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Genetic by environment interactions of two North American *Salix* species assessed for coppice yield and components of growth on three sites of varying quality

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Abstract

Key message **Field testing of different willow species, and promising genotypes within a species, can maximize biomass yield and quality traits due to strong genotype by environment interactions.**

Abstract Coppice yield and components of growth were quantified in eight clones of two widely distributed North American willows, *Salix discolor* (DIS) and *S. eriocephala* (ERI), in common-garden field tests on three sites of varying quality. Both species and clones within species varied significantly across different sites and interacted with site for plant yield and components of growth traits. At the species level, ERI was significantly more productive than DIS on the two most productive sites (85 and 57 % greater, respectively), but on the poorest site, a shale coal mine overburden, species ranking was reversed, with DIS showing a 60 % greater biomass yield than ERI. These sites had similar mean temperature, growing degree days, and seasonal precipitation. Site quality differences were thus most probably driven by soil nutrients, physical traits, and water-holding capacity quantified by 13 soil properties, 12 of which were significantly different and showed fairly consistent ranking among sites. At the clonal level, growth trends and differences were mostly consistent among clones across the three test sites, with the exception of one clone of DIS and to a lesser extent a clone of ERI, which showed abnormally strong clone by site interaction for specific growth traits. Productivity reached as high as 6.0 kg green mass in 2-year-old coppices for a clone from each of DIS and ERI on the most productive site. The strong expression of genetic by environment interactions at both the species and clonal levels suggests that biomass production can be optimized by taking advantage of such interactions and highlights the need for testing not only different species, but also a number of clones within a species before selecting clones for biomass production on different site types. Our results highlight variation in coppice form and the potential for genetic selection both among and within species.

Keywords Willows · Species and clone by site interaction · Components of growth traits · Site quality · Coppice yield

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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; Volk et al. 2006), with growing concerns over the impact of carbon emissions on climate warming (Goodale et al. 2002; Houghton 2005; Harper et al. 2007; Intergovernmental Panel on Climate Change (IPCC) 2007), and with the search for various environmental applications (Kuzovskina and Quigley 2005). With more than 350 species worldwide, willows are widespread across the northern hemisphere; Canada has 76 native willows (Argus 2010). Willows are widely distributed across every region of Canada and are adapted to a great range of site conditions. Yet, despite a rich species diversity and ecological importance, native North American willows have received limited attention as a potential biomass resource (Mosseler et al. 1988, 2014b; Kopp et al. 2001; Labrecque and Teodorescu 2005; Tharakan et al. 2005) or for land reclamation following disturbances such as mining operations (Chosa and Shetron 1976; Mosseler et al. 2014a).

Salix discolor (DIS) and *S. eriocephala* (ERI) are widespread across eastern and central Canada and appear promising as sources of biomass production (Mosseler et al. 1988). Most willows are associated with various types of wetlands and riparian zones across the northern hemisphere, where they provide important wildlife habitat (Densmore et al. 1987; Singer et al. 1994; Hood and Bayley 2009), but there are also willows adapted to well-drained and drier upland sites (Kuzovkina and Volk 2009). Both DIS and ERI are commonly found in wet areas on a wide variety of disturbed sites, but DIS is also commonly found colonizing drier, upland sites and on the highly disturbed coal mine spoils investigated here for reclamation purposes (Mosseler et al. 2014a). ERI is more commonly associated with disturbed stream banks adjacent to fast-flowing water. The primary requirements for willow establishment from seed are a moist seedbed of exposed mineral soil and full sunlight for optimal seedling growth following seed germination. The same requirements pertain to willow established from stem cuttings, but cuttings offer a more reliable means of artificial establishment for ecological restoration activities (Chosa and Shetron 1976).

Eight selected DIS and ERI clones were field tested on three sites that varied widely in soil characteristics, but shared similar climatic conditions to explore genetic responses to different sites and genetic by site interactions at two genetic levels: species and genotype within species. One of the few attempts to study genetic by environment interactions in willows was for the European species *S. viminalis* (Ronnberg-Wastljung and Thorsen 1988), which is now also commonly used in bioenergy plantations in Canada (Guidi-Nissim et al. 2013). Our objective was to quantify and understand variability in yield and components of growth of promising native willows across three sites of varying quality to assess both the economic viability of willow biomass production and the restoration value of various species, assist in making genetic selections of superior clonal material for operational biomass production, and model growth and yield.

Materials and methods

Common-garden experiments

In 2005, a common-garden experiment was established at the Montreal Botanical Gardens (MBG) in Montreal, Quebec, Canada (Lat. 45°56′N, Long. 73°57′W) that included six clones collected from each of 12 natural populations of DIS and ERI distributed across New Brunswick (NB), Quebec (QC), and Ontario (ON), for a total of 144 clones (72 clones per species) to assess population genetic variation in these two willows. In subsequent years, a selection of some of the better-performing clones from this MBG common-garden experiment were used to establish common-garden experiments at several locations in eastern Canada: at the MBG site described above and in NB at the Atlantic Forestry Centre experimental tree nursery (AFC) in Fredericton (Lat. 45°94′N, Long. 66°62′W) and on shale overburden at the nearby Salmon Harbour coal mine (SH) near Minto (Lat. 46°07′N, Long. 66°05′W), formerly operated by NB Coal Ltd. (a subsidiary of the local electrical power utility NB Power). The eight clones were selected because they had demonstrated good survival and productivity across several field tests and they were common to all three sites selected for this study (Table 1). These three common-garden sites have average annual temperatures of 5.8, 5.6, and 5.7 °C and annual precipitation of 1,046, 1,124, and 987 mm for MBG, AFC, and SH, respectively (Environment Canada 2013). Long-term 30-year averages for growing degree days (GDD above 5 °C) were 2098 GDD and 2080 GDD for Montreal and Fredericton, respectively (www.farmzone.com). Due to the prevailing westerly winds, the seasonal distribution of precipitation in the Fredericton area is similar to that of Montreal.

The MBG common-garden was established in 2005 on a deep, fertile loam soil that had been prepared for horticultural demonstrations and was lying fallow and covered with sod at the time of site preparation for willow establishment. The AFC site has an artificially constructed soil consisting of a 60-cm depth of fine-to-medium-textured sand contained within a polyethylene liner to permit experimental leachate collections. This site was covered in a thin grass sod at the time of willow establishment. The SH site consisted of crushed or broken shale coal mine overburden with very little organic matter and no soil development because the site had recently been bulldozed into a gently sloping terrain to minimize surface runoff and erosion into an adjacent watercourse following cessation of surface strip mining for coal. Soil analysis was based on six soil samples taken from the top 15 cm of the mineral soil in an equidistant grid pattern to provide a uniform or systematic coverage at each of these three sites and indicated

Table 1 Origins of clones of *Salix discolor* and *S. eriocephala* used in coppice biomass measurements

Species	Clone	Origin of clone	Latitude N	Longitude W
S. discolor	LEV-D3	Levis, QC	46°78′	71°18′
	MON-D1	Montmagny, QC	46°94′	70°60′
	MUD-D4	Mud Lake (Westmeath), ON	45°88′	76°78′
	RIC-D2	Richmond Fen, ON	45°13′	75°82′
S. eriocephala	BRI-E2	Bristol, NB	46°47′	67°58′
	FRE-E1	Fredericton, NB	45°94′	66°62′
	GRE-E1	Green River, NB	47°34′	68°19′
	SHE-E3	Shepody Creek, NB	45°71′	64°77′

Table 2 Soil properties for the three sites; Atlantic Forestry Centre (AFC), Montreal Botanical Gardens (MBG) and Salmon Harbour (SH) coal mine

Site	Organic matter (%)	Carbon (%)	Nitrogen (%)	Potassium meq 100 g^{-1}	Calcium meq 100 g^{-1}	Magnesium meq 100 g^{-1}	Phosphorus (ppm)
AFC	0.82 ± 0.40 b	0.48 ± 0.23 b	0.082 ± 0.017 b	0.142 ± 0.034 b	1.33 ± 1.31 c	0.50 ± 0.05 a	11.40 ± 2.02 b
MBG	5.72 ± 0.40 a	3.32 ± 0.23 a	0.303 ± 0.017 a	0.393 ± 0.034 a	18.15 ± 1.31 a	0.62 ± 0.05 a	40.69 ± 2.02 a
SH	0.79 ± 0.40 b	0.46 ± 0.23 b	0.102 ± 0.017 b	0.233 ± 0.034 b	7.33 ± 1.31 b	0.66 ± 0.05 a	3.98 ± 2.02 c

Site	Sand (%)	Silt (%)	Clay (%)	pH	C:N ratio	Sulfur (%)
AFC	81.8 ± 2.2 a	15.3 ± 2.2 c	2.9 ± 1.5 b	6.4 ± 0.1 b	5.8 ± 0.6 b	0.002 ± 0.006 b
MBG	50.6 ± 2.2 c	34.9 ± 2.2 a	14.5 ± 1.5 a	7.3 ± 0.1 a	10.8 ± 0.6 a	0.019 ± 0.006 b
SH	67.2 ± 2.2 b	23.4 ± 2.2 b	9.4 ± 1.5a	6.8 ± 0.1 ab	4.6 ± 0.6 b	0.079 ± 0.006 a

Sites with different letters are significantly different using Tukey's mean separation test, $\alpha = 0.05$

significant site differences in fertility and physical quality among sites, with the MBG site having the greatest percentage of total organic matter, total carbon (C), and total nitrogen (N), and the highest available potassium (K), calcium (Ca), and phosphorus (P) (Table 2). Soil analyses were conducted according to McKeague (1978) as follows: available P—#TP-CSS-MSSA 4.41 (sodium bicarbonate extraction), exchangeable cations—#TP-CSS-MSSA 4.5, FCMM 15 (ammonium acetate extraction), pH—#TP-CSS-MSSA3.13 (pH in 1:1 water), texture—#TP-CSSMSSA 2.12 (hydrometer method), organic matter, N, C and S—#TP-LFIM (total C by LECO induction furnace). AFC had the highest sand but lowest silt and clay content, whereas MBG had the lowest sand but highest silt and clay content. Soil samples from SH contained an average of 56.5 % stones that would not pass through a 2-mm sieve; the AFC and MBG sites had few stones.

Stem cuttings were collected from early to mid-December from the original MBG field test established in 2005 and stored at −5 °C until May when cuttings were removed from frozen storage and placed at refrigeration temperatures of 3–5 °C for at least 1 week (Densmore and Zazada 1978). At planting time, the cuttings were separated by clone in plastic bags and soaked in water for 48 h prior to transportation to the field for the day of planting. Both the AFC and SH common-garden experiments were established in 2008, and the eight clones of DIS and ERI were replicated in three blocks (replicates). Willows were established with unrooted stem cuttings without the use of plant rooting hormones and were approximately 20 cm in length. At SH, each genotype (clone) was represented by a single five-tree (ramet) linear row plot in each of three replicates, and ramets were spaced at 0.5 m within rows spaced 2 m apart, providing approximately 1 m^2 of growing space per plant. Plants at AFC and MBG were established as a single three-ramet linear row plot within each of three replicates, and plants were established at 1 m × 1 m spacing between plants. The AFC and MBG tests were surrounded by a border row to avoid edge effects on plant growth. The SH mine site was established as a linear ribbon that followed the waterline of a watercourse that drained the mine site. Knowledge from previous studies (Mosseler et al. 2014a, b) showing that the SH site had poor growth potential indicated that inter-plant competition would be minimal and that the site did not require border rows to minimize edge effects at the plant spacing used in this study.

In 2011, the aboveground biomass was harvested in each of the three common gardens (MBG, AFC, and SH), leaving one plant (ramet) in each row plot at AFC and SH unharvested for other (e.g., flowering and pollination) studies. In the fall of 2013, the 2-year-old coppice growth (both dead and live stems) of the first ramet in each plot was selected for harvesting, provided that the ramet was representative of the plot and had not been obviously affected by herbivory. The green mass was measured in the field to the nearest 10 g using an electronic weigh scale (Electronic Infant Scale, model ACS-20A-YE). Previous measurements of fresh and dry mass taken at the MBG common garden demonstrated that percent moisture content was approximately 50 ± 2 % regardless of species or clones (Mosseler, unpublished). The number of stems (both dead and live) per coppice was counted for each harvested plant. The length of up to 20 of the largest stems per coppice was measured to the nearest 1 cm using an aluminum meter ruler, and the basal diameter of each coppice stem was measured at the base of each stem to the nearest 0.1 mm using an electronic caliper on each of the 20 largest stems per coppice.

Statistical analysis

The data for each common-garden field test were subjected to analysis of variance (ANOVA) in which site, species, and clones were considered fixed effects. With one replicate per block, block interactions in the ANOVA model would result in no separate estimate of error variance. In such cases, it is customary to pool the block interaction terms to serve as the error variance (Hicks 1982, p: 254). We did examine species x block (site) using clone (species)*block (site) as the only blocking error term but found no significant species × block (site) effects. Thus, we also incorporated species x block (site) into the error term. The ANOVA model used is as follows:

$$Y_{ijklm} = u + B_{i(j)} + T_j + S_k + \mathrm{C}_{l(k)} + T_jS_k + T_jC_{l(k)} + e_{ijklm},$$

where Y_{ijklm} is the dependent ramet trait of the ith block, of the jth site, of the kth species, of the lth clone of the mth ramet, and u is the overall mean. B_i is the effect of the ith block ($i = 1, 2, 3$), T_j is the effect of the jth site ($j = 1, 2, 3$), S_k is the effect of the kth species ($k = 1, 2$), $C_{l(k)}$ is the effect of the lth clone ($l = 1, 2, 3, 4$) nested within the kth species, and T_jS_k is the effect of the site by species interaction, $T_jC_{l(k)}$ is the effect of the site by clone nested within species interaction, and e_{ijklm} is the random error component. Results were considered statistically significant at $\alpha = 0.05$, although individual P values are provided for all traits so that readers can make their own interpretations. The data had satisfied normality and equality of variance assumptions. The general linear model from Systat (Chicago, Illinois) was used for analysis.

Results

Both species and clones differed significantly for most biomass traits assessed: number of stems per coppice, coppice stem length, average single stem mass, and plant biomass yield (Table 3). Growth trends were also consistent across the different sites with no rank change (Figs. 1, 2, 3) and appeared to reflect site quality, with MBG producing the greatest, SH the least, and AFC intermediate yield (Fig. 1), numbers of stems per coppice (Fig. 2a), stem biomass (Fig. 2b), average stem length (Fig. 3a), and average stem diameter (Fig. 3b). Plant yield showed a significant species by site interaction (Table 3). The pattern of biomass yield (Fig. 1) showed ERI as significantly more productive than DIS on the most productive site (85 % greater on MBG) and less so on the intermediate site (57 % greater on AFC), but on the SH coal mine overburden, species rank was reversed, with DIS showing a 60 % greater biomass yield than ERI and thus, the significant species by site interaction. The average yield was 4.55 kg for ERI on the MBG site and 0.16 kg for ERI on the SH site, almost a 30-fold difference. Number of stems ANOVA also showed significant species, site, and species by site effect (Table 3); however, the interaction was due to magnitude effects, as there was no species rank change by site (Fig. 2a). ERI had a greater number of stems than DIS at MBG and AFC (approximately 2.5 times greater), whereas on the least productive site at SH, ERI had only 1.5 times the number of stems per coppice. The highest average number of stems per coppice was 19 for ERI on the MBG site, with DIS having the lowest average of 4.5 stems per coppice on both the AFC and SH sites.

At the species level, average single stem mass ANOVA showed significant species and site effects but a non-significant species by site interaction (Table 3). DIS had greater average stem mass than ERI, with an average difference of 0.06 kg (Fig. 2b). Average stem length ANOVA showed significant species and site effects (Table 3). Although there was a small and insignificant rank change (species by site, $P = 0.081$), DIS and ERI had on average 1.7 and 1.6 m average stem length over the three sites (Fig. 3a). The average stem lengths were 2.4, 1.7 and 0.8 m for MBG, AFC, and SH, respectively. Average stem diameter had no species or species by site effect, but the site effect was significant (Table 3). Average stem diameter was the only trait that did not show a species effect. Average stem diameters were 1.66, 1.54, and 0.86 cm for MBG, AFC, and SH, respectively (Fig. 3b).

Growth results on a clonal basis (Figs. 4, 5, 6, 7, 8) reflected the general growth results and trends described at the species level but also showed significant variation among clones within each species for all growth traits (Table 3) and some large and significant clone by site

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)

Source of variation	*df*	Green mass yield (kg)		Number of stems		Average single stem mass (kg)	
		MS	*P* value	MS	*P* value	MS	*P* value
Block (site)	6	0.62	0.270	15.08	0.113	0.0025	0.813
Species	1	13.50	**<0.001**	946.12	**<0.001**	0.0606	**0.001**
Site	2	67.08	**<0.001**	333.38	**<0.001**	0.4009	**<0.001**
Species x site	2	7.49	**<0.001**	133.04	**<0.001**	0.0038	0.480
Clone (species)	6	3.56	**<0.001**	62.99	**<0.001**	0.0351	**<0.001**
Clone (species) x site	12	4.21	**<0.001**	43.97	**<0.001**	0.0200	**<0.001**
Error	42	0.47		8.16		0.0051	
R^2			0.924		0.894		0.862

Source of variation	*df*	Average stem height (m)		Average stem diameter (mm)	
		MS	*P* value	MS	*P* value
Block (site)	6	0.020	0.810	0.0016	0.945
Species	1	0.159	0.055	0.0545	0.333
Site	2	15.864	**<0.001**	4.4797	**<0.001**
Species × site	2	0.110	0.081	0.0386	0.512
Clone (species)	6	0.302	**<0.001**	0.3095	**<0.001**
Clone (species) × site	12	0.119	**0.005**	0.0720	0.272
Error	42	0.041		0.0567	
R^2			0.954		0.833

P values <0.05 are in bold print

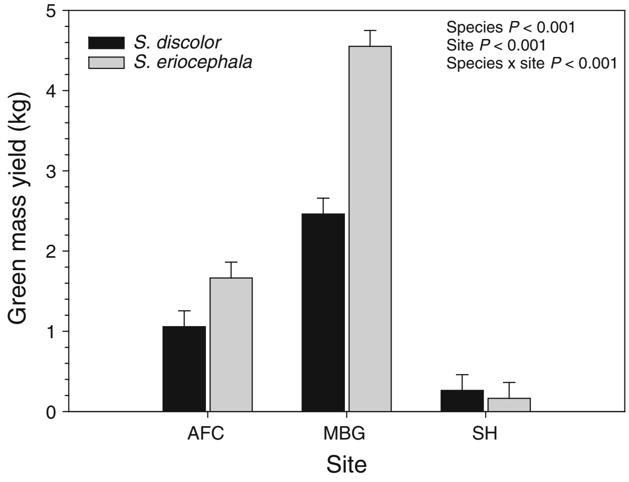

Fig. 1 Green mass yield (mean and SE) for 2-year-old coppice of *Salix discolor* and *S. eriocephala* at three sites: Atlantic Forestry Centre, Fredericton, NB (AFC), Montreal Botanical Gardens, Montreal, QC (MBG), and Salmon Harbour coal mine near Minto, NB (SH)

interactions for specific clones. For instance, DIS clone RIC-D2 showed a significant and disproportionately greater growth response at the MBG site for both yield (Fig. 4b) and average stem biomass (Fig. 6b). These growth responses were disproportionately greater than most of the other clones of both species, but the response of this clone was proportionate to other clones on the AFC and SH sites for other biomass traits (Figs. 4, 5, 6). The coppice structure for clone RIC-D2 also produced the greatest number of stems per coppice (Fig. 5b) and the longest stems (Fig. 7b) with the greatest stem basal diameter (Fig. 8b) on the most fertile MBG site. A similarly disproportionate response in number of stems per coppice was shown by the ERI clone SHE-E3 at the MBG site (Fig. 5b).

Discussion

Given the rich diversity of willow species native to North America and their widespread distribution (Argus 2010), comparatively little effort has been put into quantifying and understanding the potential of native willows for either biomass production for energy or other industrial purposes (Mosseler et al. 1988; Labrecque et al. 1993; Kopp et al. 2001; Labrecque and Teodorescu 2005; Tharakan et al. 2003, 2005) or for their potential use in reclamation of highly disturbed areas (Mosseler et al. 2014a). In northern Europe, there has been a long-standing interest in using willows for bioenergy purposes, and both biomass yields and a number of adaptive traits have been well documented (Erikson 1988; Gullberg 1993; Ledin 1996; Mola-Yudego and Aronsson 2008). The growth potential of these exotic clones has also been quantified in the northeastern USA (Kopp et al. 2001; Smart et al. 2005; Tharakan et al. 2005;

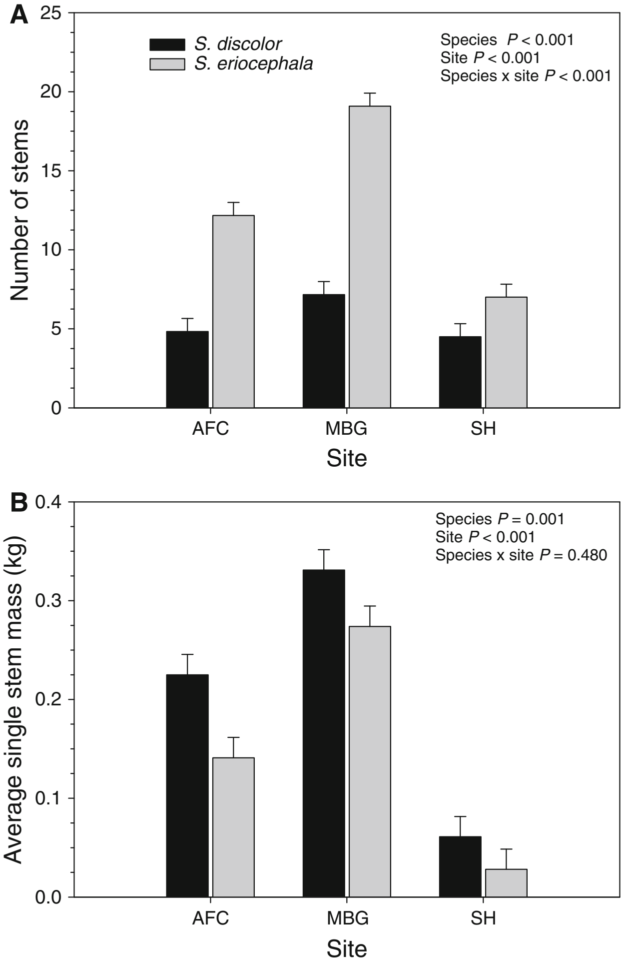

Fig. 2 **a** Average number of stems per coppice (mean and SE), and **b** average single stem green mass for 2-year-old coppice of *Salix discolor* and *S. eriocephala* at three sites: Atlantic Forestry Centre, Fredericton, NB (AFC), Montreal Botanical Gardens, Montreal, QC (MBG), and Salmon Harbour coal mine near Minto, NB (SH)

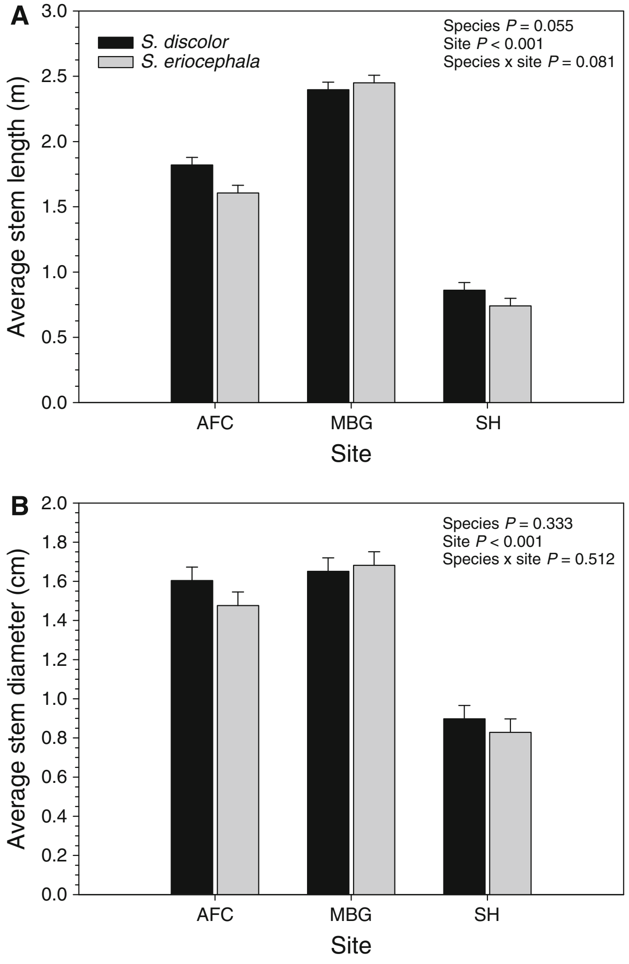

Fig. 3 **a** Average stem height (mean and SE), and (B) average basal stem diameter for 2-year-old coppice of *Salix discolor* and *S. eriocephala* on three sites: Atlantic Forestry Centre, Fredericton, NB (AFC), Montreal Botanical Gardens, Montreal, QC (MBG), and Salmon Harbour coal mine near Minto, NB (SH)

Volk et al. 2006). A number of jurisdictions have shown great concern recently about the introduction of exotic species and their potential to harm native biodiversity, which may eventually lead to increased restrictions on importation and use of exotic willows for biomass plantations. On productive sites, the biomass yields reported here for two native willows reached as high as or higher than 6.0 kg, and assuming high survival, could reach a potential of 60 t ha^{-1} green mass for both species: thus yields of DIS and ERI, appear comparable to those reported for exotic willows on similar sites and in similar climates of northeastern USA and Canada (Labrecque et al. 1993; Tharakan et al. 2005; Volk et al. 2011). Results presented here and elsewhere (Mosseler et al. 2014b) indicate that through further selection and breeding, biomass yields from native willows can equal or surpass those from highly bred introduced European clones of species such as *S. viminalis*.

What might be the driving factor(s) that produce these site productivity differences and interactions? In genetic tests such as progeny or provenance tests, which are deployed over a range of natural conditions, it can be difficult to ascertain the effects of environmental differences among sites on growth responses. Temperature and precipitation differences are generally the first and easiest site attributes to quantify. In this study, temperature, growing degree days, and seasonal precipitation are very similar among sites and thus in our case, it is most likely site quality, soil nutrients, physical traits, and water holding capacity. We measured 13 different soil property traits, 12 of which showed significant differences, and ranking was fairly consistent among sites. An examination of soil physical properties indicates that SH would most likely also have had the lowest water availability for most of the year because this site consisted of 56.5 % stone (>2 mm sieve), 67 % sand, and broken shale rock. The well-drained

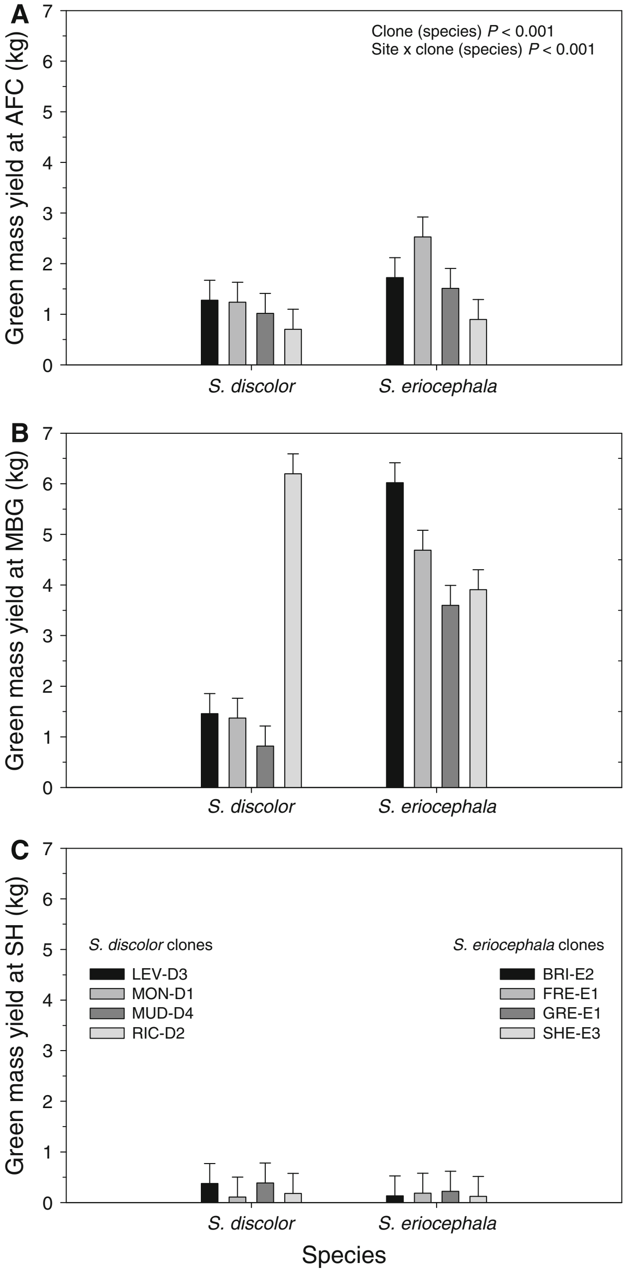

Fig. 4 Green mass yield (mean and SE) for 2-year-old coppice from eight clones of *Salix discolor* (four) and *S. eriocephala* (four) on three sites: **a** Atlantic Forestry Centre, Fredericton, NB (AFC). **b** Montreal Botanical Gardens, Montreal, QC (MBG) and **c** Salmon Harbour coal mine near Minto, NB (SH)

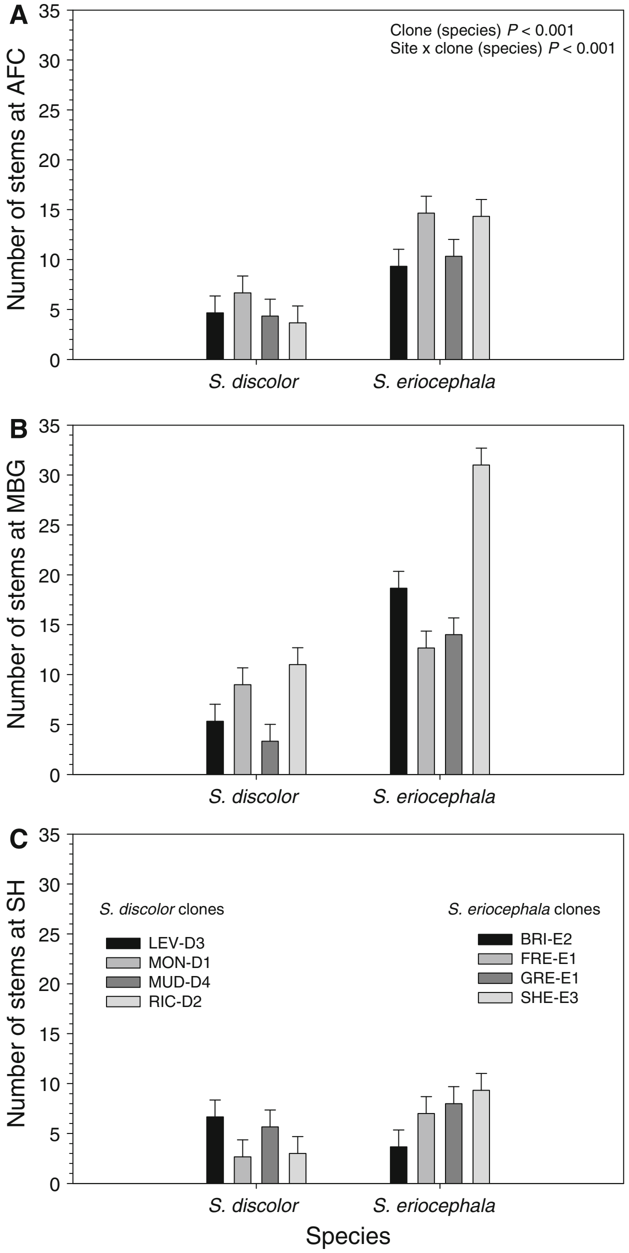

Fig. 5 Average number of stems per coppice (mean and SE) for 2-year-old coppice from eight clones of *Salix discolor* (four) and *S. eriocephala* (four) on three sites: **a** Atlantic Forestry Centre, Fredericton, NB (AFC), **b** Montreal Botanical Gardens, Montreal, QC (MBG) and **c** Salmon Harbour coal mine near Minto, NB (SH)

AFC site with 82 % sand and only 3 % clay would most likely have been intermediate in water availability, and the MBG with only 50 % sand would probably have had the best water availability properties. Thus, site quality differences, a combination of soil nutrients, physical properties, and water-holding capacity were most probably the source of the productivity differences.

Significant variation in coppicing (stem sprouting) capacity both within and between these species indicates great potential for clonal selection and breeding for coppicing ability and either increasing or decreasing the number of stems per coppice (Sennerby-Forsse and Zsuffa 1995; Ceulemans et al. 1996). European willows with high numbers of stems per coppice, such as *S. viminalis*, also experience high levels of stem mortality as plantations age

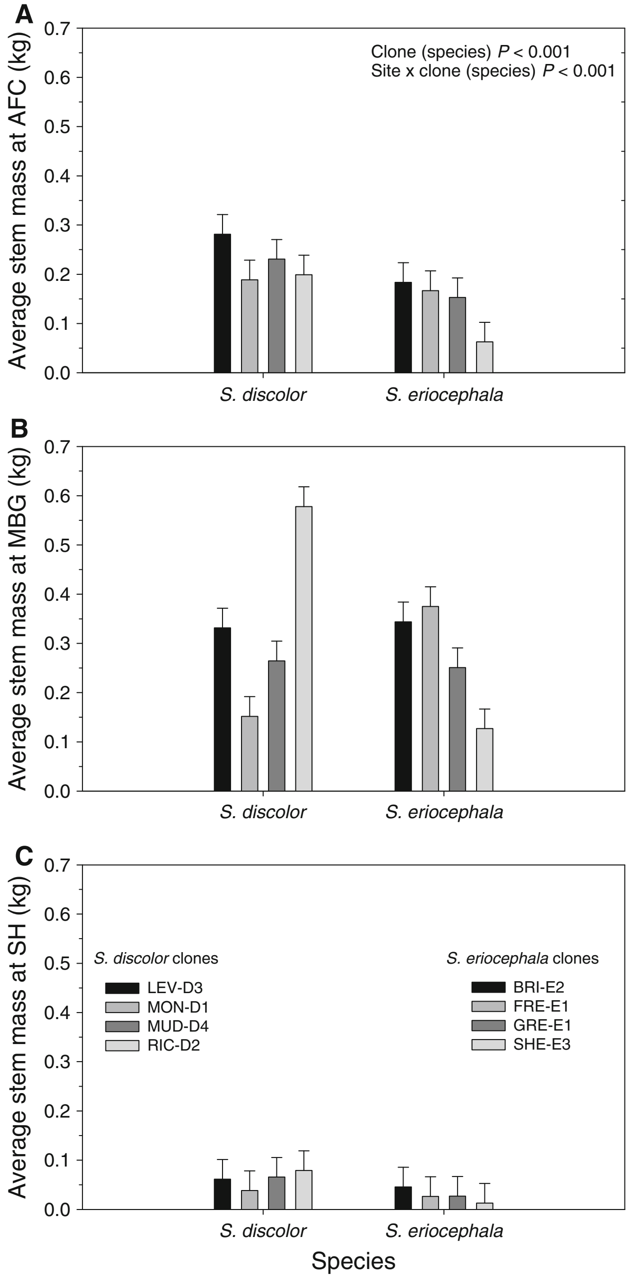

Fig. 6 Average stem green mass (mean and SE) for 2-year-old coppice from eight clones of *Salix discolor* (four) and *S. eriocephala* (four) on three sites: **a** Atlantic Forestry Centre, Fredericton, NB (AFC), **b** Montreal Botanical Gardens, Montreal, QC (MBG) and **c** Salmon Harbour coal mine near Minto, NB (SH)

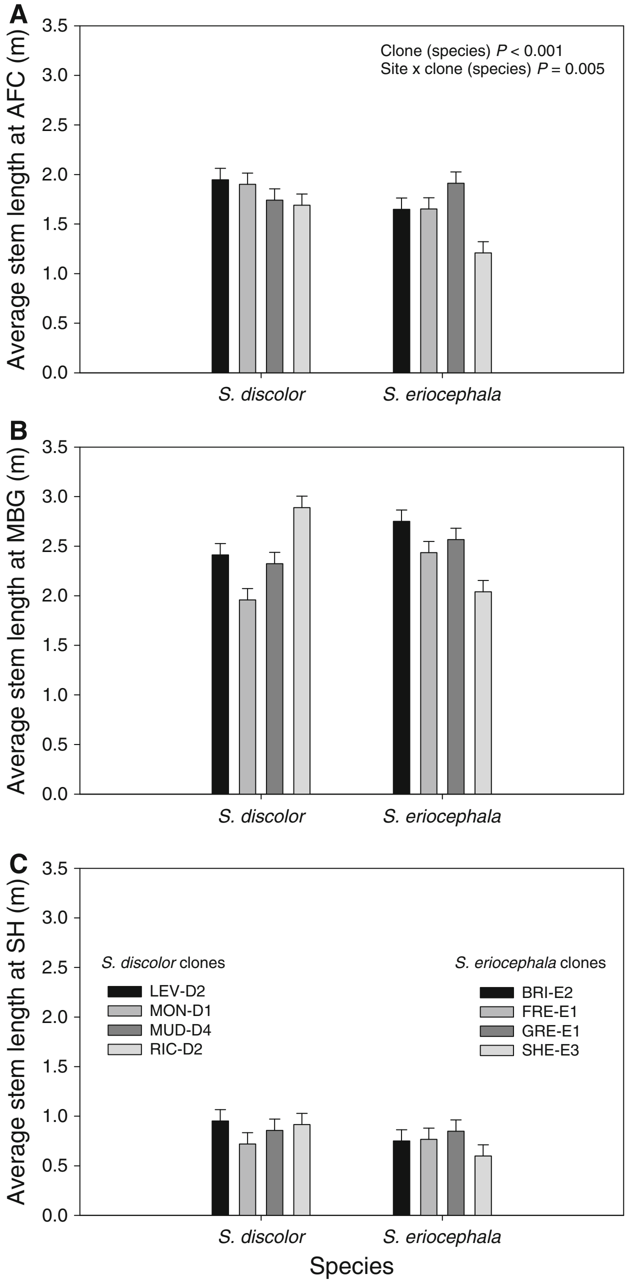

Fig. 7 Average stem height (mean and SE) for 2-year-old coppice growth from eight clones of *Salix discolor* (four) and *S. eriocephala* (four) on three sites: **a** Atlantic Forestry Centre, Fredericton, NB (AFC), **b** Montreal Botanical Gardens, Montreal, QC (MBG) and **c** Salmon Harbour coal mine near Minto, NB (SH)

(Verwijst 1991; Ceulemans et al. 1996). Some mortality of suppressed stems has also been observed within our field tests, especially in clones of ERI, which regularly produced more than 20 stems per coppice on the MBG and AFC sites, reaching a maximum of 34 stems per coppice in ERI clone SHE-E3 (Mosseler et al. 2014b). Nevertheless, shoot mortality was rare and occurred mostly in small, suppressed stems and did not appear to have a major impact on biomass yields in 2-year-old coppice plants (Verwijst 1991).

In the natural populations from which we collected the 144 genotypes of DIS and ERI for our original (2005) common-garden experiment at the MBG, ERI seemed to occur most often on fertile sites associated with small streams and agricultural fields and was rarely found on drier, less fertile upland sites such as the mine overburden

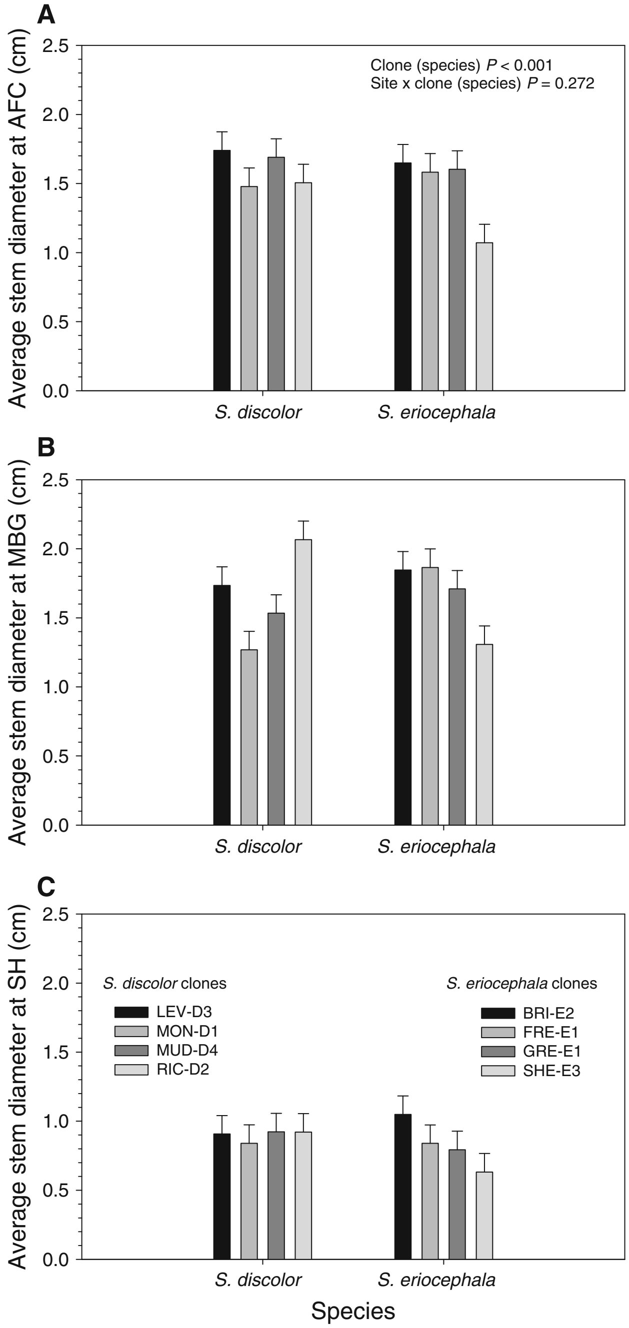

Fig. 8 Average stem basal diameter (mean and SE) for 2-year-old coppice from eight clones of *Salix discolor* (four) and *S. eriocephala* (four) on three sites: **a** Atlantic Forestry Centre, Fredericton, NB (AFC) (B) Montreal Botanical Gardens, Montreal, QC (MBG) and (C) Salmon Harbour coal mine near Minto, NB (SH)

at the SH coal mine in NB. DIS was also found in fertile sites associated with streams, but in contrast to ERI, was common on drier, less fertile, upland sites such as the SH coal mine spoils, together with *S. bebbiana* (BEB). Both BEB and DIS are commonly found together on upland sites across eastern and central Canada, especially as one moves further into boreal forest environments, and both species were among the first woody perennials to colonize the SH mine overburden via natural seed dispersal, along with birches (*Betula papyrifera* and *B. populifolia*), aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), and pin cherry (*Prunus pensylvanica*). Based on our observations of natural populations across eastern and central Canada, we speculate that ERI may be better adapted to more fertile sites, and this may explain why ERI grew comparatively better on the more productive sites at MBG and AFC, and why DIS performed comparatively better on the least productive sites on the SH coal mine overburden, demonstrating a strong species by site interaction.

As was the case with species trends, growth trends for these DIS and ERI clones were generally consistent across the three sites and allowed a clear ranking of site productivity, with MBG being the most productive, SH the least productive, and AFC having an intermediate productivity (Figs. 4, 5, 6, 7, 8). Growth responses in DIS clone RIC-D2 and ERI clone SHE-E3 showed strong genotype by site interactions that can be expected in willow biomass production plantations. However, these large genotypes by environment interactions were both difficult to predict and difficult to understand at a genetic level and only became evident following empirical field testing. Nevertheless, such interactions can alter biomass yields and economic viability on a given site and indicate that there may be benefits in testing both different species as well as a number of promising clones within a species on a given site to determine if species and/or genotype by environment interactions can be exploited for productivity and commercial gain. Use of a single clone across large areas of highly variable site conditions may not be optimal for short-rotation biomass production plantations.

Following field testing of seven willow species from natural populations on the harsh SH site, survival for ERI and DIS was 72 and 22 %, respectively (Mosseler et al. 2014a). In most species, percent survival was relatively constant after the initial establishment phase, allowing good prediction of final survival in the first or second year after establishment. After initial natural population field testing at MBG, genotypes were selected for good survival and growth. These selected genotypes grew better, and survival increased to 94 and 61 % for ERI and DIS, respectively, on the SH site, demonstrating the ability to rapidly improve survival results based on preliminary field testing. Our objective for this study was to examine the genetic by environment interaction effect on productivity and components of growth and thus on trees that survived.

There were not only differences in coppice yield and components of growth among sites and genetic interaction with site, but also a number of significantly different relationships among coppice yield and components of growth. There was no species effect on the average stem length–diameter allometric relationship, but there was a strong site effect (Mosseler et al. 2014b). Stems from MBG were much longer in relation to diameter compared with those from the AFC site, which were longer per unit diameter than the SH site. Increased number of stems within a plant slightly but significantly decreased average stem diameter but not average stem length. For every 10-stem increase in stem number, there was a 0.13-cm decrease in basal diameter. Allometric relationships among stem basal diameter, stem length, number of stems per plant, and biomass yield clearly reflected site quality differences and could be used in the same way that stem height at a specific age has been used for single stemmed trees to define site quality (site index) in forest timber production. The poor quality SH site had lower values and a lower slope for average stem length or diameter relationship to yield, whereas the more productive the site, the greater the values and the greater the average stem length or diameter slope relationship to yield (Mosseler et al. 2014b).

Components of growth may affect practical or operational aspects of the design of harvesting equipment and handling of woody biomass harvested from short-rotation plantations (Bergkvist and Ledin 1998). Harvesting short-rotation biomass consisting of a large number of smaller individual stems as opposed to the large, single-stemmed trees of traditional forestry practice has stimulated the development of alternative harvesting machinery from modified hay balers with specialized cutting heads (Savoie et al. 2009) to modified corn harvesters with more traditional saw blades, in which the gathered stems are cut, bunched, and/or chipped directly on site (Savoie et al. 2013), and then stored in large bins or trucks for transportation to municipal heating plants or wood-pellet processing facilities. For instance, ERI generally produced coppice growth consisting of significantly more stems per coppice but with stems having significantly less biomass per stem than DIS, and these differences may also affect how short-rotation woody biomass is harvested.

In summary, strong genetic, site, and genetic by site interactions have been observed both at the species level and the genotype (clone) level within species in both DIS and ERI, two promising native North American willows for biomass production (Mosseler et al. 1988, 2014a). As these sites had similar temperatures, growing degree days, and seasonal precipitation, site quality differences were most likely driven by soil nutrients, physical traits, and water holding capacity. These interactions with site quality can have important impacts on both biomass yield and components of growth and need to be considered in clonal selection and deployment to optimize commercial viability of biomass production. Productivity reached as high as 6.0 kg green mass after 2 years of coppice growth for a clone from each of DIS and ERI on the MBG site. Coppice form and growth rates may affect decisions on clonal selections at all stages of biomass production from site type to be planted, to plant spacing and rotation length, to harvesting methods and processing machinery.

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Conflict of interest The authors declare that they have no conflict of interest.

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