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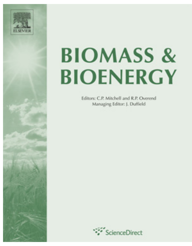

Biomass and root stem production of a colony-forming willow (*Salix interior*) on highly disturbed, low fertility sites

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ABSTRACT

This study describes biomass production, colony formation, and clonal spread via root stems (RS) of a wide-ranging North American willow species, *Salix interior* Rowlee (INT), one of the few willows that spread via vegetatively reproduced colonies, which can result in hundreds of upright stems arising from a shallow horizontal root network. Eight INT clones were tested in a common-garden experiment on two distinct site types (shale rock overburden and coarse gravel erosion sediments) with very low nitrogen and nutrients on a former coal mine site. Survival, height growth, aboveground biomass, and number of root stems (NRS) were quantified following 3 years of growth after establishment as rootless stem cuttings. Clonal differences were significant for survival and height at age 3. Survival was significantly greater on the coarse gravel outwash than shale rock overburden after the first and third year, but differences were only significant in the first year. There was a significant positive relationship between height growth and survival, both of which are indicators of plant vigor. Analysis of clonal variation in NRS showed an intermediate level of significance ($P = 0.083$), and a significant clone by site interaction ($P = 0.024$). On coarse gravel outwash, the more vigorous clones for height growth also produced more RS, indicating the absence of a potential trade-off in carbohydrate resource allocation between height growth of the ortet (mother plant) and its capacity for colony formation and spread via RS. It appears that RS quickly become independent sources of carbohydrate production. However, there was no relationship between clonal height growth and NRS on the rock overburden. Loose sand and gravel outwash deposits promoted a more rapid spread of the shallow horizontal root network than the less penetrable shale rock overburden that dominates this former coal mine site.

1. Introduction

Production of short-rotation woody biomass from coppiced willows (*Salix* spp.) and poplars (*Populus* spp.) aimed at emerging bioenergy, chemicals, and materials industries may be commercially hampered by the limited lifespan of coppice production systems following a number of harvest rotations [1–5] in which yields may decline as a result of coppice rootstock deterioration following repeated harvests [6–8]. As plantation establishment costs represent a major expense in

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Table 1 – Origins of *Salix interior* genotypes (clones) used for biomass production trait assessments made in a common-garden study at the Salmon Harbour mine site near Minto, NB, Canada.

Origin	Latitude; Longitude	Clone	Crown form description
Ottawa, ON	45°42′N; 75°69′W	LAF-I2f	Bushy, upright stems; medium density branching
		LAF-I5f	Few, tall, upright stems; sparsely branched
Roebuck, ON	44°80′N; 75°61′W	LIM-I3m	Compact, rotund bush; medium density branching
		LIM-I6f	Few, upright stems; medium density branching
Long Sault, ON	45°03′N; 74°89′W	LON-I2m	Rotund bush, medium density branching
		LON-I3m	Few, upright stems; sparse to medium density branching
		LON-I4f	Few, upright stems; sparse to medium density branching
Pembroke, ON	45°50′N; 77°07′W	PEM-I4f	Many short upright stems; rotund bush; densely branched

Note: m = male, f = female.

short-rotation biomass feedstock production, any reduction in establishment costs can add significantly to the economic viability of biomass production [9,10]. Woody biomass production based on vegetative reproduction via root stems arising from either roots (adventitious stems), rhizomes (underground stems), or stolons (superficial stems) would eliminate the need for repeated re-establishment of plants and associated costs.

Of approximately 350 willow species worldwide [11], a small group of closely related North American willows in the taxonomic section *Longifoliae* [12,13] have a unique ability among willows for vegetative spreading as clonal colonies of upright root stems (RS) that arise from an extensive network of shallow, horizontal roots [14–17]. This vegetative growth form via RS has been well described by Douglas [16,18] for *Salix setchelliana*, the only other willow outside this small group of 6–7 species in section *Longifoliae* that reproduces via RS [11]. Section *Longifoliae* includes *Salix interior* Rowlee (sandbar willow) and the closely related, if not synonymous, *Salix exigua* Nuttall (also commonly called sandbar or coyote willow. These are wide-ranging, riparian willows that occur naturally from Mexico to Alaska and across much of the interior of North America east of the Rocky Mountains [13,19–22]. In the literature, these two species are often confused and have been variously treated as synonymous, intergradations, varieties, and/or subspecies of one another [11,13,19,21–25]. As with most riparian-zone willows, *S. interior* (INT) is easily propagated *in situ* from dormant, rootless stem sections or cuttings [26] and can produce relatively high biomass yields, even on sites of low fertility, such as abandoned farmland [27] and highly disturbed mine site overburden [28]. While INT has both deep and shallow roots, root stems arise from shallow, horizontally spreading roots.

Our aims were to (i) characterize clones of INT that would be useful for both land reclamation and woody biomass production on infertile and highly disturbed sites subject to wind and water erosion and (ii) raise awareness of the potential economic advantages of vegetative reproduction and colony formation via root stem production as a way of reducing plantation establishment costs, thereby increasing the commercial viability of short-rotation woody biomass plantations. The specific objectives of this study were to (i) quantify survival, height growth, biomass production, and colony spread via RS formation among eight INT clones that had been previously selected for their survival and growth performance in multi-species, common-garden field tests established at several locations in eastern Canada [28], (ii) determine the degree to which differences in colony spread via RS were related to site/soil conditions, (iii) quantify the genetic (clonal) component of variation in biomass production traits, and (iv) identify superior performing clones for both land reclamation and commercial biomass production on highly disturbed, infertile sites.

2. Materials and methods

The field test site was located in a narrow, shallow valley that had been landscaped to minimize erosion at the former Salmon Harbour coal mine operated by NB Power (a public utility), near Minto, New Brunswick (NB), Canada (Lat. 46°07′ N; Long. 66°05′ W). The valley bottomland consisted of two distinct site types: areas characterized by the exposed broken shale rock overburden that dominates the mine site and areas covered to varying depths by coarse sand and gravel deposits formed from erosion sediments. The depth of these gravel deposits was measured to the nearest 1 cm using a steel reinforcement bar that was driven into the gravel deposit until the rock overburden was reached. These gravel depth measurements were taken at 120 locations beside plants that had been selected for height measurements as described below. The shale rock overburden consisted largely of broken rock, and therefore no attempt was made to measure soil depth. To further characterize soil properties, six soil samples were taken from each of the two site types to a depth of 15 cm along the valley bottom. Soil properties analyzed include soil texture, pH, and organic matter, carbon, nitrogen, potassium, calcium, magnesium, phosphorus, and sulfur concentrations. The Salmon Harbour mine site has an average temperature of 5.7 °C and a mean annual precipitation of 987 mm [29].

The valley bottomland used in this study consists of a series of patches where soil sediments have formed as a result of erosion from the adjacent hillsides. These patches of sediments are interspersed with patches of the broken shale rock overburden that dominates this post-mining landscape. We established 10 blocks consisting of eight clones of INT, five blocks (replicates) on patches of sediment and five blocks on patches of broken shale rock. Patches of sediments and broken shale rock occur at random across the valley bottomland. Five patches of each soil type were selected for establishing each block of the eight INT clones.

Table 2 – Soil properties for the two site types, rock overburden and loose gravel, 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)
Shale rock overburden	3.54 ± 1.12 a	0.27 ± 0.03 a	0.070 ± 0.005 a	0.198 ± 0.019 a	8.53 ± 0.52 a	1.10 ± 0.09 a	4.33 ± 0.70 a
Coarse gravel outwash	5.28 ± 1.12 a	0.15 ± 0.03 b	0.048 ± 0.005 b	0.158 ± 0.019 a	7.21 ± 0.52 a	0.96 ± 0.09 a	2.00 ± 0.70 b

Site	Sand (%)	Silt (%)	Clay (%)	pH	C:N ratio	Sulfur (%)	Rock (%)*
Shale rock overburden	28.0 ± 4.9 b	57.5 ± 4.4 a	14.4 ± 0.7 a	5.9 ± 0.3 a	4.0 ± 0.5 a	0.021 ± 0.006 a	29.7 ± 7.7 a
Coarse gravel outwash	72.2 ± 4.9 a	23.1 ± 4.4 b	4.6 ± 0.7 b	6.4 ± 0.3 a	3.1 ± 0.5 a	0.018 ± 0.006 a	32.2 ± 7.7 a

* Note: Rock percentage is taken before the sand, silt and clay percentages, which are equal to 100%.

Stem cuttings, approximately 20 cm in length were collected from eight selected clones of INT (Table 1) in December 2011 from vigorous 1- and 2-year-old stems from a common-garden field test established in 2007 at the Montreal Botanical Gardens in Montreal, Quebec, Canada. Stem sections were stored over the winter months in a freezer at −5 °C at the Atlantic Forestry Centre in Fredericton NB. Prior to field test establishment, stem cuttings were moved from frozen storage to a refrigerator held at 3 °C for several days of thawing, followed by 48 h of soaking in water immediately prior to planting at the mine site.

In April 2012, rootless stem cuttings from these eight INT clones were established at 2 m by 2 m spacing within linear clonal row plots containing up to 25 plants (ramets) per clone. Each clonal row plot appeared once within each of 10 blocks with clones randomly assigned within blocks. The clonal row plots were aligned perpendicular to the axis of the valley bottomland and ran up both sides of the valley. Clonal row plots were spaced at 2 m apart. Despite landscaping attempts to minimize erosion, the shallow valley rose steeply enough from the valley bottom to create variable site conditions with respect to erosion and soil moisture. Only the small group of plants at the center of the valley bottom received enough moisture to sustain “normal” growth over the three growing seasons (2012–2014) following establishment.

A highly bred control clone of *Salix viminalis* (VIM) clone 5027, originating from central Europe and developed by the Swedish willow breeding program [30], which has been widely used in North American biomass plantations [31], was established in similar linear clonal plots in three of the 10 blocks for comparative purposes but was not included in ANOVAs for growth traits.

Survival counts were made in August 2012, approximately 4 months after stem cuttings had been established and again in June 2014 during the third growing season following establishment by considering survival of only the original stem cutting (ortet). In late October 2014, after plants had completed 3 years of growth following establishment as rootless stem cuttings, the three tallest plants from the valley bottom of each clonal row plot were selected for height measurements to the nearest 1 cm using a flexible measuring tape. The ramet with the largest aboveground biomass per clonal plot was harvested at ground level, and the fresh weight (including stems, branches, and any remaining leaves that had not yet fallen away) was measured to the nearest 0.01 kg using an electronic infant weigh scale (Electronic Infant Scale, model ACS-20A-YE). Measurements of fresh and dry weight taken from field tests at the Montreal Botanical Garden demonstrated that percentage moisture content was approximately 50 ± 2% [48] both among and within species.

Stem colony spread was measured by counting the number of RS arising within a 2 m × 2 m square plot around each of the three plants (ramets) selected for height measurement from each clonal plot. The three sampled ramets served as the plot centers of the 2 m × 2 m plot used to count number of root stems (NRS) arising within each plot by October 2014. Although clonal differences in leaf form, coloration, and stage of leaf senescence generally allowed us to associate the RS with a particular clone, we could not be completely certain that each RS tallied within this 2 m × 2 m plot originated from the INT plant at the center of the plot (the ortet). We did not want to jeopardize future studies by pulling up roots to confirm the ortet of origin of the RS within each plot.

A simple two-way ANOVA was used to assess significance of differences among the eight clones for height, biomass yield, survival, and NRS using the following linear model:

$$Y_{ijkl} = \mu + S_i + C_j + CS_{ij} + B_{k(i)} + e_{ijkl}$$

where μ is the overall mean, Y_{ijkl} is the dependent ramet trait from the ith site type of the jth clone and the kth block nested within the ith site type and the lth ramet; S_i is the effect of the ith site type (rock overburden versus gravel erosion sediments), C_j is the effect of jth clone ($j = 1–8$), $B_{k(i)}$ is the effect of the kth block nested within the ith site type ($k = 1–5$), CS_{ij} represents the clone by site interaction, and e_{ijkl} is the random error term. Blocks, site types, and clones are all considered as fixed effects for our ANOVA model. Survival was *arcsine* transformed for normality. The general linear model from Systat (Chicago, Illinois) was used for analysis. All statistical tests were assessed for a significance level at $\alpha = 0.05$. Tukey's mean separation test was used for multiple comparisons.

3. Results

From the soil analysis, soil texture properties showed significant differences between site types; sand content was 28.0 and 72.2%, silt content was 57.5 and 23.1%, and clay content was 14.4 and 4.6% for the shale rock overburden and gravel

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.

Source of variation	df	Survival 2012[a] (%)		Survival 2014[a] (%)		Mean height (cm)		Aboveground biomass of tallest stem (g)	
		MS	P value	MS	P value	MS	P value	MS	P value
Block (site)	9	0.255	**0.009**	0.228	**0.001**	1555.0	**0.001**	0.074	**0.015**
Site type	1	0.577	**0.015**	0.102	0.163	53.6	0.707	0.009	0.568
Clone	7	0.394	**0.001**	0.192	**0.002**	867.0	**0.042**	0.033	0.331
Site type x clone	7	0.184	0.069	0.097	0.088	584.5	0.172	0.038	0.258
Error	51	0.091		0.051		373.3		0.028	
R^2			0.630		0.629		0.643		0.516

Source of Variation	df	Mean number of root stems	
		MS	P value
Block (site)	9	30.92	**0.019**
Site type	1	1046.51	**<0.001**
Clone	7	23.69	0.083
Site type x clone	7	31.68	**0.024**
Error	51	12.05	
R^2			0.787

[a] Note: arcsine square root transformation.

erosion sediments, respectively (Table 2). Soil nutrient properties were generally similar between the two site types, with some notable exceptions. Soil nitrogen, although significantly different, was very low for both site types, with 0.07 and 0.05 for the shale rock overburden and gravel sediments, respectively. Two more elements, phosphorus and carbon, were significantly greater for the shale rock overburden than gravel sediments. However, it was the depth of gravel erosion sediments – which ranged from 16 cm to 117 cm, with an overall average depth of 53 cm – that most clearly distinguished the soil between the two site types. A ranking of the eight INT clones based on ANOVA in traits of interest (Table 3; Fig. 1) showed high variability in survival rates and significant differences in survival among clones during the first and third growing season. There was also a large decline in survival over the three growing seasons from an overall average survival of 73% during the year of establishment to an overall survival of just 42% by the third growing season. For several clones, there was a significantly lower survival during the first growing season on the shale rock overburden as opposed to sites containing the coarse-textured sand and gravel erosion sediments, but by the third growing season, these survival differences – although still significant at the clonal level – had become more muted (Fig. 1b). However, by the third year, there was an intermediate clone x site type interaction ($P = 0.088$), whereby three of the eight clones (i.e., LAF-I2, LIM-I3, and LON-I4) showed significantly greater survival on the rock overburden than the sand and gravel outwash sediments. Conversely, the other five clones had greater survival on sand and gravel outwash than on the rock overburden.

There was a positive correlation between percentage survival and plant height by age 3 from establishment as rootless cuttings ($P = 0.015$, Fig 2). Both height and aboveground biomass production at age 3 were highly variable, and there were significant differences in height growth among the eight clones (Table 3; Fig. 3a). Overall, there were no significant differences in height or biomass production between site types (Table 3), but certain clones (i.e., LAF-I2, LIM-I3, LON-I2, and PEM-I4) showed significant differences in their growth performance across the different site types (Fig. 3a and b). The latter two clones grew better on the loose, coarse gravel deposits, whereas the former two clones performed better on the rock overburden. By age 3, there was a modest statistical difference among clones in the NRS arising within the 2 m × 2 m plots around each ortet ($P = 0.083$), but there was both a significant and a large difference among site types for this trait (Table 3; Fig. 4). The NRS was greater on sites containing the coarse sand and gravel outwash deposits. In addition, there was also a significant clone by site interaction ($P = 0.024$). Clone PEM-I4 showed a dramatically greater capacity in spreading via RS on the coarse gravel outwash than all other clones. Also, there were very different growth responses on the two site types in terms of the relationship between plant height and NRS (Fig. 5). On rock overburden, plant size was not correlated with RS formation, but on the coarse gravel erosion sediments, there was a strong positive correlation between clonal plant height and colony spread via RS formation.

Although we did not attempt to quantify variation in crown architecture, such variation was evident in a visual, qualitative characterization of the appearances of the different INT clones (Table 1). For instance, clones such as LAF-I5f were tall and thinly branched, whereas the crowns of clones LIM-I3m, LON-I2m, and PEM-I4f were more compact, bushy, and densely branched. These clonal differences in crown architecture were reflected in the absence of significant correlations between mean height and biomass ($R^2 = 0.165$, $P = 0.318$) or between height of the tallest plants and biomass ($R^2 = 0.004$, $P = 0.878$).

Overall survival rates of *S. viminalis* clone 5027 (VIM) were almost identical to those for the INT clones in year 1, with 74% and 75%, respectively, and for year 3, with 46% and 43%, respectively, but height growth was greater for the VIM than

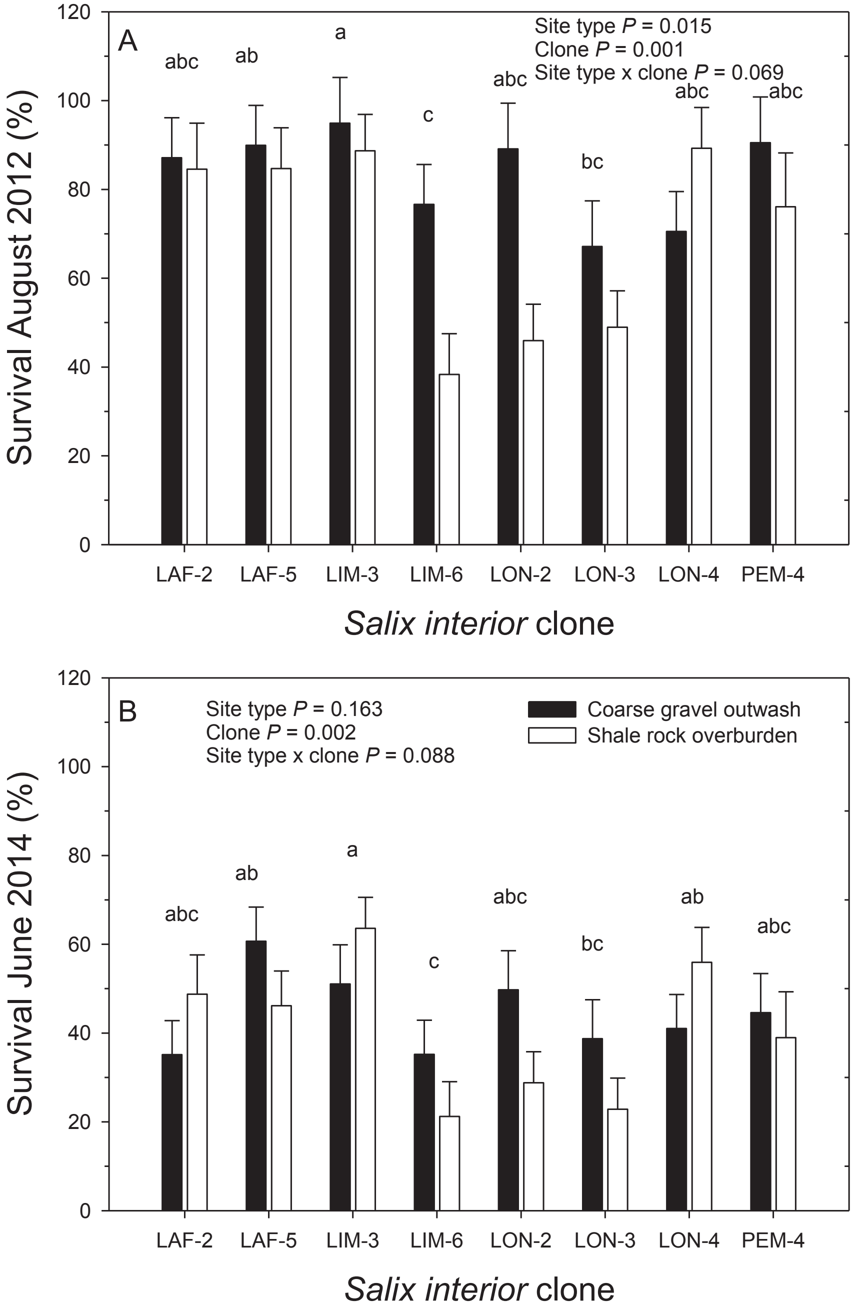

Fig. 1 – Percentage survival in eight clones of *Salix interior*, established as rootless stem cuttings, on two different site types (coarse gravel outwash versus shale rock overburden) during the year of establishment (A) and during the third growing season (B) on a former coal mine site. Clones that share a letter are not significantly different at $\alpha = 0.05$ as determined by Tukey's mean separation test. Clonal origin codes are defined in Table 1.

the overall mean for the INT clones at 160 cm and 111 cm, respectively. This VIM clone grew consistently well on these harsh, infertile mine sites.

4. Discussion

Salix interior is a pioneer, riparian species adapted to high levels of moisture and flowing water and known for its rapid invasion of fresh alluvial deposits [32–38]. Despite variable moisture conditions along the linear clonal plots that transected the narrow, shallow valley of broken shale rock overburden of this former coal mine, survival over the year of establishment was surprisingly high. However, by the third year after establishment, survival had declined dramatically, and growth was very poor, particularly along sections of each clonal row plot transect that extended up both sides of the valley. Differences in growth and survival were probably

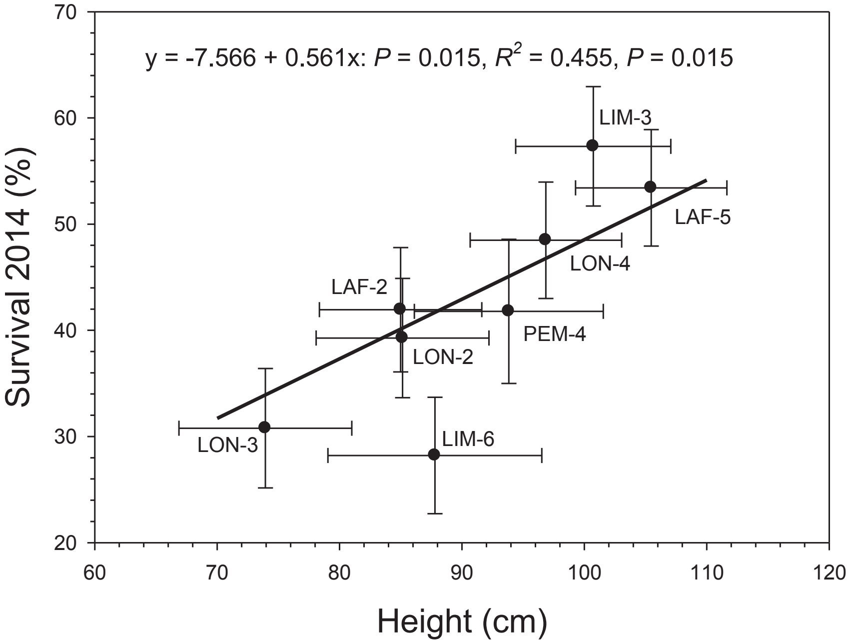

Fig. 2 – Relationship between percentage survival in the third growing season from establishment as rootless stem cuttings and third-year height growth (cm) on a former coal mine site. Clonal origin codes are defined in Table 1.

primarily related to differences in moisture availability that were evident along the entire sides of the valley on both the shale rock overburden and the coarse sand and gravel-dominated erosion sediments. Only those plants located in the moist center of the valley bottom of each transect (clonal plot) grew “normally”. Poor growth in areas outside the moist valley bottom demonstrated that riparian species, such as INT, require abundant available moisture throughout the growing season for sustained growth [32,39–41].

It appeared that the main difference between the two site types was related to presence or absence of the loose, coarse gravel erosion sediments that sometimes formed via surface water flow over the shale rock overburden that dominates the mine site. This gravel outwash deposit resulted in significant soil texture differences, but only small differences in the soil nutrients, carbon, and pH, that generally reflected the infertility of the shale rock overburden.

Using only the three best plants per clonal plot, there was a moderately strong positive relationship between height growth and percentage survival. Although both height growth and survival are expressions of plant vigor, correlations among these vigor traits have not often been reported in the literature. The two clones with the highest survival (2014) and height were LIM-I3 and LAF-I5, and thus far these would be considered the best clones for overall productivity; LON-I3 and LIM-I6, however, had among the poorest survival and height, and unless there is a dramatic change over time, these clones might be considered less favorable for overall productivity on these harsh infertile sites.

The growth pattern observed in these eight INT clones along the clonal row plots were characteristic of the response to water availability described by Smith et al. [42] as typical for riparian species adapted to arid regions, and especially for phreatophyte species such as INT, characterized by their deep root systems and capacity for accessing deep water tables. We did not try to quantify water availability in this study because it is very difficult to accurately and confidently quantify this over a growing season in which water availability varies greatly across these harsh sites. Selecting only the three best-growing ramets per clonal plot from the wettest part of the valley bottom for growth assessments under such harsh conditions provides the best way to assess site effects. Although survival was high in the year of establishment, in the early years following establishment, most INT clones appeared to lack adaptations that would enable them to tolerate extended periods of water stress. Growth was very poor in those plants that did manage to survive along the drier sides of the valley. Under natural conditions, INT usually occurs in direct association with stream channels, floodplains, and narrow riparian corridors. In other restoration plantings across this coal mine site, INT appeared to survive and thrive best when associated with active erosion stream channels that provided a source of water throughout most of the growing season [48].

The effect of moisture availability was also evident in the lack of RS formation on the drier hillsides where moisture availability restricted colony expansion up the sides of drier valley slopes. Thus, the potential invasiveness of INT colonies under drier natural conditions may be constrained by moisture availability, at least in the early years of colony formation when INT may be allocating relatively more biomass to root development in order to reach the water table [40]. This constrained colony growth has also been described in natural colonies of INT growing up the sides of river and stream banks

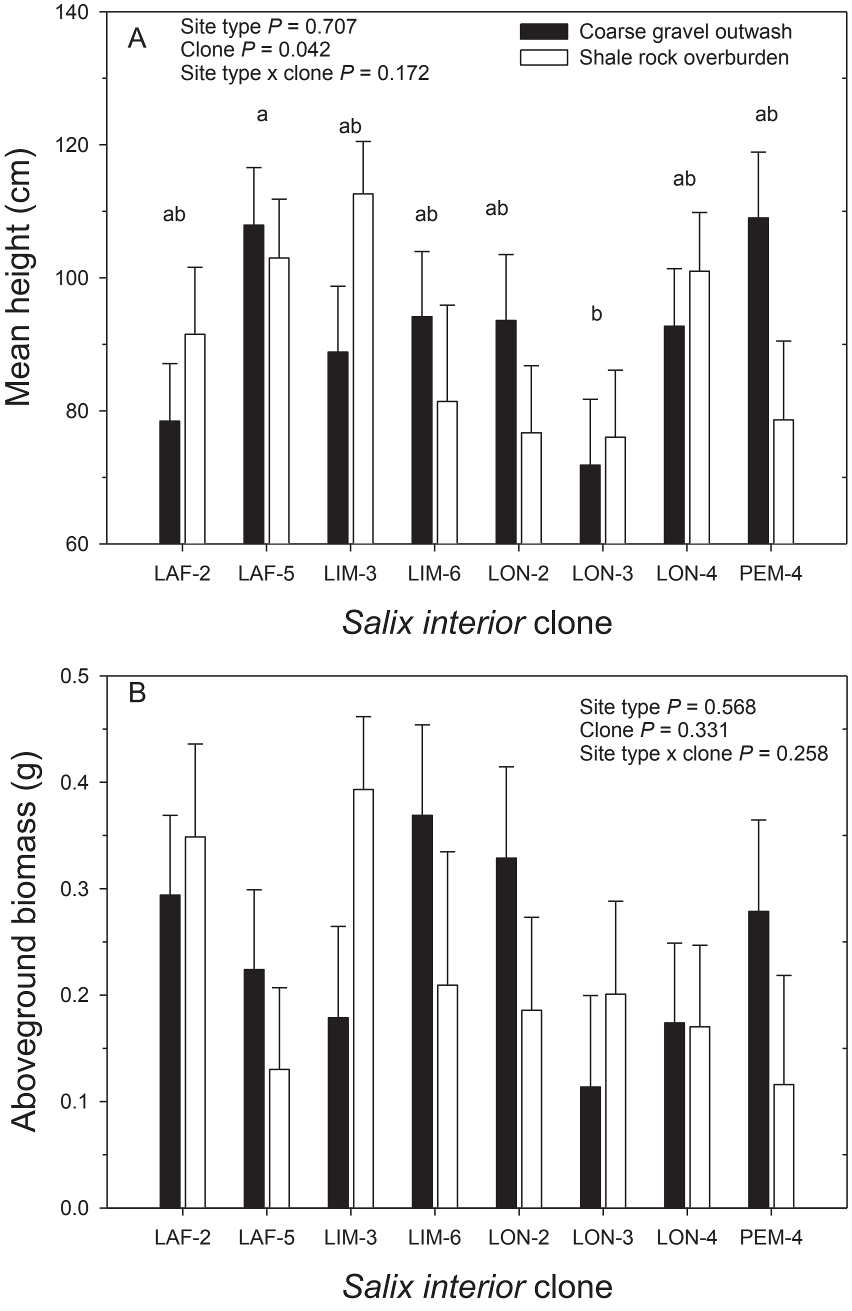

Fig. 3 – Mean height growth (cm) and aboveground fresh biomass production (g) in eight clones of *Salix interior* on two different site types (coarse gravel outwash versus shale rock overburden) at age 3 from establishment as rootless stem cuttings. Clones that share a letter are not significantly different at $\alpha = 0.05$ as determined by Tukey's mean separation test. Clonal origin codes are defined in Table 1.

in riparian zones [15]. The positive correlation between height and NRS on the gravel erosion sediments also indicated no important trade-off between plant height and RS formation and that RS become independent sources of carbohydrate production soon after they form.

Nevertheless, over a number of years, root suckering will allow INT to colonize adjacent drier sites in much the same way as balsam poplar (*Populus balsamifera* L.) uses RS formation exclusively for colonizing sites too dry for seed germination [17]. Although establishment of INT via rootless stem cuttings on sites with high seasonal variation in moisture availability was not very successful along the drier sides of the shallow valley in this experiment, we have observed older INT colonies that have developed and spread up the sides of dry embankments at various other locations on the Salmon Harbour coal mine site where INT has been used for site reclamation. However, such colony development occurred only 5 or 6 years after establishment from rootless cuttings. This indicates that INT can eventually invade drier sites but that this process takes more time as this phreatophyte

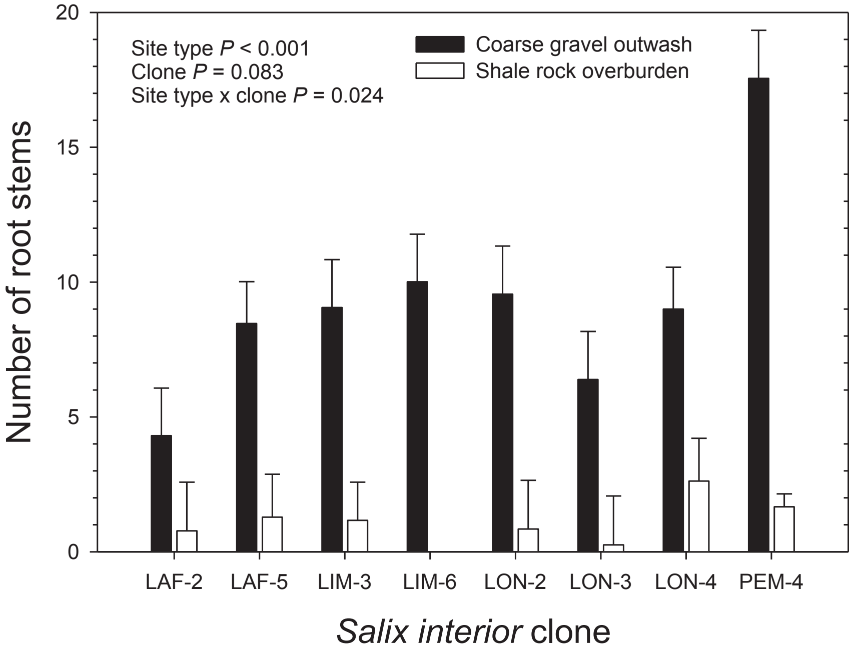

Fig. 4 – The number of root stems produced in a 2 m × 2 m plot around ortets of eight clones of *Salix interior* on two different site types (coarse gravel outwash versus shale rock overburden) at age 3 from establishment as rootless stem cuttings. Clones that share a letter are not significantly different at $\alpha = 0.05$ as determined by Tukey's mean separation test. Clonal origin codes are defined in Table 1.

develops a root system able to reach the water table [40,43]. However, this ability to invade drier soils over time via RS formation and colony spread may be an important characteristic for biomass production plantations on sites with great variation in seasonal moisture availability.

The strong clonal response in terms of genotype by environment interactions for survival (Fig. 1), height growth and biomass yield (Fig. 3), and colony spread via RS (Fig. 4) indicates potential opportunities for clonal selection for different growth forms and traits. For instance, if the rate of

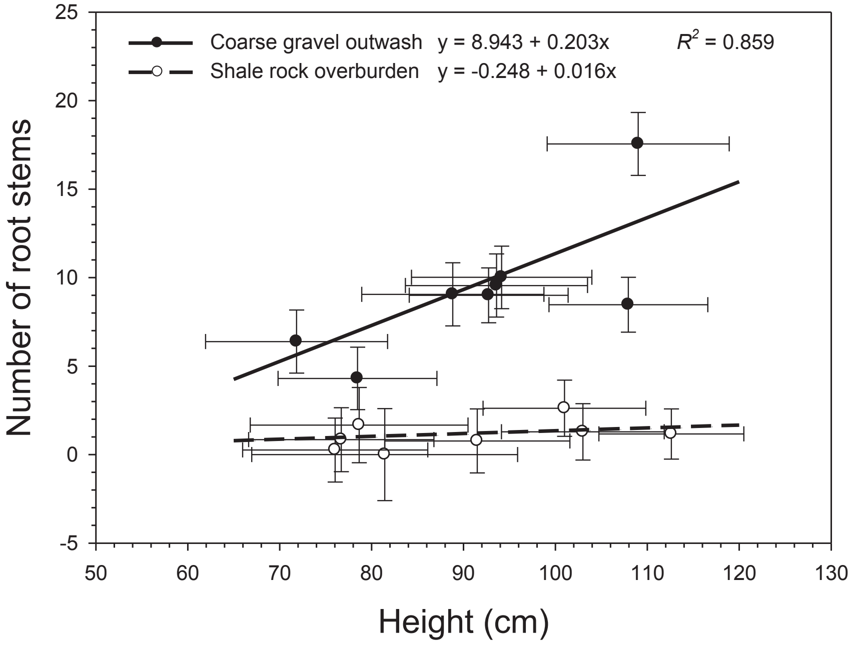

Fig. 5 – Relationship between number of root shoots produced in a 2 m × 2 m plot around ortets of eight clones of *Salix interior* and height (cm) on two different site types (coarse gravel outwash versus shale rock overburden) at age 3 from establishment as rootless stem cuttings.

clonal spread via RS formation is considered as the most important growth trait for stream bank stabilization and erosion control, clones such as PEM-I4 may be a preferred growth form. If biomass production for bioenergy purposes on better sites is of primary interest, then higher-yielding clones such as LAF-I2, LIM-I6, or LON-I2 might be best. For creation of a diffuse canopy that both protects underplanted conifers from wind and snow abrasion, yet allows good light penetration, then the taller, more thinly branched crown architecture of clone LAF-I5 might be the preferred growth form for use as a nurse crop in forest restoration activities [44].

There are very few studies available on relationships between soil texture and riparian plant growth [45]. The best growth of INT colonies in this study was found on sites with loose, coarse sand and gravel outwash sediments as opposed to the shale rock overburden that dominates this mine site. Others, working with either INT or the closely related *S. exigua* [38,46], have also found that INT was generally restricted to coarser-textured sandy soils rather than on heavier-textured clay soils, although in arid areas, a component of finer-textured materials can improve the moisture-holding capacity of coarse-textured substrates [45]. By the end of the year of establishment, some plants had already reached heights of 1 m on these gravel outwash deposits and had started to produce RS. Colony spread was also most rapid on loose, gravel deposits created by erosion sediments as opposed to the less penetrable rock sites or where ponding had developed deposits of fine-textured clay and silt. Root impedance on heavy-textured soils has been described by Desrochers and Tremblay [47] in poplars.

In this study, INT flowered as early as the second growing season after establishment from rootless stem cuttings, but abundant and sustained flowering throughout the season did not occur until the third growing season when plants had attained a height of 1–2 m and had developed more substantial branching and crown biomass [17]. Although we haven't yet quantified flower production, anecdotal observations from ongoing pollination studies [48] show that some clones have more prolific flower production than others. Therefore, flower production for promotion of insect pollinators in various agricultural applications, such as berry or fruit production, may also be a trait for genetic selection and clonal development. Willow plants are usually dioecious, producing unisexual staminate and pistillate flowers on different plants. Male INT clones might be especially effective for promotion of insect pollinators in agricultural applications where avoidance of potential invasiveness via prolific seed dissemination is important.

Some INT clones grew very well despite seasonal flooding throughout 2014 and appeared to tolerate extended periods of inundation [32,49,50]. These inundated INT clones developed dense masses of adventitious roots along portions of stems that had remained underwater for extended periods [51]. A similar phenomenon has also been observed in other riparian willows, such as *Salix amygdaloides* Andersson and *Salix nigra* Marsh., that have also been established in common-garden field tests at other locations on the Salmon Harbour mine site [28].

Plants of the European *S. viminalis* clone 5027 [31] produced consistently high yields on the wetter areas of the three blocks in which it was established as a check clone. However, on an area basis, much of the biomass yield in INT may be dispersed across the RS arising from the extensive horizontal root network [52]. This rapidly expanding root network may also be a major carbohydrate sink, especially in the early years following establishment, and the real potential of harvestable, aboveground biomass may not be realized until later in the life cycle of willows that form colonies via RS [16,18]. Nevertheless, *S. viminalis* has shown a remarkable consistency both in survival and growth on these infertile mine sites, which is surprising given its origins on the more fertile sites of its native range in central and eastern Europe, and in the Swedish breeding program from which this clone originates [30].

5. Conclusions

Strong genetic (clonal) variation within INT has become apparent even at this early growth stage, and we expect some of these clonal differences to strengthen over time, indicating ample opportunity for genetic selection for survival and various growth traits, including height, biomass yield, rates of clonal spread via RS formation, and crown architecture. The ability of INT colonies to continuously renew themselves through vegetative reproduction as new stems arise from shallow, spreading, horizontal roots may obviate the need to periodically replace plants in biomass production plantations. Such vegetative colony formation may represent a major cost advantage in plantation establishment, maintenance, and management for the commercialization of woody biomass production. Plantations of INT could theoretically be harvested continuously and annually, through a long succession of biomass harvests without relying on extant rootstocks for regrowth via coppicing. This eliminates the need to periodically re-establish biomass plantations as coppicing rootstocks deteriorate over time and repeated harvests. Other studies have shown that biomass yields from INT clones have been comparable to both highly bred clones from exotic species such as *S. viminalis* as well as several other native willow species being field tested on this former mine site [28]. However, much of the aboveground biomass production from INT plants results from the process of colony formation. Therefore, it may be difficult to compare biomass yield in INT with that of non-colony-forming willows except on an area basis [52]. Nevertheless, INT provides one of the most promising and compelling opportunities in the genus *Salix* for commercialization of short-rotation woody biomass production because of its mode of clonal spread via root stems. In the context of land reclamation and forest restoration in Canada's oil sands development, INT has been observed as a natural colonizer of mine tailings in northern Alberta. This species appears to be well adapted to these mine tailings and could play a central and useful role in future land reclamation activities as well as provide a source of woody biomass for emerging energy, chemicals and materials industries.

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