficient of determination (R^2). *P* values < 0.05 are in bold print

		Green mass (t ha^{-1})		Number of stems		Average single stem mass (kg)		Average stem height (m)	
Source of Variation	df	MS	*P* value	MS	*P* value	MS	*P* value	MS	*P* value
Block (site)	4	0.96	0.466	5.06	0.123	0.0008	0.219	0.097	**0.033**
Species	1	0.25	0.628	229.29	**< 0.001**	0.0052	**0.002**	0.179	**0.026**
Site	1	7.08	**0.012**	34.50	**< 0.001**	0.0005	0.315	0.071	0.157
Species × site	1	0.40	0.544	3.30	0.270	0.0002	0.517	0.078	0.137
Genotype (species)	13	2.43	**0.017**	8.51	**0.001**	0.0011	**0.030**	0.076	**0.021**
Genotype (species) × site	13	2.63	**0.010**	6.98	**0.006**	0.0006	0.314	0.089	**0.001**
Error	56	1.06		2.66		0.0005		0.034	
R^2			0.565		0.767		0.517		0.597

		Average stem diameter (cm)		Survival 2013		Overall yield (t ha^{-1})	
Source of Variation	df	MS	*P* value	MS	*P* value	MS	*P* value
Block (site)	4	0.081	0.064	0.016	0.800	0.584	0.521
Species	1	0.100	0.093	4.380	**< 0.001**	5.210	**0.009**
Site	1	0.143	**0.046**	0.325	0.006	6.220	**0.005**
Species × site	1	0.042	0.274	0.782	**< 0.001**	0.017	0.880
Genotype (species)	13	0.069	**0.036**			1.880	**0.006**
Genotype (species) × site	13	0.033	0.501			2.144	**0.002**
Error	56	0.034				0.716	
R^2			0.501		0.630		0.623

own interpretations. The data had satisfied normality and equality of variance assumptions. The general linear model from Systat (Chicago, IL) was used for analysis. Survival was arc sine square root transformed for normality.

RESULTS

Species and Site Variation

Although the soils of both study sites were composed from similar shale rock overburden, the clay deposit was highly acidic ($\mathrm{pH} = 3.6$) compared with the adjacent overburden with comparatively little clay ($\mathrm{pH} = 6.8$) (Table 2). The clay deposit also had a much higher proportion of organic matter, C, N, Ca, Mg, P and 10 times the S than the overburden site. The overburden had 44% greater green mass than the clay site with 1.83 and 1.27 t ha^{-1}, respectively ($P = 0.012$, Table 3, Fig. 1). With the exception of survival, there were no species by site interactions. The overburden site had 25% more stems per coppice than the clay site with 6.1 and 4.9 stems per coppice, respectively ($P < 0.001$, Fig. 2A). *Salix eriocephala* had 82% more stems per coppice than DIS, with 7.1 and 3.9 stems per coppice, respectively ($P < 0.001$). There were no site differences in average stem mass, but there were species differences, with DIS and ERI weighing 0.039 and 0.024 kg, respectively ($P = 0.002$, Fig. 2B). Average stem height was 0.87 and 0.78 m for DIS and ERI, respectively ($P = 0.026$, Fig. 3A). Average stem diameter was slightly greater on the clay vs. overburden, with 0.85 and 0.79 cm, respectively, ($P = 0.046$, Fig. 3B).

The shale overburden had greater overall survival than the clay site at 81 and 77%, respectively (Fig. 4A) and survival differences were significant between species ($P < 0.001$, Table 3) and for a species × site interaction ($P < 0.001$) effect. Overall, ERI and DIS had 93% and 65% survival, respectively. The significant species × site interaction was a result of rank change with site. *Salix eriocephala* had greater survival on overburden vs. the clay site, with 99 and 86%, respectively, whereas, DIS had lower survival on the overburden vs. clay at 62 and 68%, respectively. Overall yield, which includes a survival factor, shows that the overburden had 51% greater overall yield than the clay site, with 1.54 and 1.01 t ha^{-1}, respectively ($P = 0.005$, Fig. 4B). In contrast to plant green mass yields, overall yield (yield × survival) had a species effect with 48% greater yield for ERI compared with DIS, with 1.52 and 1.03 t ha^{-1}, respectively.

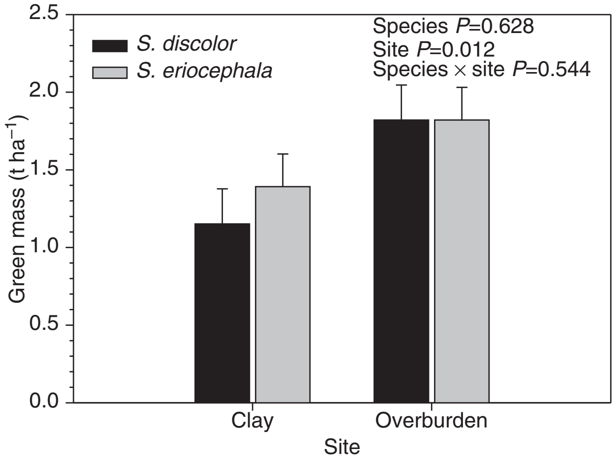

Fig. 1. Green mass yield (mean and SE) for 2-yr-old coppice of *Salix discolor* and *S. eriocephala* on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

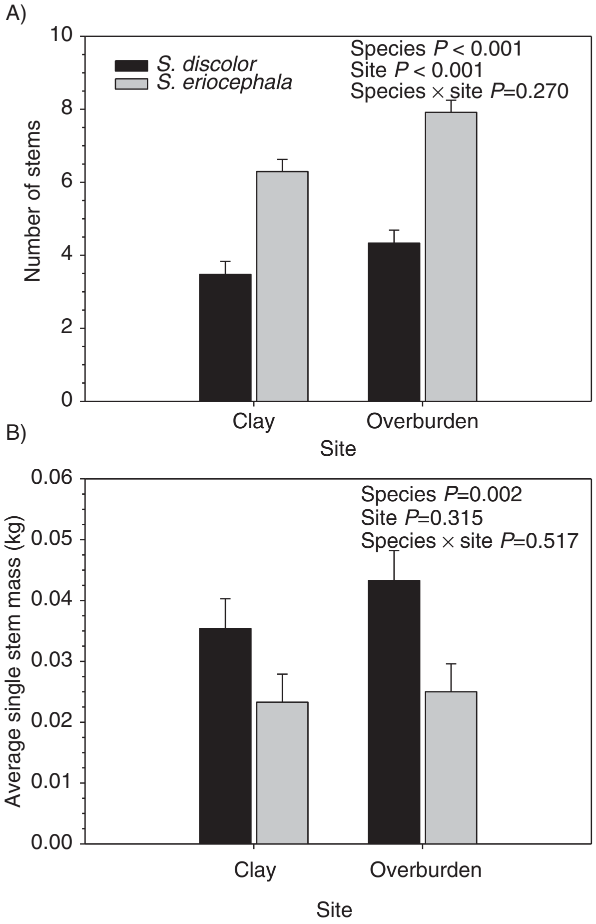

Fig. 2. (A) Average number of stems per coppice (mean and SE), and (B) average single stem green mass for 2-yr-old coppice of *Salix discolor* and *S. eriocephala* on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

Genotype and Site Variation

Only one DIS clone, MON-D1, grew significantly better on the clay than the overburden (site × clone (species), $P = 0.010$, Table 3, Fig. 5A). There were two *DIS* clones that grew exceptionally better on the overburden than the clay site, LEV-D3 and MUD-D4. There were two

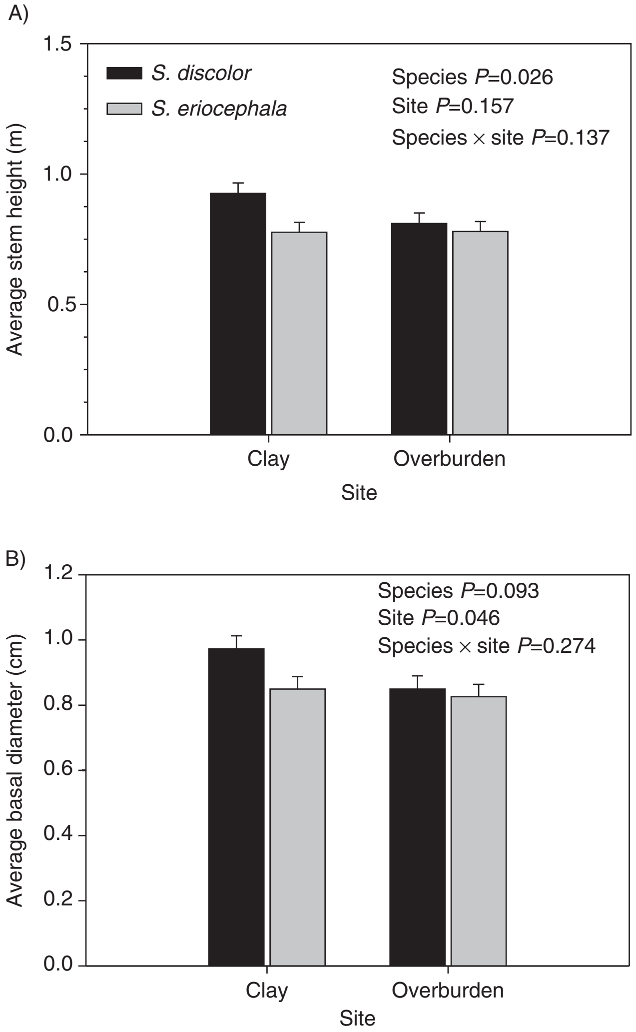

Fig. 3. (A) Average stem height (mean and SE), and (B) average basal stem diameter for 2-yr-old coppice of *Salix discolor* and *S. eriocephala* on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

ERI clones, BRI-E2 and SHE-E3 that grew better on the clay than the overburden (Fig. 5B). There were no exceptionally better ERI clones growing on the overburden compared with the clay site. The two DIS clones that had exceptionally better green mass on the overburden site, LEV-D3 and MUD-D4 (Fig. 5A), also had greater stem numbers per coppice on the overburden; whereas, the other DIS clones had similar stem numbers per coppice between sites (Fig. 6A). The two ERI clones that had greater yield on the clay site, BRI-E2 and SHE-E3 (Fig. 5B), had either a greater or the same number of stems per coppice as on the overburden, whereas for the other six clones, the stem number was always greater on the overburden vs. the clay site (Fig. 6B).

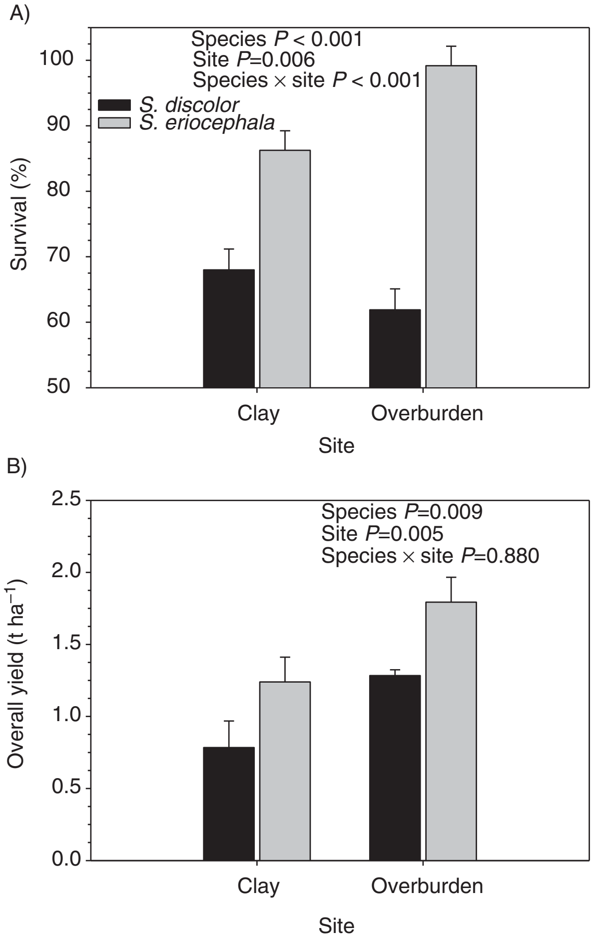

Fig. 4. (A) Survival (mean and SE), and (B) overall yield for 2-yr-old coppice of *Salix discolor* and *S. eriocephala* on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

Variation in survival among genotypes within species was low, with DIS at 62.5% + 2.2 (mean ± SE), with a 55–72% range in survival, and 92.7% ± 2.1, with 85–100% range in survival for ERI, respectively. As a result, the effect on overall yield, which includes survival, was similar across clones and shows a very similar result as yield (Fig. 5).

Allometry

Covariate analysis of average stem height using average stem diameter as covariate and testing for species effect showed no significant species or species × diameter interactive effects. Covariate analysis of average stem height and testing for site effect showed no significant site or site × diameter interactive effects. Thus, neither site nor species affect the average stem height to diameter relationship, which was $y = 0.0079 \times 0.9278x$; $R^2 = 0.691$ (Fig. 7).

Covariate analysis of green mass using stem number per coppice as covariate and testing for site effect had a significant species × stem number interaction ($P = 0.030$). Thus, there was a positive, but different, slope for

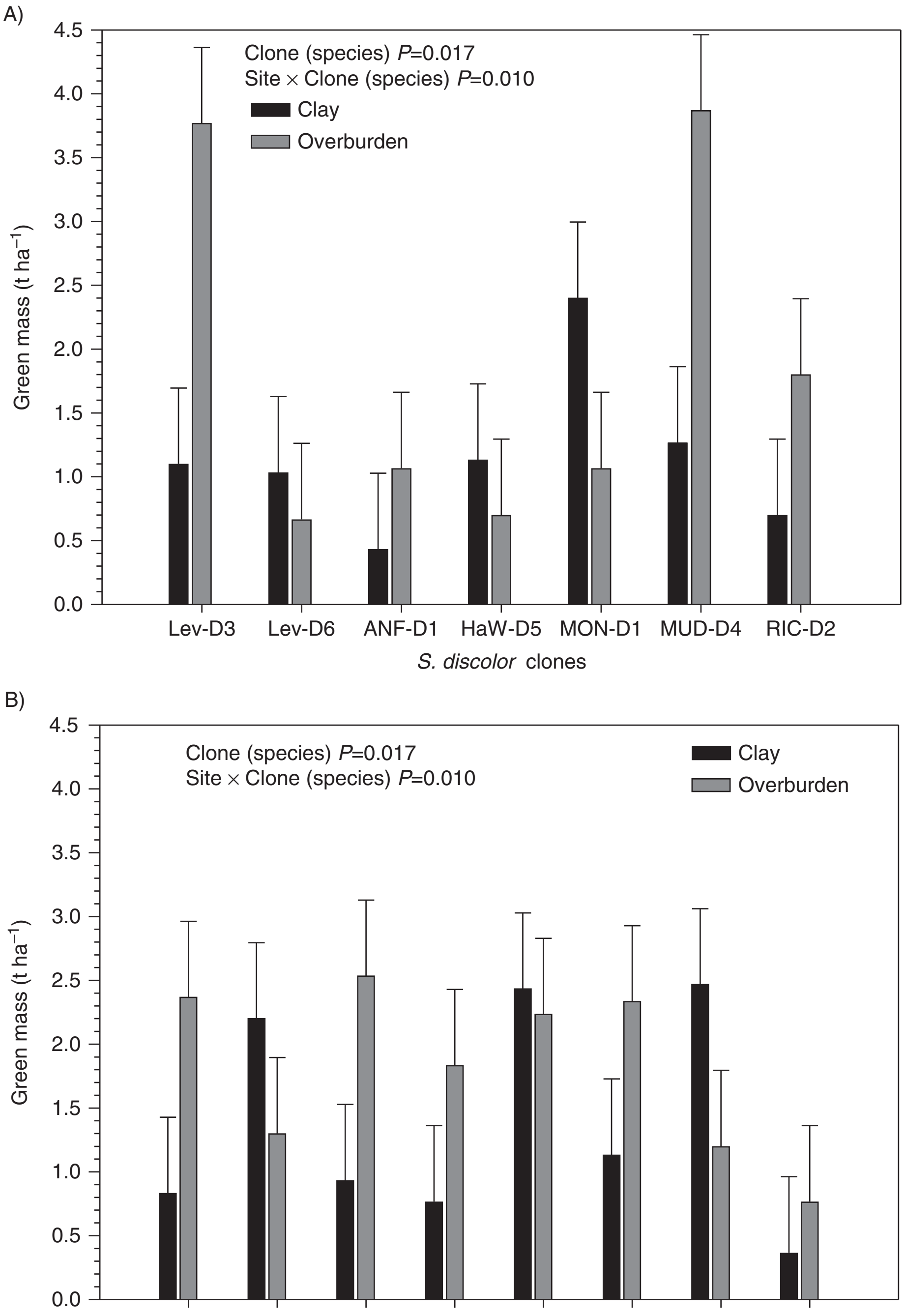

Fig. 5. Green mass (mean and SE) for 2-yr-old coppice from 15 genotypes of (A) *Salix discolor* (seven) and (B) *S. eriocephala* (eight) on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

the relationship between green mass and stem number per coppice, with DIS having twice the slope as ERI (Fig. 8).

Covariate analysis showed a positive green mass relationship to average stem height, with a significant interaction with site ($P = 0.002$). Despite having a similar stem green mass by site, as average stem height increased, there was a greater increase in green mass on the overburden vs. the clay site (Fig. 9A). Covariate analysis of green mass showed no significant site × average stem diameter interaction ($P = 0.200$). There was a significant site effect and a positive relationship

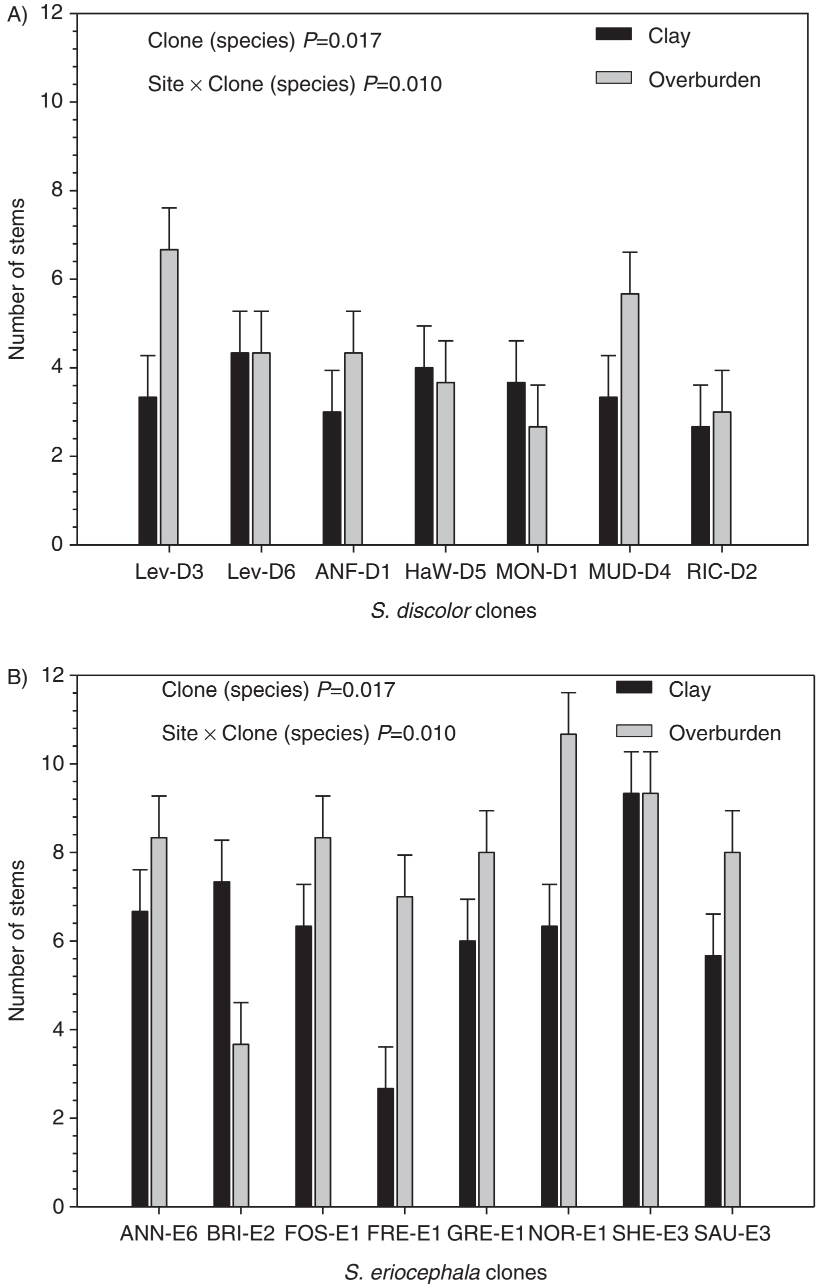

Fig. 6. Number of stems (mean and SE) for 2-yr-old coppice from 15 genotypes of (A) *Salix discolor* (seven) and (B) *S. eriocephala* (eight) on a clay and overburden site at Salmon Harbour coal mine near Minto, NB (SH).

to average stem diameter. Given the same diameter, the overburden site produced greater green mass than the clay site (Fig. 9B).

Comparison of *S. viminalis* with North American Willow Clones

Average values for biomass yields and growth responses in 2-yr-old coppice traits for the best genotype of DIS and ERI were comparable to the highly selected check clone of VIM #5027 on the clay site, but less so on the overburden (Table 4). On the overburden, the best DIS genotype exceeded the biomass yield of VIM by approximately 12%, whereas the best ERI genotype underperformed VIM by 27%. The average number of stems per coppice was always highest in ERI regardless of site type, exceeding the VIM clone by approximately three stems per coppice. The heights of the coppice stems were comparable on the overburden, but on the

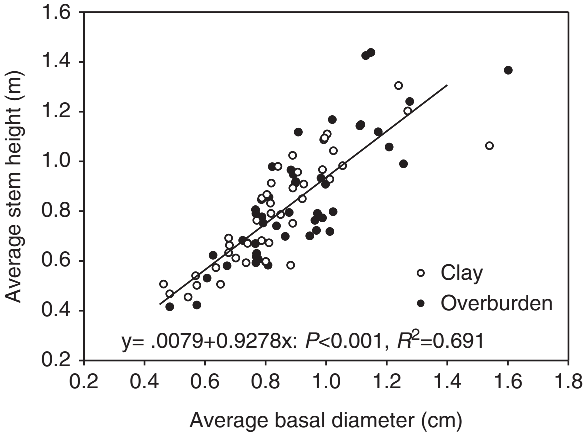

Fig. 7. Covariate analysis of average stem height vs. average stem basal diameter showing no effect by site type.

clay site, the best DIS genotype was approximately 28% taller than VIM.

DISCUSSION

Mine tailings from base metal mining operations can be acidic (Bagatto and Shorthouse 1999), but the pH of the clay deposit at the Salmon Harbour coal mine was extremely low (pH = 3.6, vs. pH = 6.8 on mine overburden). The mineral nutrition or status of foliage from willows planted in these two field tests was not tested because no symptoms of foliar toxicity were evident.

Nevertheless, plants growing in highly acidic soil conditions can experience Al toxicity (Delhaize and Ryan 1995; Larcheveque et al. 2013) and elevated levels of copper (Cu) and nickel (Ni) in both foliage and roots (Bagatto and Shorthouse 1999). The most easily recognized symptom of Al toxicity is inhibition of root growth (Delhaize and Ryan 1995). We did not test for potential metal toxicity effects due to acidity. Root growth can also be physically inhibited in heavy clay soils (Desrochers and Tremblay 2009). However, growth effects attributable to impedance of root growth by heavy soil texture are not easily distinguished from the impacts of acidity and/or related metal toxicity (Andersson 1988; Klang-Westin and Eriksson 2003). Nevertheless, it should also be noted that willows were vegetatively propagated from dormant stem cuttings without the presence of an a priori developed root system. It is remarkable that these cuttings not only survived but produced sufficient roots to permit growth into reproductively mature plants. Although significant impacts on biomass yields and numbers of stems per coppice were detected, these differences were not large, and both DIS and ERI survived and grew relatively well despite low soil pH, demonstrating that both species have a reasonably high tolerance for these extreme site conditions and appear to be adapted to them. Nevertheless, the covariance analysis (Fig. 9) clearly indicates that some factor on the clay deposit, whether it was root impedance due to heavy soil texture or acidity and related metal toxicities, was suppressing growth on

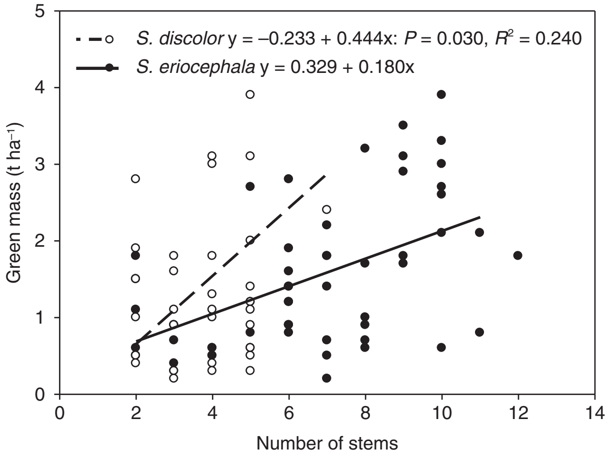

Fig. 8. Covariate analysis of green mass vs. number of stems by species.

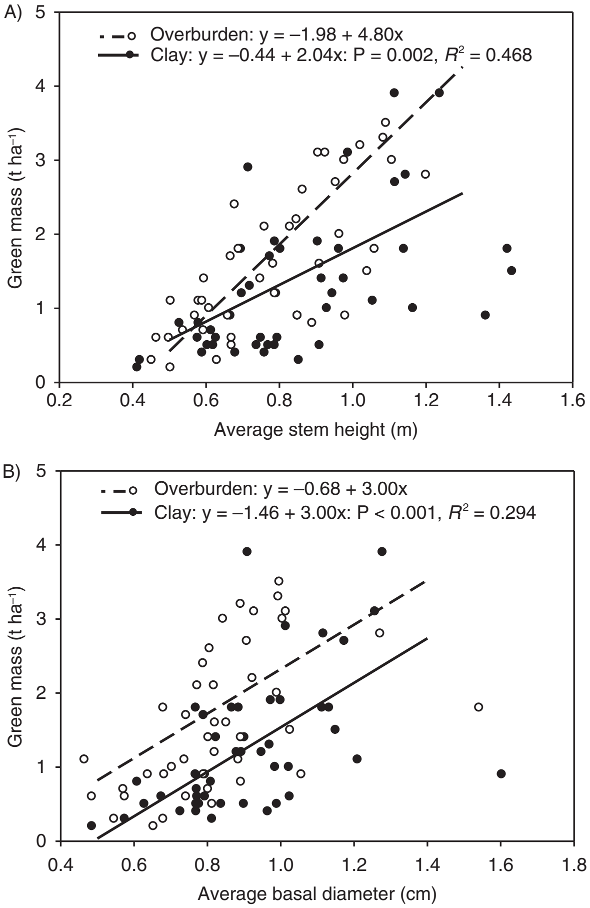

Fig. 9. Covariate analysis of green mass vs. (A) average stem height and (B) average basal diameter by site type.

Table 4. Willow productivity trait comparisons of *Salix viminalis* to the best *S. discolor* and *S. eriocephala* clones by site (mean ± SE)

Site and species	Green mass (Mt ha^{-1})	Number of stems	Average single stem mass (kg)	Average stem height (m)	Average basal diameter (cm)
Clay					
S. discolor, MON-D1	2.40 ± 0.60	3.67 ± 0.94	0.066 ± 0.013	1.36 ± 0.04	1.19 ± 0.11
S. eriocephala, SHE-E3	2.47 ± 0.60	9.33 ± 0.94	0.026 ± 0.013	0.90 ± 0.04	0.82 ± 0.11
S. viminalis	2.47 ± 0.75	6.00 ± 1.53	0.040 ± 0.005	1.06 ± 0.10	0.84 ± 0.04
Overburden					
S. discolor, MUD-D4	3.87 ± 0.60	5.67 ± 0.94	0.066 ± 0.013	0.86 ± 0.04	0.93 ± 0.11
S. eriocephala, FOS-E1	2.53 ± 0.60	8.33 ± 0.94	0.030 ± 0.013	0.93 ± 0.04	0.93 ± 0.11
S. viminalis	3.47 ± 1.02	5.67 ± 1.20	0.060 ± 0.007	1.05 ± 0.04	1.08 ± 0.06

this clay deposit compared with the overburden site. From a land reclamation perspective, perhaps the most important finding is that there are willow species, and genotypes within these species, that can grow roots and also maintain reasonably good growth under the highly acidic soil conditions commonly associated with mine overburden or mine tailings.

The existence of genotype × environment interactions both at the species level (e.g., survival) and at the individual genotype (clone within species) level (e.g., biomass yield and number of stems per coppice) indicates that there is significant scope for selection of both species and genotypes within species that show increased tolerance for high soil acidity and/or extreme soil textural variations. Therefore, further field testing of the same and different willow species and a representative sample of genotypic variation within species can be used to identify superior genotypes for survival and biomass yield on specific site types.

Species differences in allometric relationships can also be used to enhance biomass yields. In this regard, the relationship between coppice stem number and biomass yield is especially interesting from a biomass production perspective and for yield improvement, especially for certain species. Our results show that increases in coppice stem number can have twice the effect on yield in DIS than in ERI, demonstrating important differences among species in the impact of coppice structure itself. As well as yield improvement, difference in coppice structure (e.g., stem size and number of stems per coppice) can affect the design and efficiency of harvesting and processing equipment for biomass production plantations (Mosseler et al. 2014a). Finally, but to a lesser extent, site quality differences may also affect allometric relationships within species in their coppice structure (e.g., average stem height), which in turn can affect yields differently in different willow species (Mosseler et al. 2014b).

Comparison of growth and yield among the best genotypes of DIS and ERI and the VIM check clone showed that native North American willow species can compete favorably in terms of biomass production with arguably the most highly bred and selected willow clones from European willow breeding programs (Guidi-Nissim et al. 2013) and that, with greater efforts at selection and breeding, well-adapted, superior genotypes can also be developed from native North American willow species both for bioenergy production as well as for mine site reclamation.

ACKNOWLEDGMENTS

We are grateful to Moira Campbell, Ted Cormier, John Malcolm, Don Ostaff, and Peter Tucker for their assistance in collection of material from natural populations, establishment of field tests, and assistance with data collection. We also thank Michele Coleman at Mine Restoration Inc., a subsidiary of NB Power for providing space for the common gardens used for the field tests described here, Michel Labrecque at the Montreal Botanical Gardens for making sites available for willow clonal testing and selection, and Jim Estey of the Laboratory for Forest Soils and Environmental Quality at the University of New Brunswick for analysis of soil samples from the sites described here.

Andersson, M. 1988. Toxicity and tolerance of aluminium in vascular plants. Water Air Soil Pollut. **39**: 439–462.

Argus, G. W. 2010. *Salix* L. Pages 23–162 *in* Flora of North America Editorial Committee, eds. Flora of North America north of Mexico. Volume 7. *Magnoliophyta: Salicaceae* to *Brassicaceae*. Oxford University Press, Oxford, UK, and New York, NY, USA.

Bagatto, G. and Shorthouse, J. D. 1999. Biotic and abiotic characteristics of ecosystems on acid metalliferous mine tailings near Sudbury, Ontario. Can. J. Bot. **77**: 410–425.

Berti, W. R. and Cunningham, S. D. 2000. Phytostabilization of metals. Pages 71–88 *in* I. Raskin and B. D. Ensley, eds. Phytoremediation of toxic metals-using plants to clean up the environment. John Wiley and Sons, New York, NY.

Delhaize, E. and Ryan, P. R. 1995. Aluminum toxicity in plants. Plant Physiol. **107**: 315–321.

Densmore, R. and Zazada, J. C. 1978. Rooting potential of Alaskan willow cuttings. Can. J. For. Res. **8**: 477–479.

Desrochers, A. and Tremblay, F. 2009. The effect of root and shoot pruning on early growth of hybrid poplars. For. Ecol. Manage. **258**: 2062–2067.

Gage, E. A. and Cooper, D. J. 2004. Constraints on willow seedling survival in a Rocky Mountain montane floodplain. Wetlands **24**: 908–911.

Green, C. H., Heil, D. M., Cardon, G. E., Butters, G. L. and Kelly, E. F. 2003. Solubilization of manganese and trace metals in soils affected by acid mine runoff. J. Environ. Qual. **32**: 1323–1334.

Guidi-Nissim, W. G., Pitre, F. E., Teodorescu, T. I. and Labrecque, M. 2013. Long-term biomass productivity of willow bioenergy plantations maintained in southern Quebec, Canada. Biomass Bioenerg. **56**: 361–369.

Klang-Westin, E. and Eriksson, J. 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil **249**: 127–137.

Kuzovkina, Y. A., Knee, M. and Quigley, M. F. 2004. Cadmium and copper uptake and translocation in five willow species. Int. J. Phytoremed. **6**: 269–287.

Kuzovkina, Y. A. and Quigley, M. F. 2005. Willows beyond wetlands: uses of *Salix* L. species for environmental projects. Water Air Soil Pollut. **162**: 183–204.

Kuzovkina, Y. A. and Volk, T. A. 2009. The characteristics of willow (*Salix* L.) varieties for use in ecological engineering applications: co-ordination of structure, function and autecology. Ecol. Eng. **35**: 1178–1189.

Labrecque, M. and Teodorescu, T. I. 2005. Field performance and biomass production of 12 willow and poplars in short-rotation coppice in southern Quebec (Canada). Biomass Bioenerg. **29**: 1–9.

Landberg, T. and Greger, M. 1996. Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl. Geochem. **11**: 175–180.

Landberg, T. and Greger, M. 2002. Differences in oxidative stress in heavy metal resistant and sensitive clones of *Salix viminalis*. J. Plant Physiol. **159**: 69–75.

Larcheveque, M., Desrochers, A., Bussiere, B., Cartier, H. and David, J.-S. 2013. Re-vegetation of non-acid-generating, thickened tailings with boreal trees: a greenhouse study. J. Environ. Qual. **42**: 351–360.

Ledin, S. 1996. Willow wood properties, production and economy. Biomass Bioenerg. **11** (2–3): 75–83.

Mosseler, A., Zsuffa, L., Stoehr, M. U. and Kenney, W. A. 1988. Variation in biomass production, moisture content and specific gravity in some North American willows (*Salix* L.). Can. J. For. Res. **18**: 1535–1540.

Mosseler, A., Major, J. E. and Labrecque, M. 2014a. Growth and survival of seven native willow species on highly disturbed coal mine sites in eastern Canada. Can. J. For. Res. **44**: 1–10.

Mosseler, A., Major, J. E., Labrecque, M. and Larocque, G. R. 2014b. Allometric relationships in coppice biomass production for two North American willows (*Salix* spp.) across three different sites. For. Ecol. Manage. **320**: 190–196.

Niiyama, K. 1990. The role of seed dispersal and seedling traits in colonization and coexistence of *Salix* species in a seasonally flooded habitat. Ecol. Res. **5**: 317–331.

Punshon, T. and Dickinson, N. M. 1997. Acclimation of *Salix* to metal stress. New Phytol. **137**: 303–314.

Punshon, T. and Dickinson, N. M. 1999. Heavy metal resistance and accumulation characteristics in willows. Int. J. Phytorem. **4**: 361–385.

Rout, G. R., Samantaray, S. and Das, P. 2001. Aluminium toxicity in plants: a review. Agronomie **21**: 3–21.

Shanahan, J. O., Brummer, J. E., Leininger, W. C. and Paschke, M. W. 2007. Manganese and zinc toxicity thresholds for mountain and geyer willow. Int. J. Phytorem. **9**: 437–452.

Volk, T. A., Abrahamson, L. P., Nowak, C. A., Smart, L. B., Tharakan, P. J. and White, E. H. 2006. The development of short-rotation willow in the northeastern United States for bioenergy and bio-products, agroforestry and phytoremediation. Biomass Bioenerg. **30**: 715–727.

Vyslouzilova, M., Tlustos, P. and Szakova, J. 2003. Cadmium and zinc phytoextraction of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ. **49**: 542–547.

Zsuffa, L. 1990. Genetic improvement of willows for energy plantations. Biomass **22**: 35–47.