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ARTICLE

Growth and survival of seven native willow species on highly disturbed coal mine sites in eastern Canada

A. Mosseler, J.E. Major, and M. Labrecque

Abstract: Significant differences were apparent in seven native North American willow (*Salix*) species compared and assessed in common garden field tests for survival, biomass production, and coppice structure on former coal mine sites in New Brunswick, Canada. In most species, percentage survival was relatively constant after the initial establishment phase, allowing good prediction of final survival in the first or second year after establishment. Unrooted dormant stem sections collected from clones of five willow species previously field-tested and selected for survival and growth, survived and grew better on the mine site to be reclaimed than those collected directly from natural populations, demonstrating the ability to rapidly improve survival results based on prior field testing. Survival at ages 5 and 6 improved from an average of 70% to 94% for *S. eriocephala* Michx. and from 42% to 84% for *S. interior* Rowlee. The best clones in both species had over 95% survival and had approximately 5–6 t·ha^{-1} (t = tonne) fresh mass after 2 years of coppice growth. We recommend these two species for use in mine reclamation activities, because they grew best overall and had the highest survival rates. Despite poor average rooting ability in *S. bebbiana* Sarg., *S. discolor* Muhl., and *S. humilis* Marshall, some genotypes of these species showed good survival and growth, and further selection for these traits is warranted.

Key words: willow, *Salix*, common garden test, land reclamation, biomass production.

Résumé : D'importantes différences étaient apparentes parmi sept espèces indigènes de saule (*Salix*) d'Amérique du Nord comparées et évaluées dans des essais sur le terrain en plantation comparative pour la survie, la production de biomasse et la structure en taillis sur le site d'anciennes mines de charbon du Nouveau-Brunswick, au Canada. La plupart des espèces avaient un taux de survie relativement constant après la phase initiale d'établissement; ce qui permettait de faire de bonnes prédictions de survie finale durant la première ou la deuxième année après l'établissement. Des sections de tige dormante non racinées, prélevées parmi les clones de cinq espèces de saule précédemment testés pour leur croissance, ont davantage survécu et mieux poussé sur le site minier à réhabiliter que celles qui ont été prélevées directement dans des populations naturelles. Cela démontre qu'on peut rapidement améliorer le taux de survie en s'appuyant sur les résultats d'essais antérieurs sur le terrain. La survie à cinq et six ans s'est améliorée en moyenne de 70 à 94 % dans le cas de *S. eriocephala* Michx. et de 42 à 84 % dans le cas de *S. interior* Rowlee. Les meilleurs clones chez les deux espèces avaient un taux de survie supérieur à 95 % et produisaient approximativement 5-6 t·ha^{-1} (t = tonne) de biomasse après deux ans de croissance en taillis. Nous recommandons ces deux espèces pour la réhabilitation des sites miniers parce qu'elles ont dans l'ensemble la meilleure croissance et le taux de survie le plus élevé. Malgré une capacité d'enracinement en moyenne faible chez *S. bebbiana* Sarg., *S. discolor* Muhl. et *S. humilis* Marshall, certains génotypes de ces espèces avaient un taux élevé de survie et une bonne croissance, ce qui justifie la poursuite de la sélection pour ces caractères. [Traduit par la Rédaction]

Mots-clés : saule, *Salix*, essais en plantation comparative, réhabilitation d'un terrain, production de biomasse.

Introduction

Over the past 40 years, interest in the use of willows (*Salix* spp.) as a source of biomass for energy has increased concomitantly with the search for alternative energy sources (Zsuffa 1990; Labrecque et al. 1993; Tharakan et al. 2005; Volk et al. 2006), with growing concerns over the impact of carbon emissions on climate warming and possible environmental applications (Kuzovkina and Volk 2009). In North America, much of the initial interest in willow as a biomass and bioenergy crop focused on the use of willow clones and species imported from Europe, where interest in willow biomass production has a longer history (Erikson 1988; Gullberg 1993). However, environmental concerns about the potential invasiveness of exotic species and the possibility of introducing pests have prompted provincial governments to legislate against using exotic species. Furthermore, North America has a rich diversity of native willows, with approximately 108 native willow species (Argus 2010). Canada alone has some 76 native willows, which are widely distributed across Canada, adapted to a large range of site conditions, fast growing, and generally easily propagated vegetatively from unrooted stem sections (cuttings). Nevertheless, exploration of native North American willows as a potential biomass resource has received limited attention despite species richness and their ecological importance in North America (Mosseler et al. 1988; Labrecque et al. 1993; Kopp et al. 2001; Smart et al. 2005).

Willows are relatively short-lived species that invade disturbed sites, often occupying such sites for short periods during early stages of natural succession along with herbaceous, flowering species (Kuzovkina and Quigley 2005). Although most willows are associated with wetlands and riparian zones across the northern hemisphere, there are also willows adapted to well-drained, drier

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Table 1. Native North American willow (*Salix*) species being field tested for biomass production and restoration of highly disturbed areas.

Salix species	Height and habit	Natural habitat
S. amygdaloides	8–15 m, tree	Poorly drained, standing wetlands
S. bebbiana	4–6 m, shrub	Seepage slopes, ditches, upland sites
S. discolor	4–10 m, shrub/small tree	Seepage slopes, wetlands, and ditches
S. eriocephala	4–6 m, shrub	Fast-flowing stream banks
S. interior	4–6 m, shrub	River banks, sandbars, floodplains
S. humilis	2–3 m, shrub	Well-drained upland sites, forest openings
S. nigra	10–12 m, tree	River banks and floodplains

upland sites (Kuzovkina and Volk 2009). These adaptive traits make them potentially useful for reclaiming (re-vegetating) and restoring highly disturbed areas affected by various human activities, such as mining operations aimed at coal, metal, oil, and gas extraction (Kuzovkina and Quigley 2005; Kuzovkina and Volk 2009; Bissonnette et al. 2010). Our goal was to assess some of the more promising, common native willows of eastern and central North America for re-vegetating and restoring areas in which habitat quality has been degraded through human activities.

Areas disturbed by mining activities are often susceptible to flash flooding, erosion, and surface runoff into adjacent watercourses. Such erosion and runoff degrade water quality and aquatic habitat, and regulatory concerns about the effects of mining on water resources have been the primary driver of reclamation practices across the coal-mining regions of the Appalachian region (Zipper et al. 2011). Willows play a natural role in mitigating such erosion and maintaining riparian habitats throughout the northern hemisphere and can play an important role in the artificial reclamation and restoration of highly disturbed sites (Kuzovkina and Volk 2009). Our objective was to assess genetic variation within and among natural populations of seven willow species for survival, yield, and components of yield on highly disturbed coal mine sites. The aim was to select a set of clones from the seven species tested that would be suitable for reclaiming (sensu Bradshaw 1984) and restoring highly disturbed sites to promote succession to the natural forest climax stage. The seven species tested include both tree and shrub willows adapted to a wide range of moisture conditions, thereby providing land managers with a range of plant forms adapted to a range of site types.

By assembling a representative sample of clones from each of the seven species (Table 1) from natural populations scattered over a large area of the botanical range in Ontario and Quebec, Canada (Fig. 1), we used a common garden and population genetics approach to assess variation within and among these species for survival, yield, and components of yield. Our objective was to identify which of these species and clones performed best on these infertile coal-mining overburden.

Material and methods

Common garden experiments

A common garden field test using seven species was established at the Salmon Harbour coal mine site near Minto, New Brunswick (NB), Canada (Lat. N46°07′; Long. W66°05′) on a property operated by NBCoal Ltd., a subsidiary of the local electrical utility NBPower on 20–21 May 2008. This site has an average temperature of 5.7 °C and a mean annual precipitation of 987 mm (Environment Canada). The seven willow species used for this study were *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). Species were represented by collecting five clones from each of four natural populations (20 clones per species, resulting in a total of 140 clones), located in southern and eastern Ontario (ON) and adjacent areas of the Ottawa River Valley in the province of Quebec (QC) (Fig. 1, Table 2). The common garden was established with unrooted stem cuttings collected during the dormant season from vigorous 1 and (or) 2 year old stem sections from plants originating in natural populations (Densmore and Zazada 1978).

A 5-species common garden field test of 48 clones selected from two common gardens established in 2005 and 2007 at the Montreal Botanical Gardens (MBG; in Montreal, Quebec, Canada), was also established at the Salmon Harbour mine site (Minto, NB), adjacent to the 7-species test described above, on 7–8 May 2009. Unrooted stem cuttings were collected during the dormant season from the current year's growth of plants growing in the 2 MBG test sites. These 48 clones were among the best-performing clones from 5 of the willows listed in Table 1: DIS, ERI, INT, NIG, and AMY (Table 3). The first MBG common garden test from which DIS and ERI cuttings were collected was established in 2005 and contained 6 clones collected from each of 12 natural populations of DIS and ERI from across NB, QC, and ON, for a total of 144 clones (72 clones per species). The 2007 common garden at the MBG contained 16 clones of AMY and INT (4 clones from each of four natural populations for each species) and 63 clones of *S. nigra* (NIG) (4 to 6 clones from each of 10 natural populations originating in southwestern and eastern ON and 4 genotypes from a highly disjunct natural population of NIG from the St. John River Valley near Gagetown, NB (Lat. N45°78′; Long. W66°15′) (Table 3). This NB population may have survived in geographic isolation in New Brunswick over several millennia of climatic change since the warmer Hypsithermal Period of 5000 years ago. Each of the two common garden studies established at the Salmon Harbour mine site included a check clone of *S. viminalis* clone #5027 (VIM), a highly selected clone introduced from the Swedish willow breeding program (Gullberg 1993) and one of the more common, introduced clones used in biomass plantations established in Canada (Labrecque and Teodorescu 2005).

Both common gardens at the Salmon Harbour mine site were established with 20 cm long cuttings planted in a randomized complete block design, consisting of single rows with 2 m between the rows and 0.5 m between plants along the row replicated 3 times in the 7-species test and 5 times in the 5-species test. Five individuals of each clone were planted in each replicate (block). These two common gardens were adjacent to each other on similar crushed shale rock mine overburden that had been bulldozed and landscaped into gently sloping terrain to minimize erosion from surface runoff following cessation of strip surface mining operations for coal extraction. Both tests ran parallel to each other along a watercourse that drained the mine site. An analysis of six soil samples taken along the length of these tests indicated the following averages: organic matter content of 0.79%; total nitrogen (N) of 0.10%; a pH of 6.85; exchangeable cations (meq/100 g) of 0.23 for potassium (K), 7.33 for calcium (Ca), and 0.66 for magnesium (Mg); available phosphorus (P) of 4 ppm; clay, silt, and sand contents were 9.4%, 23.4%, and 67.2%, respectively. Soil samples also contained an average of 56.5% rocks that would not pass through a 2 mm sieve.

Survival counts were conducted in 2009, 2011, and 2013. In 2011, the aboveground biomass was harvested for up to 4 of the 5 ramets in each plot, and in 2013, the 2 year old coppice growth of one plant per 5-tree plot was harvested on 10 September for the 5-species test of selected MBG clones and on 16 September for the 7-species common garden test. The fresh mass of one plant per plot was measured in the field to the nearest 10 g using an electronic mass scale (Electronic Infant Scale, model ACS-20A-YE). Mean fresh yield in $t \cdot ha^{-1}$ (t = tonne) was calculated by multiplying by 10 the harvested aboveground fresh mass (e.g., multiplying by 10 000 plants per ha divided by 1000 kg per tonne) multiplied by

Fig. 1. Locations of natural populations sampled for seven native North American willows.

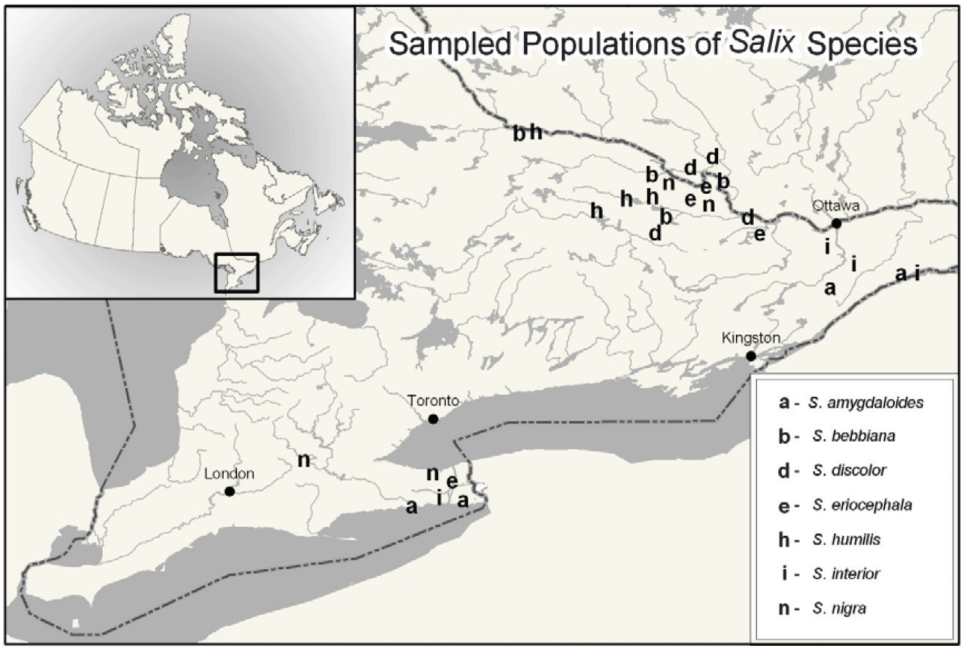


Table 2. Natural populations of seven willow species sampled for common garden studies.

Species	Population	Latitude N	Longitude W
S. amygdaloides	Hanlon Marsh, ON	44°52′	75°45′
	Long Sault, ON	44°60′	74°58′
	Port Maitland, ON	42°52′	79°35′
	Wainfleet, ON	42°55′	79°20′
S. bebbiana	Achray Road, ON	45°49′	77°23′
	Fort Coulonge, QC	45°59′	76°46′
	Foymount, ON	45°26′	77°18′
	Klock Road, ON	46°19′	78°30′
S. discolor	Allumette Island, QC	45°54′	77°06′
	Fort Coulonge, QC	45°59′	76°46′
	Foymount, ON	45°26′	77°18′
	Norway Bay, ON	45°32′	76°25′
S. eriocephala	Allumette Island, QC	45°54′	77°06′
	Norway Bay, ON	45°32′	76°25′
	Wainfleet, ON	42°55′	79°20′
	Westmeath, ON	45°49′	76°54′
S. humilis	Achray Road, ON	45°49′	77°23′
	Aylen Lake, ON	45°34′	77°53′
	Bonnechere, ON	45°40′	77°37′
	Klock Road, ON	46°17′	78°30′
S. interior	Ottawa, ON	45°04′	75°32′
	Long Sault, ON	44°60′	74°58′
	Moodie Pond, ON	45°14′	75°47′
	Wainfleet, ON	42°55′	79°20′
S. nigra	Nith River, ON	43°17′	80°34′
	Pembroke, ON	45°50′	77°07′
	Wainfleet, ON	42°55′	79°20′
	Westmeath, ON	45°49′	76°54′

survival to facilitate overall yield comparison among species and clones on an area basis. Previous measurements of fresh and dry mass taken at the MBG common gardens demonstrated that percent moisture was approximately 50 ± 2% regardless of species or clones (Mosseler, unpublished). The number of coppice stems was recorded for each harvested plant. In the 5-species test, a sixth block was eliminated from further study after 2011 because of damage from prolonged flooding.

Statistical analysis

The data for the seven species test were subjected to analyses of variance (ANOVA). Species, populations, and clones were considered fixed effects. Populations were nested within species, and clones were nested within populations, which were nested within species. The ANOVA model used was as follows:

$$Y_{ijklm} = u + B_i + S_j + \mathrm{P}_{k(j)} + C_{l(k(j))} + e_{ijklm}$$

where Y_{ijklm} is the dependent ramet trait of the ith replicate, of the jth species, of the kth population, of the lth clone, of the mth ramet, and u is the overall mean, B_i is the effect of the ith block ($i = 1, 2, 3$), S_j is the effect of the jth species ($j = 1, 2, 3, 4, 5, 6, 7$), $P_{k(j)}$ is the effect of the kth population ($k = 1, 2, 3, 4$) nested within the jth species, C_l is the effect of the lth clone ($l = 1, 2, 3, 4, 5$) nested within the kth population nested within the jth species, and e_{ijklm} is the random error component. Survival was arcsine square root transformed for normality. The general linear model from Systat (Chicago, Illinois, USA) was used for analysis. Tukey's post hoc mean separation test was used to assess differences among species All statistical tests were assessed at a significance level of $\alpha = 0.05$.

The 5-species data set was also subjected to ANOVA. Species and clone were considered fixed effects. Clones were nested within species The ANOVA model used was as follows:

$$Y_{ijkl} = u + B_i + S_j + \mathrm{C}_{k(j)} + e_{ijkl}$$

where Y_{ijkl} is the dependent ramet trait of the ith block, of the jth species, of the kth clone, of the lth ramet, and u is the overall mean, B_i is the effect of the ith block ($i = 1, 2, 3, 4, 5$), S_j is the effect of the jth species ($j = 1, 2, 3, 4, 5$), $C_{k(j)}$ is the effect of the kth clone ($k = 1, 2, 3, 4, 6, 7, 8$) nested within the jth species, and e_{ijkl} is the random error component. The same statistical analysis procedure was used as in the first ANOVA analysis.

Results

The 7-species, natural population, common garden experiment showed significant differences in survival, yield, and number of stems (Table 4), with ERI having the best survival at approximately

Table 3. Origins of clones of *Salix amygdaloides*, *S. discolor*, *S. eriocephala*, *S. interior*, and *S. nigra* selected from the Montreal Botanical Garden (MBG) for use in a five-species common garden established in 2009 at the Salmon Harbour mine site near Minto, New Brunswick.

Species	Population	Latitude N	Longitude W	Selected MBG clones
S. amygdaloides	Cobden, ON	45°63′	76°88′	COB-A1, COB-A2
	Golden Lake, ON	45°58′	77°24′	GOL-A2, GOL-A3
	Bishops Mills, ON	44°87′	75°70′	HAN-A4
	Richmond, ON	45°19′	75°83′	RIC-A3, RIC-A5, RIC-A6
S. discolor	Fredericton, NB	45°94′	66°62′	FRE-D6
	Lévis, 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-D4
	Montmagny, QC	46°94′	70°60′	MON-D1
	Mud Lake, ON	45°88′	76°78′	MUD-D4
	Richmond, ON	45°19′	75°83′	RIC-D2
S. eriocephala	Ste. Anne de la Pérade, QC	46°56′	72°20′	ANN-E4, 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, FRE-E6
	Green River, NB	47°34′	68°19′	GRE-E1
	Montmagny, QC	46°94′	70°60′	MON-E3
	Norton, NB	45°67′	65°81′	NOR-E7, NOR-E9, NOR-E10
	Shepody Creek, NB	45°71′	64°77′	SHE-E3, SHE-E5, SHE-E7
	Rivière au Saumon, QC	47°21′	70°35′	SAU-E3, SAU-E5
S. interior	Ottawa, ON	45°42′	75°69′	LAF-I2, LAF-I5,
	Roebuck, ON	44°80′	75°61′	LIM-I1, LIM-I3, LIM-I6
	Long Sault, ON	45°03′	74°89′	LON-I1, LON-I2, LON-I4
S. nigra	Dunnville, ON	42°90′	79°62′	BYN-N3
	Wainfleet, ON	42°92′	79°37′	FEE-N1, FEE-N2, FEE-N3, FEE-N6
	Wardsville, ON	42°66′	81°76′	BEN-N2
	Guelph, ON	43°54′	80°25′	GUE-N1
	Gagetown, NB	45°78′	66°15′	TCH-N4

Table 4. Seven species survival, mass and yield ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), *P* values, and coefficient of determination (R^2).

		Survival 2013[a]		Green mass (kg)		Stem number		Single stem mass (kg)		Yield ($t \cdot ha^{-1}$)	
Source of variation	df	MS	*P* value	MS	*P* value	MS	*P* value	MS	*P* value	MS	*P* value
Block	2	0.085	0.432	0.051	0.468	25.750	0.117	0.0032	0.043	5.395	0.118
Species	6	6.381	**<0.001**	1.190	**<0.001**	290.059	**<0.001**	0.1056	**<0.001**	95.357	**<0.001**
Pop (Spp)	21	0.333	**<0.001**	0.223	**<0.001**	20.594	**0.036**	0.0020	**0.015**	3.774	0.075
Clone (Pop (Spp))	103	0.233	**<0.001**	0.108	**0.010**	13.135	0.295	0.0014	0.068	2.350	0.643
Error	136	0.102		0.067		11.806		0.0010		2.508	
R^2			0.688		0.686		0.466		0.608		0.466

Note: Spp = species, Pop = population. *P* values < 0.05 are in bold print.

[a] Arcsine square root transformation.

70% and BEB and HUM having the lowest survival at approximately 3% and 10%, respectively, after 6 years (Fig. 2). The greatest number of coppice stems per plant and greatest biomass production were produced by ERI, whereas AMY and NIG produced the least green biomass and among the lowest number of stems per coppice (Fig. 3A). These survival results contrasted quite sharply with those demonstrated by clones of species that had already undergone prior selection based on field testing for survival and growth in the MBG common garden tests (Fig. 4), where survival at age 5 was much higher. Selected clones of these 5 species also showed significant differences in survival, plant biomass, coppice structure, and yield/ha (Table 5). The relative rankings and patterns for growth and survival remained largely the same by species (Fig. 5), except that INT clones showed the best overall performance. In these selected MBG clones of DIS, ERI, INT, NIG and AMY, ERI and INT had both the greatest and most consistent rate of rooting success and survival under field conditions. The pattern of decline in survival over time was also reasonably low and consistent among species, except for a rapid early decline in survival of NIG (Figs. 2 and 4).

Mean yield per hectare from the natural population experiment was significantly and many times greater for ERI than any of the other 6 species (Fig. 6A). The highest yielding clone (WAI-E1) also belonged to ERI (Fig. 6B), with BEB, HUM, NIG, and AMY all showing very poor yields on an area basis. Among the selected MBG clones in the 5-species common garden test, INT had significantly higher yields than DIS, NIG, and AMY on an area basis (Fig. 7A) and also had the highest yielding individual clone (i.e., LAF-I5) (Fig. 7B). Although yields of ERI from the 5-species test were somewhat lower on an area basis, these differences were not statistically significant, and the yield of the best clone of ERI (SHE-E7) was not much less than the yield of the best INT clone (LAF-I5).

There were significant differences in the distribution of biomass per stem, with ERI producing more stems per coppice but significantly less biomass per stem than INT (Fig. 5C) in the common garden test of selected MBG clones. AMY, DIS, and NIG produced the fewest number of coppice stems but also had relatively high biomass per stem, consistent with their natural plant form as taller tree willows.

Fig. 2. Percentage survival in 20 clones collected from natural populations from each of 7 native North American willows on a coal mine spoil site near Minto, NB. Different letters signify significant differences using Tukey's mean separation test at $P = 0.05$.

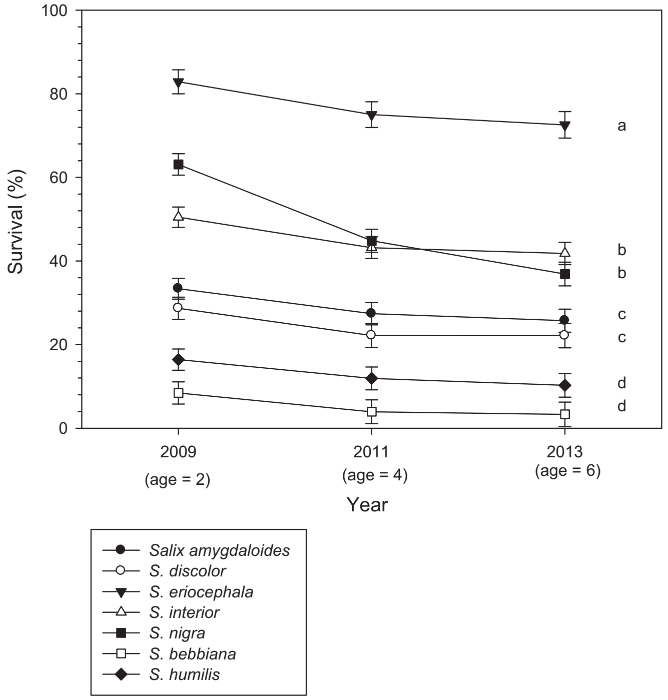
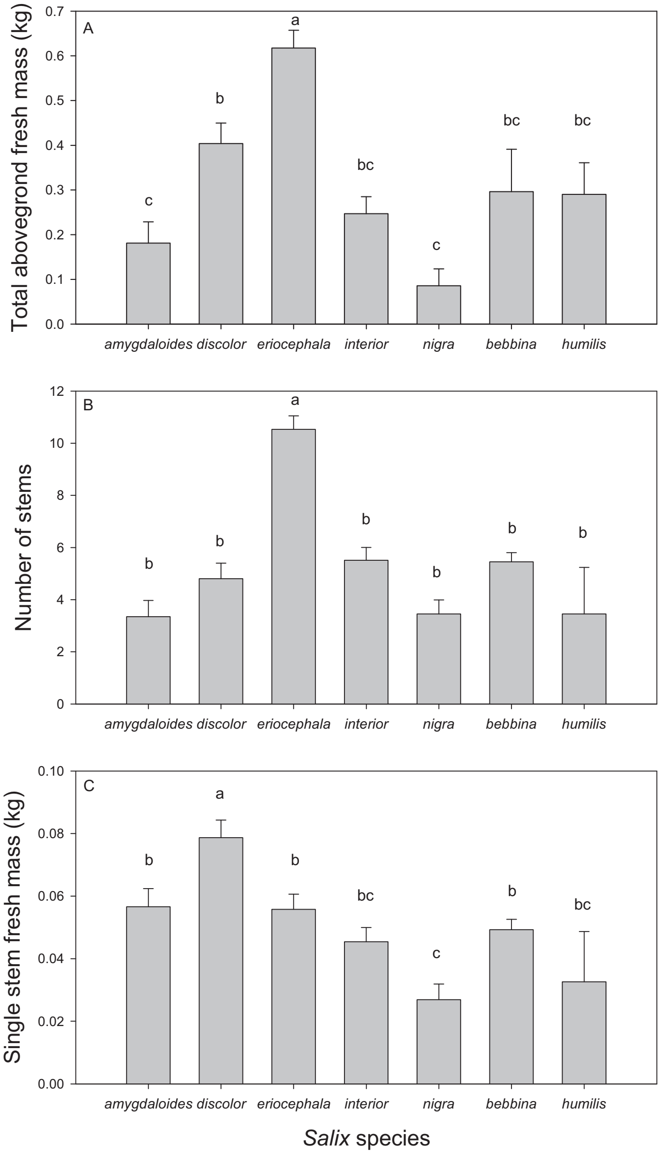


Table 6 presents the number of stems per coppice and the green weight per coppice from 2 year old coppice growth (resulting from the 2011 harvest) from the best-growing clone of each species in each of 5 replications (blocks) from the 5 species MBG selected clone test. Among the 5 native species, individual INT clones produced the greatest biomass per plant in 4 of the 5 blocks (Table 6), and INT generally produced the best biomass yields overall (Fig. 7A). The highly selected exotic test clone (VIM 5027), produced the greatest biomass yield in one of the 5 blocks at 2.25 kg/plant. This VIM clone produced some of the most consistent yields (between 0.46 and 0.56 kg/plant) of any clone in the 5-species common garden because of its rapid root development and high survival rate of 92% at age 5. This VIM clone 5027 also produced between 5 and 11 stems per coppice.

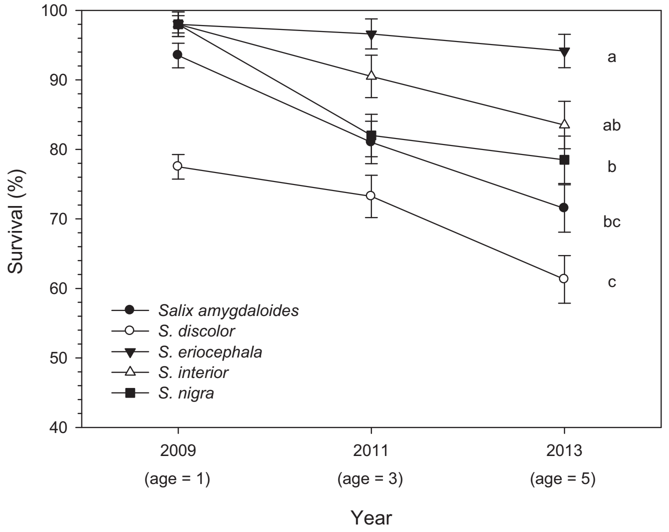
Fig. 3. (A) Total aboveground biomass (leaves and stems), (B) number of coppice stems, and (C) average mass (kg) per coppice shoot in 2 year old coppice growth from a 6 year old rooted stem cutting for 20 clones collected from natural populations for each of 7 native North American willows. Different letters signify significant differences using Tukey's mean separation test at $P = 0.05$.



Discussion

As elsewhere in the northern hemisphere, willows are a natural colonizer of highly disturbed sites and are ideally adapted to colonize mine sites because of their abundant annual seed production and effective long-distance seed dispersal by wind (Kuzovkina and Quigley 2005; Mudrak et al. 2010). Among the willows tested here, BEB and DIS were common natural colonizers of these New Brunswick coal mine sites. All the shrub willows described here (BEB, DIS, ERI, HUM, and INT) flowered and produced viable seeds in the second growing season following establishment from unrooted stem cuttings. Therefore, by establishing small patches of several dozen individuals on wetter areas dispersed across a disturbed landscape, it may be possible to quickly capture available, suitable sites via abundant natural seed dispersal with these highly fecund shrub willows. The main requirements for willow establishment from seed are exposed mineral soil, a constant moisture supply during seed germination and early seedling development, and full sunlight for developing seedlings. Site fertility does not appear to be a major limiting factor for willow establishment even on rocky sites with very little soil organic matter or soil development. Coal mine spoils across the Appalachian region of eastern North America usually contain little N and P in forms available for plant growth (Zipper et al. 2011). Nevertheless, on the barren, impoverished, rocky mine sites described here, each of the seven species was able to grow reasonably well from unrooted stem cuttings provided that rooting took place quickly enough to sustain early growth during seasonal periods when moisture is most available. However, these seven willow species showed large variation in their ability to produce roots from unrooted stem cuttings (Figs. 2 and 4; Tables 4 and 5), and such variation in rooting ability has been noted in other willows (Chmelar 1974; Densmore and Zazada 1978). Riparian willows such as ERI, INT, AMY, NIG showed much greater rooting ability than the non-riparian, upland willows such as BEB and HUM. This relationship between rooting success and riparian versus non-riparian willows was noted by Densmore and Zazada (1978), and they discussed this important ecological and evolutionary adap-

Fig. 4. Percentage survival in 8–16 clones from 5 native North American willow species selected for survival and growth at the Montreal Botanical Gardens and then field tested on a coal mine spoil site near Minto, NB. Different letters signify significant differences using Tukey's mean separation test at $P = 0.05$.



tation in relation to reproductive success in different habitat types (riparian versus non-riparian). This variation in rooting ability is a key species characteristic for survival and growth following establishment of unrooted stem cuttings, especially on harsh, exposed mine sites, and is important for successful plant establishment for reclamation purposes (Kuzovkina and Volk 2009). Rooting success may be the key criterion for determining which species may be best, or most cost effective, for reclaiming highly disturbed areas such as mine overburden. The most consistent and rapid root development from unrooted dormant stem sections was demonstrated by ERI and INT (Figs. 2 and 4), and these two species should be considered the best of the native species evaluated here for mine reclamation purposes.

As Kuzovkina and Quigley (2005) observed, willows can provide an "anchor" for the establishment of early successional plant communities. The extensive root development for which willows are well known (Kuzovkina and Volk 2009; Guidi et al. 2013), combined with their abundant annual leaf litter fall, create an improved environment for increased microbial activity that promotes soil development and increases soil organic matter and fertility. The potential contribution of willows to rapidly increase soil organic matter has been observed on these mine sites as well as on abandoned farmland in Ontario in earlier studies (Mosseler et al. 1988). *Salix eriocephala* often develops as a prostrate coppice form and has a special ability to trap abundant leaf litter fall around the base of its coppice growth. This leaf litter fall trapping function was less evident in the more upright plant forms typical of the other species tested here. Leaf litter fall can make an important contribution to soil N and fertility (Chabbi et al. 2008).

A unique feature of INT is its ability to spread as multi-stemmed colonies that develop from an extensive lateral root system. In the 7-species test, 9 of the surviving 16 INT clones have already spread 3–4 m from the original plant (stem cutting) and are developing into small, multi-stemmed colonies around many of the study plots within these common gardens. This spreading and stem suckering habit from lateral roots close to the soil surface is quite surprising on the dry, compacted shale mine overburden of these surface-mined coal mines. Biomass measurements were based only on the coppice stems arising from the root collar of the original stem cutting following the 2011 harvest. Therefore, it should be noted that biomass yield measurements from spreading

Table 5. Five species survival, mass and yield ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), P values, and coefficient of determination (R^2).

		Survival 2013[a]		Green mass (kg)		Stem number		Single stem mass (kg)		Yield ($t \cdot ha^{-1}$)	
Source of variation	df	MS	P value	MS	P value	MS	P value	MS	P value	MS	P value
Block	4	0.610	**<0.001**	0.631	**<0.001**	24 848.8	**<0.001**	0.011	**0.002**	45.99	**<0.001**
Species	4	2.059	**<0.001**	0.567	**<0.001**	59 618.8	**<0.001**	0.021	**<0.001**	37.16	**<0.001**
Clone (Species)	43	0.147	0.110	0.097	0.414	5652.0	**0.014**	0.004	**0.018**	6.65	0.496
Error	188	0.112		0.093		4711.2		0.002		6.71	
R^2			0.449		0.345		0.454		0.402		0.337

Note: P values < 0.05 are in bold print.

[a] Arcsine square root transformation.

Fig. 5. (A) Total aboveground biomass (leaves and stems), (B) number of coppice stems, and (C) average mass (kg) per coppice shoot in 2 year old coppice growth from a 5 year old rooted stem cutting for 8–16 clones selected for survival and growth at the Montreal Botanical Gardens for 5 native North American willows. Different letters signify significant differences using Tukey's mean separation test at $P = 0.05$.

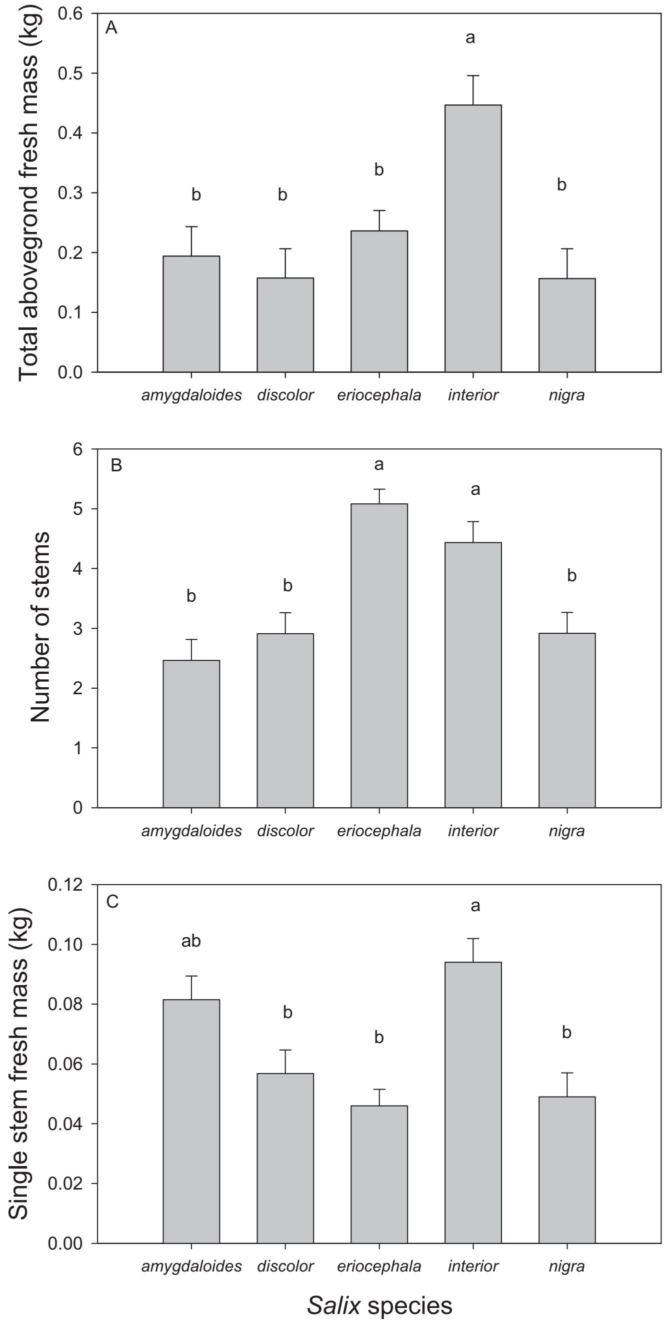
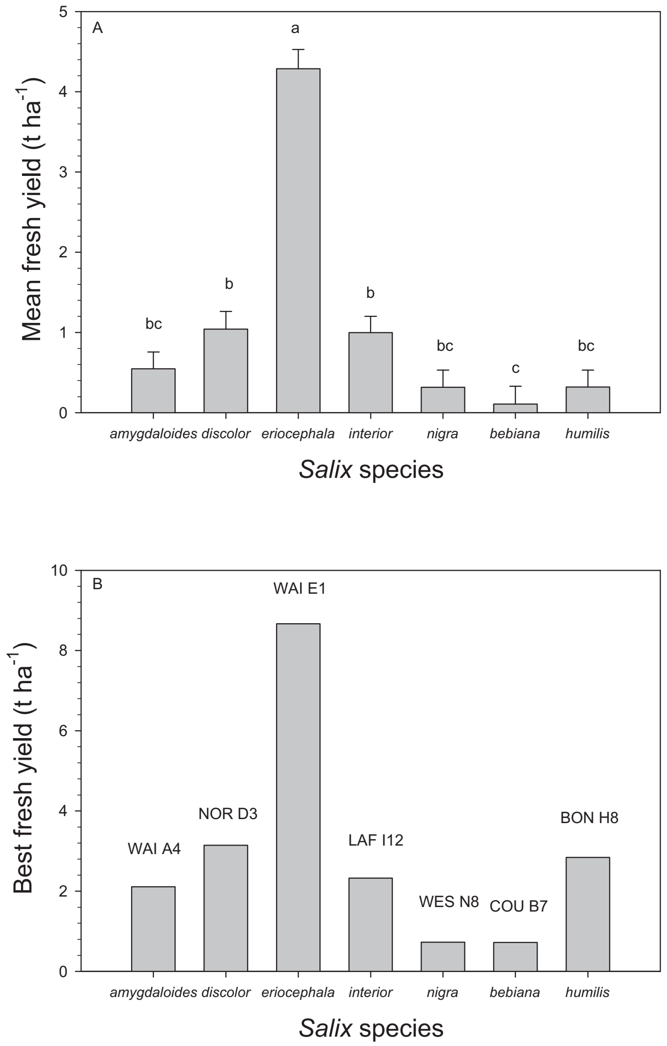


Fig. 6. (A) Mean fresh biomass yield in t·ha^{-1}, and (B) mean green biomass yield in t·ha^{-1} for the best producing clone from 2 year old coppice growth from a 6 year old rooted stem cutting for 20 clones collected from natural populations for each of 7 native North American willows planted on a coal mine site. Different letters signify significant differences using Tukey's mean separation test at $P = 0.05$.



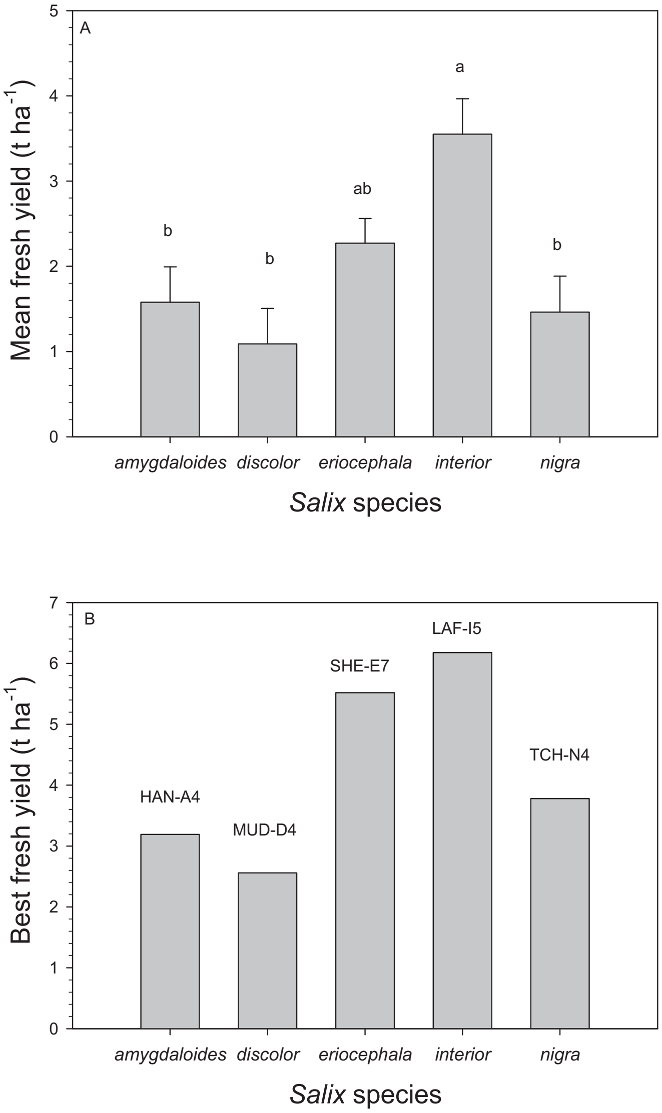
INT plants very likely underestimate true biomass production on an area basis, as it is difficult to assign aboveground biomass in these multi-stemmed colonies to an individual plant or ramet because of the rapidly spreading lateral root network of this species. This rapid spread and colony formation gives INT clones a special ability to rapidly "capture" a site to prevent erosion and mitigate surface runoff.

On average, BEB and HUM did not develop roots rapidly enough to survive conditions on these harsh mine sites (Figs. 2 and 6). However, we did identify several genotypes from these species that were able to develop roots reasonably quickly and survive to produce high biomass yields, demonstrating that further testing and selection efforts aimed at identifying superior clones of these species that have both good root development and the capability for high biomass production have value and should be pursued. These two non-riparian species are especially important for their adaptation to drier site conditions. They are often found associated with small openings in forest cover on dry upland sites. BEB is by far the most common willow colonizing these mine sites through natural seed dispersal.

Among the 8 selected NIG genotypes in the 5-species common garden test at the Salmon Harbour mine site, the clone (TCH-N4) originating from the geographically disjunct St. John River Valley population near Gagetown, NB, had the highest mean yield of 3.02 t·ha^{-1}. Although the percent survival was somewhat lower compared to ON origin clones, biomass yield per plant was highest for this Gagetown clone, suggesting that local NIG genotypes may be the best adapted for growth at the Salmon Harbour mine site.

The soil texture on the mine site was coarse, consisting largely of broken shale rock. Nevertheless, there were areas where silt-

Fig. 7. (A) Mean fresh biomass yield in t·ha^{-1}, (B) and mean green biomass yield in t·ha^{-1} for the best producing clone for 2 year old coppice growth from a 5 year old rooted stem cutting for 8–16 clones selected for survival and growth at the Montreal Botanical Gardens for five native North American willows.



ation occurred following repeated flash flooding events and where the pooling of water produced heavy clay deposits. Generally, willows did not grow well in areas where fine silts and clays were deposited, confirming observations by Ledin (1996) and Schaff et al. (2003) that heavier textured soils may not be suitable for good growth in some willow species. Willows prefer coarser textured soils. This became clear from observing very poor growth over the past 4 years following establishment of a clone bank with selected clones of ERI and DIS on an area of heavy clay deposit on the Salmon Harbour mine site. Ledin (1996) also suggested that unrooted stem cuttings should be planted as early as possible in spring to maximize survival success, but we had excellent survival with ERI cuttings planted anytime between mid-April and mid-August in 2013, which was a wetter than normal summer. Survival success will depend on soil moisture conditions during the normally dry summer months of July and August, and the inherent ability of a species for rapid root development. We had much less success with late-season plantings with poor-rooting species, such as DIS (Figs. 2 and 4). Nevertheless, with the inherently good rooting ability of ERI and INT, our experience suggests that one can expect reasonably good success establishing these species anytime from mid-April to mid-August. Willows retain their leaves until very late in the season and often appear to be growing actively until mid-November when frosts become more frequent and severe, giving species such as ERI and INT ample time for substantial shoot development before plants lose their leaves and enter dormancy.

There was significant variation in coppice structure following harvest (Figs. 3 and 5), with shrub-form willows generally producing coppices with more stems per coppice, whereas tree-form willows such as DIS, NIG, and AMY tend to concentrate more biomass within fewer coppice stems. The shrub willow ERI highlights this phenomenon and coppice form appears to be a relatively strong species characteristic (Sennerby-Forsse and Zsuffa 1995) (Figs. 3 and 5).

Reclamation of mine sites with the aim of eventually restoring the natural forest cover has proven difficult on coal mines across the Appalachian region of eastern North America (Holl 2002; Evans et al. 2013; Zipper et al. 2011). It is difficult to establish trees directly on exposed, highly disturbed areas, and simply sowing grasses for pasture may delay the process of natural succession to forest cover by further inhibiting tree growth (Wade 1989; Myster 1993; Holl and Cairns 1994; Ashby 1997; Torbert et al. 2000; Holl 2002; Skousen et al 2009; Zipper et al. 2011). Natural primary succession often takes many years, and restoration of the native forest cover can take many decades (Holl 2002). On the surface coal mines near Minto, NB, the initial colonization of herbaceous and flowering plants (many of the same species and genera described by Holl and Cairns (1994) as colonizing coal mine sites in Virginia), is normally followed by invasion of hardwood tree species, such as birches (*Betula papyrifera* and *B. populifolia*), alders (*Alnus* spp.), willows (mostly *S. bebbiana* and *S. discolor*, but *S. lucida* was also observed), aspen (*Populus tremuloides*), balsam poplar (*P. balsamifera*), pin cherry (*Prunus pensylvanica*), and black locust (*Robinia pseudoacacia*). Black locust was introduced many years ago for reclamation purposes and is now spreading on disturbed sites throughout the area. Within several decades after cessation of mining operations, the first local conifers, including white spruce (*Picea glauca*), black spruce (*P. mariana*), eastern tamarack (*Larix laricina*), and white pine (*Pinus strobus*), begin to infiltrate under the early successional hardwoods.

Both ERI and INT grew very well on these mine sites and both species have features that recommend them as useful, productive, and cost-effective species for reclamation and restoration on highly disturbed sites based on their rapid rooting ability, high survival, biomass productivity, and plant morphology. One of our longer-term objectives in testing native willows on these sites was to develop a cost-effective protocol for using willows to both capture (re-vegetate) a site to prevent invasion by grasses or ericaceous shrubs detrimental to establishment of trees (Mallik 2003; Zeng and Mallik 2006; Walker and Mallik 2009) and to prepare a more fertile soil for forest succession. Willows are normally short-lived species and could serve as a compatible nurse crop for the artificial introduction of longer-lived tree species, thereby shortening the decades-long natural succession process toward forest cover. Ultimately, our aim is to develop a "best practices plant system" to help restore not only coal mine sites, but also large areas being disturbed by oil and gas exploration such as the oil sands development in western Canada. Among the seven willow species tested here, we have determined that ERI and INT may be the best species for a relatively rapid improvement of soil conditions and for creating the required structure and protective cover for both natural and artificial introduction of some of the native spruces (e.g., black spruce and white spruce) and white pine. We began this artificial succession process in 2012 with the establishment of our first larger-scale multispecies plantations of ERI, INT, and DIS, and within 5–6 years, we hope to begin the first series of

Table 6. Biomass green weight (0.01 kg) for the best clone in each of five experimental blocks in five native willows, plus a check clone of the exotic, *S. viminalis* (clone 5027), commonly used in biomass plantations across Canada.

		Number of stems per coppice and green weight (0.01 kg)									
Species	Sample size	Rep 1		Rep 2		Rep 3		Rep 4		Rep 5	
S. amygdaloides	40	2 Han-A4	0.25	3 Cob-A1	0.55	4 Ric-A5	0.99	**3** Cob-A1	**0.56**	2 Ric-A3	0.45
S. discolor	40	2 Ric-D2	0.18	6 Mud-D4	0.57	3 Mud-D4	0.31	3 Mud-D4	0.27	4 Haw-D4	0.40
S. eriocephala	80	9 Sau-E3	0.48	21 She-E7	2.17	9 Nor-E9	0.41	8 Ann-E3	0.36	6 Sau-E3	0.51
S. interior	40	**2** Laf-I2	**0.50**	**9** Laf-I5	**2.62**	**4** Lon-I4	**1.65**	5 Lim-I6	0.46	**8** Lim-I6	**0.59**
S. nigra	40	2 Gue-N1	0.08	4 Fee-N1	0.14	10 Tch-N4	1.24	2 Tch-N4	0.21	5 Byn-N3	0.09
S. viminalis (5027)	5	4	0.18	1	0.17	**10**	**2.25**	6	0.08	3	0.13

Note: Sample size represents the number of clones × five replications (Rep).

conifer under-planting experiments to test this nurse crop concept in our continuing efforts to re-establish the natural forest cover that existed on these sites before land clearing for mining operations.

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