

# ARTICLE

# Distribution of genetic variation in five coppice growth traits among natural populations of seven North American willow (*Salix*) species

A. Mosseler, J.E. Major, and D. Ostaff

Abstract: Genetic variation and population structure in biomass yield and coppice growth traits were assessed in seven native North American willow species (*Salix amygdaloides* (AMY), *Salix bebbiana* (BEB), *Salix discolor* (DIS), *Salix eriocephala* (ERI), *Salix humilis* (HUM), *Salix interior* (INT), and *Salix nigra* (NIG)) established together in common-garden field tests on two sites. Differences in biomass yield, coppice stem number, and average single-stem mass were significant at the site, species, population, and genotype (clonal) levels. There were also species × site interactions. Analyses of variance components for these traits showed that only 3%–5% of the total variation in these traits was due to site differences, whereas genetic variation at the species, population, and genotype levels accounted for approximately 10%–39%, 5%–13%, and 12%–23%, respectively. Populations were a significant source of variation in some willow species (e.g., AMY, DIS, ERI, and INT) but not in other species. Tree willows were less prolific in stem sprout production than shrub willows, and ERI coppices produced by far the highest number of stem sprouts per coppice. This multispecies investigation demonstrated strong species and clonal differences, but variation among populations within a species, although significant, was relatively small, indicating that major growth and yield gains can be made through proper species selection and clonal selection within local populations.

Key words: biomass yield, coppice structure, genetic diversity, population genetics, willows.

**Résumé**: Les auteurs ont déterminé la variation génétique et la structure de populations pour des caractères de rendement en biomasse et de croissance en cépée chez sept espèces indigènes de saules d'Amérique du Nord (*Salix amygdaloides* (AMY), *Salix bebbiana* (BEB), *Salix discolor* (DIS), *Salix eriocephala* (ERI), *Salix humilis* (HUM), *Salix interior* (INT) et *Salix nigra* (NIG)) établies ensemble en test comparatif au champ sur deux sites. Les différences de rendement en biomasse, en nombre de tiges par cépée, et en masse moyenne des tiges individuelles étaient significatives pour les effets reliés aux sites, aux espèces, aux populations et aux génotypes (clones). Des interactions espèce × site ont également été observées. L'analyse des composantes de la variance pour ces caractères a démontré que seulement de 3 à 5 % de la variation totale chez ces caractères était dûe aux différences entre les sites, alors que la variation génétique chez les espèces, les populations et les génotypes comptait pour respectivement environ 10–39 %, 5–13 % et 12–23 %. Les populations constituaient une source significative de variation chez certaines espèces de saules (p. ex., AMY, DIS, ERI et INT), mais pas chez les autres. Les saules arborescents étaient moins prolifiques en ce qui a trait à la production de rejets que les saules arbustifs, et les cépées d'ERI produisaient de loin le plus grand nombre de rejets par cépée. Cette étude de plusieurs espèces a mis en lumière de fortes différences entre les espèces et les clones. Mais la variation parmi les populations d'une même espèce, quoique significative, était relativement faible, indiquant que des gains majeurs en croissance et en rendement peuvent être obtenus via la sélection d'espèces et de clones appropriés au sein des populations locales. [Traduit par la Rédaction]

Mots-clés : rendement en biomasse, structure des cépées, diversité génétique, génétique des populations, saules.

# Introduction

Plant biomass production is seen as an opportunity to promote sustainable biofuels and reduce the effects of greenhouse gas emissions through carbon sequestration (Killeen et al. 2011; Searle and Malins 2014; Harris et al. 2015; Margaritopoulou et al. 2016), and short-rotation intensively cultured (SRIC) willows (*Salix* spp.) may play an important role in emerging biomass and bioenergy industries due to the rapid early growth and ease of vegetative propagation of some species (Tharakan et al. 2005; Helby et al. 2006; Rytter 2012; Sevel et al. 2012; Vigl and Rewald 2014; Rytter et al. 2015). There is growing interest in North America in understanding variation patterns in native willows as potential sources of fast-growing woody biomass (Mosseler et al. 1988; Labrecque et al. 1993; Lin and Zsuffa 1993; Labrecque and Teodorescu 2005; Volk et al. 2006), for rapid reclamation and restoration of highly disturbed areas affected by various industrial activities (Kuzovkina and Quigley 2005; Shanahan et al. 2007; Boyter et al. 2009; Mosseler et al. 2014*a*, 2014*b*, 2014*c*), and to explore the use of native, as opposed to, introduced willows.

Canada has 76 native willows (Argus 2010), yet very little is known about genetic variation among and within North American willows, despite their ecological importance as pioneer colonizers and invaders of highly disturbed sites (Reisch et al. 2007; Grady et al. 2011; Mosseler et al. 2014*a*). Currently, clonal selection

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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 or small tree	Seepage slopes, wetlands, and ditches
S. eriocephala	4–6 m, shrub	Banks of fast-flowing streams
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

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

programs are aimed at identifying faster growing and better adapted planting stock for mine tailings and rock overburden characterized by very poor soil development, low fertility, and low water-holding capacity (Mosseler and Major 2014, 2015, 2016). Most studies aimed at understanding genetic variation and population structure have used various molecular genetic markers (Brunsfeld et al. 1991; Barker et al. 1999; Lian et al. 2003; Palmé et al. 2003; Reisch et al. 2007; Lin et al. 2009; King et al. 2010; Puschenreiter et al. 2010; Mosner et al. 2012; Trybush et al. 2012; Berlin et al. 2014; Perdereau et al. 2014; Huang et al. 2015). Relatively fewer studies have used common-garden field testing to quantify variation among different willow species and subpopulations for growth performance, coppice structure, phenology, or other physical traits that characterize biomass quality (Rönnberg-Wästljung and Thorsén 1988; Telenius and Verwijst 1995; Grady et al. 2011; Mosseler et al. 2014b).

Salix amygdaloides Andersson (AMY), Salix bebbiana Sarg. (BEB), Salix discolor Muhl. (DIS), Salix eriocephala Michx. (ERI), Salix humilis Marshall (HUM), Salix interior Rowlee (INT), and Salix nigra Marshall (NIG) (Table 1) are native to much of eastern and central Canada, with some of these species extending as far west as the Rocky Mountains and beyond (e.g., AMY, BEB, DIS, ERI, and INT). These seven willow species are all associated with seasonally wet areas and riparian zones, but some of them are also adapted to a wider range of ecological conditions and can colonize highly disturbed drier sites. For instance, BEB, DIS, and HUM can be found as natural invaders and colonizers of drier upland areas and of highly disturbed mine sites in eastern Canada (Mosseler et al. 2014a), whereas ERI, INT, and NIG are most commonly associated with the fast-flowing water of riparian habitats, and AMY is most often found along the edges of more stagnant wetlands and swamps. Despite the ability of some willows (e.g., BEB, DIS, HUM, and INT) to grow on sites experiencing seasonal drought and some ability to both acclimate and adapt to water stress (Weih and Nordh 2002; Wikberg and Ogren 2007; Kuzovkina and Volk 2009; Mosseler et al. 2014a), growth performance is likely to be limited by water availability (Larsson 1998; Weih and Nordh 2002; Linderson et al. 2007), especially on former mine tailings and overburden characterized by high proportions of coarse sand and rock, with low moisture-retention capacity (Tordoff et al. 2000; Bussière 2007; Maiti 2007)

The seven willows being tested include both tree-forming species that can reach heights of 15 m across their geographic range in Canada (e.g., AMY and NIG) and shrub-forming species that normally attain heights of 2–6 m (e.g., BEB, DIS, ERI, HUM, and INT). However, natural populations of DIS reaching heights of 10 m have been found in New Brunswick (NB), Canada. These willows were selected as potentially promising species for SRIC woody biomass production based on field observation and past studies (Mosseler et al. 1988) and for land reclamation purposes because some of these willows are among the first colonizers of highly disturbed mine sites (Russell and La Roi 1986; Strong 2000; Bourret et al. 2005; Mosseler et al 2014*a*) and appear to tolerate exposed, low-fertility sites such as the shale rock overburden that dominates coal-mining operations in eastern Canada (Mosseler et al. 2014*a*; Mosseler and Major 2014, 2015).

Our objective was to grow 20 clones from each of seven North American willows using a common-garden, population genetics approach to compare species, populations within a species, and up to five clones within each population to assess genetic variation in biomass yields and several coppice growth traits on two different sites: a coal mine overburden of exposed shale bedrock and a sandy-loam nursery soil used for growing tree planting stock. Understanding genetic variation patterns within species can be an important guide to sampling natural populations when collecting and propagating material for selection of plants suitable for commercial biomass production, land reclamation, and conservation of genetic resources (Graudal et al. 2014). We hypothesized that there might be important differences among species, among natural populations within a species, and among genotypes (clones) within natural populations in biomass growth and yield traits, and we wanted to quantify these differences for the selection of clones with superior growth performance and adaptability to conditions of low soil fertility.

### Material and methods

#### Common-garden experiments

During the winter of 2008, stem sections (cuttings) were collected from five clones from each of four natural populations located in southern and eastern Ontario (ON) and adjacent areas of the Ottawa River Valley in the province of Quebec (QC), Canada (Fig. 1; Table 2). Stem cuttings approximately 20 cm long were collected from vigorous 1- and 2-year-old branches from plants located in natural populations and then stored in a freezer at -5 °C at the Atlantic Forestry Centre (AFC) in Fredericton, New Brunswick (NB), Canada. In preparation for field establishment, stem cuttings were removed from frozen storage to a refrigerator at 3 °C for several days of thawing, followed by 48 h of soaking in water immediately prior to establishment in two common-garden field tests. The study sites were located at the AFC tree nursery (hereafter, AFC) site in Fredericton, NB (45°94'N, 66°62'W), and at the former Salmon Harbour (SH) coal mine site near Minto, NB (46.07°N, 66.05°W). Fredericton and Minto share a similar climate, with an average annual temperature of 5.6 °C and an annual precipitation of 1124 mm (Environment Canada, 2013, http:// climate.weather.gc.ca/index\_e.html).

The AFC site consists of an artificially constructed soil of 60 cm of fine- to medium-textured, washed sand, devoid of stones or rocks, contained within a polyethylene liner to permit experimental leachate collections. This site was covered in a sparse grass sod at the time of willow establishment. Six soil samples were taken in an equidistant grid across this flat, uniform site for a standard soil analysis that included percent sand, silt, clay, and organic matter; percent carbon (C), sulfur (S), and nitrogen (N); available potassium (K), calcium (Ca), and magnesium (K); phosphorus (P) concentration (in ppm); pH; and C:N ratio (Table 3). The SH mine site consisted of the recently landscaped shale rock overburden remaining after coal strip-mining operations. Six soil samples were

Fig. 1. Locations of natural populations sampled for establishment of common-garden studies of seven native North American willows on two sites.



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

Salix species	Population	Population no.	Latitude (N)	Longitude (W)	Elevation (m)
S. amygdaloides	Hanlon Marsh, ON	1	44°52′	75°45′	110
	Long Sault, ON	2	44°60′	74°58′	76
	Port Maitland, ON	3	42°52′	79°35′	179
	Wainfleet Bog, ON	4	42°55′	79°20′	182
S. bebbiana	Achray Road, ON	1	45°49′	77°23′	223
	Fort Coulonge, QC	2	45°59′	76°46′	287
	Foymount, ON	3	45°26′	77°18′	525
	Klock Road, ON	4	46°19′	78°30′	197
S. discolor	Allumette Island, QC	1	45°54′	77°06′	114
	Fort Coulonge, QC	2	45°59′	76°46′	287
	Foymount, ON	3	45°26′	77°18′	525
	Norway Bay, ON	4	45°32′	76°25′	87
S. eriocephala	Allumette Island, QC	1	45°54′	77°06′	114
-	Norway Bay, ON	2	45°32′	76°25′	87
	Wainfleet Bog, ON	3	42°55′	79°20′	182
	Westmeath, ON	4	45°49′	76°54′	112
S. humilis	Achray Road, ON	1	45°49′	77°23′	223
	Aylen Lake, ON	2	45°34′	77°53′	298
	Bonnechere, ON	3	45°40′	77°37′	186
	Klock Road, ON	4	46°17′	78°30′	202
S. interior	Ottawa, ON	1	45°04′	75°32′	100
	Long Sault, ON	2	44°60′	74°58′	76
	Moodie Pond, ON	3	45°14′	75°47′	104
	Wainfleet Bog, ON	4	42°55′	79°20′	180
S. nigra	Nith River, ON	1	43°17′	80°34′	299
0	Pembroke, ON	2	45°50′	77°07′	107
	Wainfleet Bog, ON	3	42°55′	79°20′	173
	Westmeath, ON	4	45°49′	76°54′	103

Note: ON, Ontario; QC, Quebec.

also taken from each site for a standard soil analysis conducted by the Laboratory for Forest Soils and Environmental Quality at the University of New Brunswick. The mine overburden had a much higher proportion of coarser shale rock, with an average of 56.5% stone content that would not pass through a 2 mm sieve.

Both common-garden studies were established in May 2008, using 20 cm long rootless stem cuttings and containing the same

20 clones from each of four natural populations (Table 2) per species (140 clones in total). Clones (genotypes) were replicated in three blocks at each location (AFC and SH) in a randomized complete block test design. Plants in the AFC test were established at 1 m  $\times$  1 m spacing using a three-tree (ramet) linear clonal plot, with each clone replicated once within each of the three blocks (replicates). The SH common garden was established on shale

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Table	3. Soil proper	ties for the tw	o sites: Atlantic	Forestry Centr	e (AFC) and Sa	lmon Harbour (	(SH).						
	Organic			Potassium	Calcium	Magnesium	Phosphorus						
Site	matter (%)	Carbon (%)	Nitrogen (%)	(mdd)	(mqq)	(mdd)	(mqq)	Sand (%)	Silt (%)	Clay (%)	рН	C:N ratio	Sulfur (%)
AFC	0.82±0.40a	0.48±0.23a	0.082±0.017a	55.5±3.3b	266±262b	60.8±6.1b	11.40±2.02a	81.8±2.2a	15.3±2.2b	2.9±1.5b	6.4±0.1a	5.8±0.6b	0.002±0.006b
SH	0.79±0.40a	0.46±0.23a	0.102±0.017a	91.1±13.3a	1466±262a	80.2±6.1a	3.98±2.02b	67.2±2.2b	23.4±2.2a	9.4±1.5a	6.8±0.1a	4.6±0.6b	0.080±0.006a
Not	:: Sites with diffe	erent letters are	significantly differ	tent $\alpha = 0.05$ .									

overburden along a gentle slope, landscaped to minimize soil erosion and surface runoff into an adjacent watercourse. Clonal plots at the SH site were established as linear, five-ramet clonal row plots, with each plant spaced at 0.5 m within the clonal plot and 2 m between each row plot. Thus, each plant on both the AFC and the SH sites had 1 m<sup>2</sup> of growing space. In 2011, the aboveground biomass was harvested at both sites, and in 2013 the 2avar-old compice represents of the largest plant

And in 2013, the 2-year-old coppice regrowth of the largest plant per clonal plot was harvested (September at SH and October at AFC), and the fresh mass of the aboveground biomass was measured in the field to the nearest 10 g using an electronic infant weigh scale (Electronic Infant Scale, model ACS-20A-YE). The number of coppice stems per plant was counted for each harvested plant at both sites. At the AFC site, the length of up to 20 of the largest stems per harvested plant was measured to the nearest 1 cm using a flexible aluminum metre ruler, and the basal diameter of each stem was measured to the nearest 0.1 mm using an electronic caliper on each of up to 20 of the largest stems per plant harvested.

# Statistical analysis

Coppice growth trait data for the seven species were subjected to analyses of variance (ANOVA). Site, species, populations, and clones were all considered as fixed effects to allow us to make specific recommendations regarding them. Populations were nested within species, and clones were nested within populations, which were nested within species. The following ANOVA model was used:

$$Y_{ijklm} = u + B_i + A_j + S_k + AS_{jk} + P_{l(k)} + C_{m(l(k))} + e_{ijklm}$$

where  $Y_{ijklm}$  is the dependent ramet trait of block *i* from site *j* of species *k* of population *l* of clone *m*; *u* is the overall mean;  $B_i$  is the effect of block *i* (*i* = 1, 2, 3);  $A_j$  is the effect of site *j* (*j* = 1, 2);  $S_k$  is the effect of species *k* (*k* = 1, 2, 3, 4, 5, 6, 7);  $AS_{jk}$  is the interaction effect of site *j* and species *k*;  $P_{l(k)}$  is the effect of clone *m* (*m* = 1, 2, 3, 4, 5), nested within species *k*;  $C_m$  is the effect of clone *m* (*m* = 1, 2, 3, 4, 5), nested within species *k*; and  $e_{ijklm}$  is the random error component.

We also analyzed the population structure within each species separately using the following ANOVA model:

$$Y_{ijlm} = u + B_i + A_j + P_l + AP_{jl} + C_{m(l)} + e_{ijlm}$$

where  $Y_{ijlm}$  is the dependent ramet trait of block *i* from site *j* of population *l* of clone *m*; *u* is the overall mean;  $B_i$  is the effect of block *i* (*i* = 1, 2, 3);  $A_i$  is the effect of site *j* (*j* = 1, 2);  $P_1$  is the effect of population l (l = 1, 2, 3, 4);  $AP_{jl}$  is the interaction effect of site j and population *l*;  $C_m$  is the effect of clone *m* (*m* = 1, 2, 3, 4, 5), nested within population l; and  $e_{ijlm}$  is the random error component. The data satisfied normality and equality of variance assumptions. The general linear model from Systat Software Inc. (Chicago, Illinois) was used for analysis. Tukey's post hoc mean separation test was used to assess differences among species and populations. All statistical tests were assessed at a significance level of  $\alpha = 0.05$ . Note that average stem length and average basal diameter traits were only measured on the AFC site; thus their ANOVAs do not have site or interaction with site as sources of variation. The variance component analysis was carried out using the sum of squares as outlined by Hicks (1982, pp. 55-57).

# Results

Both AFC and SH sites had similar pH, organic matter content, C, N, and C:N ratios, but the SH site had significantly higher levels of Ca, K, Mg, and S, and the AFC site had higher P content (Table 3). The AFC site had higher sand content, but the SH site had higher

**Table 4.** Productivity trait ANOVAs for willow coppices established at two sites (AFC and SH), including source of variation, degrees of freedom (df), mean square values (MS), variance component (VC), *P* values, and coefficient of determination (R<sup>2</sup>).

		Green	mass (kg)		Coppice	e stem nu	mber	Average s	ingle stem	ı mass (g)
Source of variation	df	MS	VC (%)	P value	MS	VC (%)	P value	MS	VC (%)	P value
Block	2	0.516	0.8	0.004	45.4	0.5	0.040	274.8	<0.1	0.832
Species	6	6.769	30.5	<0.001	1227.7	38.8	<0.001	21 803.8	10.5	<0.001
Site	1	6.697	5.0	<0.001	521.4	2.7	<0.001	48 816.8	3.9	<0.001
Species × site	6	1.481	6.7	<0.001	148.8	4.7	<0.001	5 172.1	2.5	0.002
Pop.(species)	21	0.841	13.3	<0.001	70.1	7.8	<0.001	2 959.5	5.0	0.006
Clone(pop.(species))	107	0.150	12.0	<0.001	20.3	11.5	0.005	2 677.2	23.0	<0.001
Error	460	0.092	31.7		14.0	34.0		1 490.4	55.0	
R <sup>2</sup>				0.723			0.698			0.478

Note: *P* values <0.05 are in bold type. Pop., population.

silt and clay contents, as well as a much higher content of shale rock (56.5%).

A species comparison of total aboveground green biomass on the two sites showed significant differences among species (P = < 0.001; Table 4; Fig. 2A): ERI showed the greatest aboveground biomass on both sites, followed by INT and DIS and then a grouping of BEB and HUM. The least productive were the tree willows AMY and NIG. The productivity of the AFC site was twice that of the SH site, with an overall average of 0.56 and 0.28 kg aboveground biomass, respectively. The significant species × site interaction for aboveground biomass (P < 0.001) was a result of magnitude (no rank changes between sites) differences. All species showed better productivity on the AFC site; the significant interaction indicates that some species grew much better on the AFC site, where ERI and INT showed 2.4× and 2.7× higher productivity than on the SH site.

The coppice stem number showed a similar pattern to that of aboveground biomass described previously (Fig. 2B); however, clone numbers were much more similar between sites, with an overall average number of 7.8 and 5.3 for AFC and SH, respectively. ERI had by far the greatest stem number, with an average of 15 stems per coppice; one ERI coppice at the AFC site produced 67 stems. Next were a group of four willows, INT, DIS, HUM, and BEB, with an average of between five and six stems per coppice. The tree willows AMY and NIG had the lowest number of stems, with an average of 4.1 and 3.4 stems per coppice, respectively. The significant species × site interaction was again largely a result of magnitude differences among species. AFC had greater stem numbers for all species except AMY, which had the same number of coppice stems on both sites.

Average single stem mass was 76.3 g and 52.2 g for the AFC and SH sites, respectively (Table 4; Fig. 2C). Average single stem mass was greatest for DIS at 86.8 g, followed by INT and BEB at 73.0 g and 71.0 g, respectively, and was lowest for NIG at 34.9 g. A significant species × site interaction was once again due to magnitude effects as there were no rank changes between sites for any of these species. The greatest difference in average stem mass was demonstrated by INT at a factor of 2.2 between the SH to AFC sites.

The variance component analysis for green mass, coppice stem number, and average single stem mass was significant for sites (all P < 0.001). All three traits showed that site variance accounted for between 2.7% and 5.0% of the total variation in these traits (Table 4). Species differences were also significant for these three traits and accounted for between 10.5% and 38.8% of the total variance. The effect of populations (nested within species) was also significant for each of these three traits, accounting for between 5.0% and 13.3% of the total variation in these traits. Clonal variation (nested within population and species) was also significant for all three traits and accounted for between 11.5% and 23.0% of the total variation. The species × site interaction was again **Fig. 2.** (A) Total aboveground biomass, (B) number of stems, and (C) single stem mass for 2-year-old coppiced plants from seven willow species on two sites (AFC tree nursery and SH mine site). Different letters among species indicate significant differences according to Tukey's mean separation test,  $\alpha = 0.05$ .



		AMY		BEB		DIS		ERI		HUM		INT		NIG	
Source of variation	df	VC (%)	P value												
Block	2	1.2	0.518	9.9	0.047	1.3	0.467	1.3	0.207	2.5	0.342	8.8	0.001	1.8	0.315
Site	1	2.0	0.150	8.8	0.020	3.3	0.049	23.6	<0.001	6.4	0.022	30.0	<0.001	19.8	<0.001
Pop.	3	0.5	0.915	0.6	0.799	4.9	0.124	28.1	<0.001	3.0	0.462	1.7	0.404	1.1	0.686
Site × Pop.	3	8.0	0.043	2.3	0.455	6.9	0.047	7.3	0.001	5.0	0.234	3.6	0.104	2.7	0.327
Clone(Pop.)	15	30.6	0.031	42.7	0.018	29.0	0.026	10.5	0.039	36.7	0.018	10.7	0.293	17.2	0.136
Error	74	57.7		35.6		54.5		29.0		46.4		46.2		57.4	
R <sup>2</sup>		0.	407	0.	642	0.	451	0.	731	0.	598	0.	565	0.	422

**Table 5.** Aboveground green mass (kg) ANOVAs by willow species, including source of variation, degrees of freedom (df), variance component (VC), P values, and coefficient of determination (R<sup>2</sup>).

**Note:** BEB had only three populations on the mine site. *P* values <0.05 are in bold type. Pop., population.

**Table 6.** Coppice stem number ANOVAs by willow species, including source of variation, degrees of freedom (df), variance component (VC), P values, and coefficient of determination ( $R^2$ ).

		AMY		BEB		DIS		ERI		HUM		INT		NIG	
Source of variation	df	VC (%)	P value												
Block	2	1.9	0.370	5.6	0.039	4.8	0.057	<0.1	0.983	3.6	0.298	12.9	<0.001	4.2	0.083
Site	1	<0.1	0.928	9.4	0.002	9.8	0.001	18.1	<0.001	2.7	0.182	6.2	0.003	11.5	<0.001
Pop.	3	14.0	0.004	2.7	0.189	3.6	0.225	14.9	<0.001	5.2	0.325	5.8	0.038	4.6	0.141
Site × Pop.	3	1.5	0.669	6.2	0.028	1.7	0.546	4.3	0.109	6.8	0.211	2.8	0.245	2.2	0.441
Clone(Pop.)	15	24.8	0.102	57.2	<0.001	27.3	0.031	14.1	0.114	22.3	0.385	18.9	0.043	17.8	0.141
Error	74	57.8		18.9		52.7		48.5		59.4		53.4		59.8	
R <sup>2</sup>		0.4	405	0.	836	0.	489	0.	556	0.4	467	0.4	466	0.	410

Note: BEB had only three populations on the mine site. P values <0.05 are in bold type. Pop., population.

**Table 7.** Average coppice stem mass (g) ANOVAs by willow species, including source of variation, degrees of freedom (df), variance component (VC), P values, and coefficient of determination ( $R^2$ ).

		AMY		BEB		DIS		ERI		HUM		INT		NIG	
Source of variation	df	VC (%)	P value												
Block	2	0.3	0.867	5.9	0.291	8.2	0.004	1.1	0.517	0.7	0.682	0.5	0.634	0.1	0.955
Site	1	4.6	0.041	0.5	0.624	0.7	0.312	9.0	0.002	10.0	0.002	30.2	<0.001	4.6	0.040
Pop.	3	3.0	0.431	2.6	0.518	10.5	0.003	12.0	0.004	5.1	0.142	1.6	0.403	2.3	0.534
Site × Pop.	3	5.0	0.205	1.1	0.794	7.3	0.019	1.2	0.701	5.4	0.125	2.2	0.264	1.5	0.704
Clone(Pop.)	15	20.7	0.340	32.5	0.287	28.6	0.006	18.2	0.085	42.7	0.001	21.4	0.004	14.8	0.515
Error	74	66.3		57.3		44.7		58.5		36.2		44.1		76.8	
R <sup>2</sup>		0.3	349	0.	456	0.	532	0.	425	0.	612	0.	585	0.	236

Note: BEB had only three populations on the mine site. *P* values <0.05 are in bold type. Pop., population.

significant for all three traits, accounting for between 2.5% and 6.7% of the total variation.

The patterns of population variation in average total aboveground green mass (Table 5), average coppice stem number (Table 6), and average single stem mass (Table 7) at both sites are shown in Figs. 3, 4, and 5, respectively, for each of the seven willow species. Among the seven willows studied, only ERI showed significant differences among natural populations and produced by far the highest total aboveground green mass, with clones from the Wainfleet Bog, in southern ON, producing the highest yields (Table 5). ERI also produced by far the highest number of 2-year-old coppice stems per plant among these seven species. However, AMY, ERI, and INT all showed significant differences among populations for coppice stem number (Table 6). Once again, the Wainfleet Bog population had a significantly greater number of stems than other ERI populations. One particular clone (clone WAI-E1m) from the Wainfleet Bog population has consistently shown superior results across several different field tests. Only DIS and ERI showed significant differences in average single stem mass among populations (Table 7), with the DIS population at Allumette Island, QC, producing stems with the largest average stem mass. All species, with the exception of INT and NIG, showed significant clonal variation in aboveground green mass (Table 5). Only BEB, DIS, and INT showed significant variation in coppice stem number (Table 6), and only DIS, HUM, and INT showed significant clonal variation for average coppice stem mass (Table 7).

The ANOVA for the more intensive study of coppice traits at the AFC site included measurements of stem basal diameter and stem length on up to 20 stems per coppiced plant that showed significant genetic differences (P < 0.001) at each level of the genetic hierarchy among species, among populations within species, and among genotypes (clones) within populations (Table 8). The species effect accounted for 9.6% and 26% of total variation in average basal stem diameter and average stem length, respectively. The effect of populations (nested within species) accounted for between 9.5% and 10.0%, and clonal variation accounted for 34.1% and 35.1% % of total variation in average stem basal diameter and average stem length, respectively. The only species showing significant differences in average stem length at the population level were AMY, DIS, and ERI (Table 9). The INT populations were remarkably uniform for this trait but produced the longest stems on average (Fig. 6). Only DIS and ERI showed significant differences in average stem basal diameter at the population level, with the DIS

**Fig. 3.** Population variation in average total aboveground (AG) green mass (kg) over two sites for 2-year-old coppiced plants from seven willow species: *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). For each species, the presence of a P value indicates significant differences among populations. Population (Pop.) number links to locations are found in Table 2.



**Fig. 4.** Population variation in average stem number over two sites for 2-year-old coppiced plants from seven willow species: *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). For each species, the presence of a P value indicates significant differences among populations. Population (Pop.) number links to locations are found in Table 2.



populations at Foymount, ON, and from Allumette Island in the Ottawa River (along the border between QC and ON) producing populations with the largest diameter stems (Fig. 7). With the exception of NIG, all other species showed significant clonal variation in coppice stem length (Table 9). Only BEB, HUM, and INT showed significant clonal variation in basal stem diameter (Table 10).

# Discussion

Coppice structure in willows can be a defining characteristic of the species (Mosseler and Major 2014, 2016). The two tree willows, AMY and NIG, had the lowest biomass yields and the fewest stems **Fig. 5.** Population variation in average stem mass (g) over two sites for 2-year-old coppiced plants from seven willow species: *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). For each species, the presence of a *P* value indicates significant differences among populations. Population (Pop.) number links to locations are found in Table 2.



per coppice. The shrub willows generally showed much more prolific stem sprouting following harvesting of the aboveground plant and higher biomass yields, with ERI producing by far the largest coppices with the highest number of coppice stems. The greatest response to site differences was also shown by ERI and INT, which had much better growth on the better quality sandyloam AFC site than on the rocky shale overburden of the former SH coal mine site. In contrast, AMY, DIS, and NIG usually showed the least differentiation in growth and coppice structure in relation to site quality. In their species comparison of coppicing in three willows, Sennerby-Forsse and Zsuffa (1995) also found that shrub willows such as ERI and *Salix viminalis* (VIM) had a more prolific stem sprouting habit than AMY, a tree willow.

Despite the importance of biomass yields and coppice growth traits such as stem number, average stem mass, stem diameter, and stem length for woody biomass production systems, very little is known about the pattern and distribution of genetic variation of these important traits among and within North American willow species. Although we found large and significant differences both among species and among clones within species for these physical traits, population differences were surprisingly small for most species, suggesting that extensive gene flow among populations may be minimizing genetic structure at the population level. Alternatively, genetic selection within populations and (or) genetic drift among geographically distributed populations may not be strong enough to create a more pronounced population genetic structure for the quantitative traits assessed in our study (Loveless and Hamrick 1984; Ellstrand and Elam 1993; Alvarez-Buylla and Garay 1994).

Willows are largely insect pollinated (Faegri and van der Pijl 1966; Kevan 1972; Argus 1974; Sacchi and Price 1988; Karrenberg et al. 2002*b*; Füssel et al. 2007; Dötterl et al. 2014). Population genetic structure could be influenced by the shorter distances normally associated with insect-mediated pollen flow, and this is particularly true for the more limited foraging neighborhoods of smaller bee species (Loveless and Hamrick 1984; Gathmann and Tscharntke 2002; Greenleaf et al. 2007; Osborne et al. 2008; Zurbuchen et al. 2010; Wright et al. 2015). Although some larger bee species can travel longer distances of more than a kilometre,

**Table 8.** Average coppice stem basal diameter and stem length ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), variance component (VC), P values, and coefficient of determination ( $R^2$ ) for populations of seven willow species established at the AFC nursery site.

		Average	stem lengt	h (m)	Average	Average basal diameter (mm)					
Source of variation	df	MS	VC (%)	P value	MS	VC (%)	P value				
Block	2	0.071	0.4	0.174	4.60	0.4	0.337				
Species	6	1.444	26.0	<0.001	34.30	9.6	<0.001				
Pop.(species)	21	0.158	10.0	<0.001	9.67	9.5	0.001				
Clone(pop.(species))	103	0.144	35.1	<0.001	7.09	34.1	0.001				
Error	236	0.040	28.5		4.21	46.3					
R <sup>2</sup>				0.724			0.545				

Note: *P* values <0.05 are in bold type. Pop., population.

**Table 9.** Average coppice stem length (m) ANOVAs by willow species, including source of variation, degrees of freedom (df), variance component (VC), P values, and coefficient of determination ( $R^2$ ) for the AFC site only.

		AMY		BEB		DIS		ERI		HUM		INT		NIG	
Source of variation	df	VC (%)	P value												
Block	2	2.7	0.287	0.8	0.768	12.4	0.004	5.4	0.040	2.6	0.383	0.1	0.945	7.0	0.103
Pop.	3	15.6	0.006	6.7	0.239	13.2	0.009	42.1	<0.001	4.6	0.337	2.5	0.381	4.2	0.489
Clone(Pop.)	16	48.2	0.005	50.6	0.023	40.6	0.009	30.1	0.003	54.4	0.007	70.2	<0.001	38.1	0.084
Error	32	33.5		41.9		33.9		22.4		38.3		27.3		50.6	
R <sup>2</sup>		0.	670	0.	574	0.	664	0.	776	0.	620	0.	725	0.	488

Note: P values <0.05 are in bold type. Pop., population.

**Fig. 6.** Population variation in average coppice stem length from the AFC site for 2-year-old coppiced plants from seven willow species: *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). For each species, the presence of a *P* value indicates significant differences among populations. Population (Pop.) number links to location are found in Table 2.



**Fig. 7.** Population variation in stem basal diameter from the AFC site for 2-year-old coppiced plants from seven willow species: *Salix amygdaloides* (AMY), *S. bebbiana* (BEB), *S. discolor* (DIS), *S. eriocephala* (ERI), *S. humilis* (HUM), *S. interior* (INT), and *S. nigra* (NIG). For each species, the presence of a *P* value indicates significant differences among populations. Population (Pop.) number links to locations are found in Table 2.



foraging ranges and foraging neighborhoods for most insect pollinators may be limited to several hundred metres when food patches are close at hand.

The willows collected for this study originated from very small, patchy populations, normally consisting of less than 10 individuals. Such small, patchy populations were also described by Argus (1974). Limited interpopulation gene flow via insect pollination (Lin et al. 2009) could be counterbalanced by the expected effects of long-distance seed dispersal by wind, for which the very light, tufted seeds characteristic of willows are specifically adapted. We have estimated that individual 2-year-old coppice plants of ERI from the AFC common garden were capable of producing several million seeds annually (A. Mosseler, unpublished data). Such high annual seed production and the potential for long-distance dispersal could result in extensive interpopulation gene flow. However, willow seeds require a moist, exposed mineral seedbed for germination and early seedling growth (Karrenberg and Suter 2003). Most of the small patches of willow populations from which we collected our plant material were even-aged and would probably have been established either simultaneously or over very short

		AMY		BEB		DIS		ERI		HUM		INT		NIG	
Source of variation	df	VC (%)	P value												
Block	2	2.7	0.456	1.0	0.739	13.4	0.004	10.5	0.040	1.6	0.549	1.1	0.636	2.1	0.554
Pop.	3	12.6	0.074	0.9	0.911	19.1	0.002	21.9	0.006	3.8	0.408	5.5	0.231	4.0	0.535
Clone(Pop.)	16	31.8	0.319	50.3	0.045	31.0	0.063	23.9	0.235	57.3	0.004	50.3	0.010	31.6	0.326
Error	32	52.9		47.7		36.6		43.8		37.3		43.0		62.3	
R <sup>2</sup>		0.	487	0.	521	0.	638	0.	562	0.	619	0.	560	0.	371

**Table 10.** Average coppice stem basal diameter (mm) ANOVAs by willow species, including source of variation, degrees of freedom (df), variance component (VC), P values, and coefficient of determination ( $R^2$ ) for the AFC site only.

Note: P values <0.05 are in bold type. Pop., population.

time spans of no more than several years following a local site disturbance. Furthermore, our observations over many years of willow field-test establishment have demonstrated that willows are very poor competitors with the many herbaceous species that quickly invade, colonize, and capture the highly disturbed seedbeds required for willow establishment (Karrenberg et al. 2002a). The patchiness and small patch sizes characteristic of willows suggest that local populations may be of very limited genetic origin, perhaps often originating from a single female parent. This could promote genetic drift and strong population genetic structure, and the potential for extensive gene flow may have little effect in reducing population genetic structure in willows that form such small, patchy populations arising in response to very localized disturbance events. For typical riparian species such as ERI, INT, and NIG and European willows such as Salix alba, Salix fragilis, and VIM, associated with larger, fast-flowing river systems, vegetative propagation via rooting of broken stems and branches flowing along river systems could also promote interpopulation gene flow (Brunsfeld et al. 1991; Shafroth et al. 1994; Karrenberg et al. 2002b: Mosner et al. 2012).

Most of the seven willows studied here, with the exception of ERI, showed relatively little differentiation among populations for quantitative variation in the physical traits that we studied (Figs. 3-7). Molecular-based studies have shown a comparatively high allelic and genotypic diversity in ERI, but most of this genetic variation resides within subpopulations, and genetic structure among populations of ERI and many other willows is comparatively weak (Brunsfeld et al. 1991; Purdy and Bayer 1995; Lascoux et al. 1996; Aravanopoulos et al. 1999; Triest et al. 2000; Palmé et al. 2003; Lin et al. 2009). Therefore, the high variation among populations of ERI observed in this study may be an anomaly for which we have no good explanation. Nevertheless, with the exception of ERI, the observed pattern of quantitative trait variation indicates that selection at the species and clonal levels may provide the best sources of variation for clonal selection and breeding purposes. For instance, most species show high genetic variation at the clonal level within populations (Tables 4–10).

The variance components analysis from the site comparison study (e.g., AFC vs. SH) showed that site differences, although statistically significant, were not great, accounting for only 3%–5% of total variation in biomass yield and coppice structure traits (but see Larsen et al. 2014). Genetic differences attributed to variation among species (10%–39%), populations (5%–13%), and clones (12%– 23%) generally accounted for much higher proportions of the total variation in these traits, suggesting that willows may be less responsive to site differences than might be expected, or that the genetic components of variation were much higher and overwhelmed the significant site variation.

The variance components analysis of average basal stem diameter and average stem length in coppiced plants at the AFC site, which considered only genetic sources of variation, also demonstrated the importance of both the species (26%–34%) and the clonal (34%–35%) components of variation compared with the relatively small population component (9%–10%). Some of the superior clone selections such as clone WAI-E1m mentioned earlier and clone VIM-5027 (often used as a check clone in our field tests) are consistently high performers regardless of site quality and illustrate the importance of clone selection. Although we often found species × site interactions, they were all a result of magnitude effects, not changes in rank. Practical experience with growing clones of VIM, introduced into Canada from the Swedish willow breeding program (Gullberg 1993), has demonstrated that VIM clones do comparatively well on a wide variety of sites across eastern and central Canada (Labrecque and Teodorescu 2005; Guidi Nissim et al. 2013; Mosseler et al. 2014*a*; Mosseler and Major 2014), and some of our clone selections from native North American willows also demonstrate this feature of wide adaptability across a variety of different site types.

#### Conclusions

Population genetic structure reflects the state of genetic diversity within species and is an important consideration for the conservation and use of genetic resources. Variance components analysis based on common-garden field studies of coppice biomass trait variation can also have important implications for sampling natural willow populations for clonal selection, genetic improvement breeding, and assisted migration of species affected by global change, as noted by Grady et al. (2011). In willows, our field studies have demonstrated that proper species testing and selection are most likely to result in the greatest genetic gains in growth and yield traits important both for commercial biomass production and for selecting superior plant material adapted to specific site conditions for ecological purposes such as land reclamation and habitat creation. Clonal selection within willow populations can also result in large genetic gains in traits of interest (Larsen et al 2014; Mosseler et al. 2014a, 2014b; Mosseler and Major 2014). However, genetic gains from selection at the population level may be more modest, but potentially important nonetheless, as demonstrated in ERI.

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