Examining the salt tolerance of willow (*Salix* spp.) bioenergy species for use on salt-affected agricultural lands

R. D. Hangs[1], J. J. Schoenau[1], K. C. J. Van Rees[1], and H. Steppuhn[2]

[1]Department of Soil Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A2; and [2]Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Swift Current, Saskatchewan, Canada S9H 3X2. *Received 16 June 2010, accepted 21 January 2011.*

Hangs, R. D., Schoenau, J. J., Van Rees, K. C. J. and Steppuhn, H. 2011. **Examining the salt tolerance of willow (*Salix* spp.) bioenergy species for use on salt-affected agricultural lands**. Can. J. Plant Sci. **91**: 509–517. Dryland salinity is a significant limitation on crop production across the Canadian prairies, with an estimated 4 million ha of salt-affected land. The potential exists to make better use of saline marginal lands by developing them into willow (*Salix* spp.) plantations as a bioenergy feedstock; however, relatively little is known about the salt tolerance of willow. The objective of this study was to compare the relative salt tolerance of 37 different native and exotic willow varieties grown under controlled environment conditions on soils of varying salinity. The soils were collected from a farm field in south-central Saskatchewan along a hillslope catena influenced by saline seep salinity, containing high concentrations of sulfate salts, which commonly occurs within western Canada. Most willow varieties tested in this study were able to tolerate moderately saline conditions ($EC_e \leq$ 5.0 dS m^{-1}). In addition, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with severe salinity ($EC_e \leq 8.0$ dS m^{-1}). These results indicate that some willow varieties are quite salt-tolerant and suitable for establishment on salt-affected soils in Saskatchewan and abroad.

Key words: Biomass energy, electrical conductivity, *Salix*, salt tolerance, sulfate salts, willow

Hangs, R. D., Schoenau, J. J., Van Rees, K. C. J. et Steppuhn, H. 2011. **Tolérance au sel du saule (*Salix* spp.), une espèce bioénergétique susceptible d'être cultivée sur les terres agricoles salinisées**. Can. J. Plant Sci. **91**: 509–517. La salinité des terres arides limite passablement l'agriculture dans les Prairies canadiennes. On estime que quatre millions d'hectares sont ainsi affectés. On pourrait néanmoins faire un meilleur usage des terres marginales salinisées en y cultivant le saule (*Salix* spp.), qu'on utiliserait comme matière première pour produire de la bioénergie. Malheureusement, on connaît relativement mal la tolérance du saule au sel. La présente étude devait comparer la tolérance au sel relative de 37variétés indigènes et exotiques de saule cultivées en milieu contrôlé dans un sol de salinité variable. Les sols ont été recueillis dans le champ d'une exploitation agricole du centre-sud de la Saskatchewan, le long d'une caténa en pente qui subissait l'influence d'une remontée saline. Le sol renfermait une grande quantité de sels de sulfate, comme c'est couramment le cas dans l'ouest du Canada. La plupart des variétés testées dans le cadre de cette étude toléraient modérément le sel (ECe $\leq$5,0 dS par mètre); plusieurs (Alpha, India, Owasco, Tully Champion et 01X-268-015) n'ont cependant pas ralenti leur croissance malgré la forte salinité (ECe $\leq$8,0 dS par mètre). Ces résultats indiquent que certaines variétés de saule tolèrent considérablement le sel et pourraient s'établir sur les sols salinisés de la Saskatchewan et d'ailleurs.

Mots clés: Bioénergétique, tolérance au sel, *Salix*, sels de sulphate, saule

The use of biomass-derived energy accounts for approximately 10% of the global energy requirement (Berndes et al. 2003). However, with growing desire worldwide for secure and environmentally acceptable energy sources, there is increased interest in developing biomass production systems for use as a dedicated or "purpose-grown" feedstock for biomass energy. Canada is no exception, with its high per-capita energy consumption and the majority of its energy demand used for transportation and building utilities (Cuddihy et al. 2005). Natural Resources Canada, along with a number of Canadian provinces, declares bioenergy to be a legitimate and sustainable source of energy that will constitute a significant portion of future energy production. The establishment of purpose-grown shrub willow (*Salix* spp.) plantations represents a viable bioenergy feedstock, especially if the willow can be successfully grown on unproductive land that is marginal for annual crop production, such as saline land. With escalating public concern over the displacement of arable land from food production into bioenergy production, a great opportunity exists to realize economic and environmental benefits, through the development of non-consumable woody crops, like willow, for marginal land that is deemed unsuitable for annual crop production (Van Rees 2008).

Abbreviations: EC_e, electrical conductivity of saturated paste extract; $EC_{1:2}$, electrical conductivity of 1:2 (soil:water) extraction

Dryland salinity is a significant agronomic problem across the Canadian prairies (Acton and Gregorich 1995). According to Eilers et al. (1995), the incidence of salinity can be summarized as follows: (i) the majority (62%) of arable land in the prairies contains less than 1% saline soil; (ii) 36% of the arable land contains 1–15% saline soil; and (iii) 2% of the arable land contains more than 15% saline soil. Generally speaking, soil salinity affects around 10% of the cultivated land within the prairies, or approximately 4 million ha, translating into farm income losses of approximately $250 million annually (Dumanski et al. 1986). A number of studies have examined salinity in Saskatchewan soils (Hogg and Henry 1984; Henry et al. 1985; Keller and Van der Kamp 1988), but accurate estimates of the saline-affected area are difficult to establish due to its large areal extent and inherent variability caused by the ephemeral nature of salts moving through the soil profile. Nevertheless, it is has been estimated that there are approximately 1.6 million ha of saline soils in Saskatchewan alone (Rennie and Ellis 1978) and these lands are either being used to grow low-return forage crops or have been abandoned altogether. The potential exists, therefore, to make better use of these saline lands by developing them into short-rotation intensive culture willow plantations, which is not only economically positive for the farmer, but also may provide environmental benefits, such as precluding the build-up of surface salts given willow's phreatophytic nature, along with promoting increased biodiversity within the agricultural landscape. To our knowledge, no empirical work has been done to examine the growth of different willow varieties on soils with varying salinity. The objective of this study was to determine the relative growth response of numerous native and exotic willow varieties grown in saline soils. Identifying salt-tolerant varieties could promote the use of willow plantations to revitalize these unproductive agricultural lands; thereby supporting agricultural diversification in Saskatchewan and abroad.

MATERIALS AND METHODS

Collection and Preparation of Saline Soils

The saline soils used in this study were collected from a continually cropped field (pea-wheat-barley rotation), located approximately 7 km southeast of Central Butte, SK (UTM Co-ordinates: 13U 400114 5620205). The soils were predominantly Solonetzic loam soils of the Kettlehut Association, with an Agricultural Capability Classification rating of Class Four (Saskatchewan Centre for Soil Research 1985). Soils of varying salinity were collected along a hillslope catena influenced by saline seep salinity, containing high concentrations of sulfate salts, which commonly occurs within western Canada (Wiebe et al. 2007). The development of saline seeps along such hillslopes is primarily due to the effects of a semi-arid climate and local hydrogeology on the translocation and subsequent concentration of naturally occurring salts within near-surface discharge soil layers downslope. Briefly, the soils at this site are greatly influenced by the relatively thin glacial till parent material, derived from the underlying Cretaceous marine clay-shale bedrock rich in Na, Ca, and Mg sulphate salts. Saline seeps typically develop wherever saline groundwater occurs within 1.5 m of the surface, coupled with a local recharge zone, such as upland areas with slopes of 0–2% (Miller et al. 1981; Daniels 1987). Excess soil water (i.e., beyond evapotranspirative demand) in the upland recharge area infiltrates beyond the root zone, through thin shale-modified salt-rich parent material and contacts the impermeable shale, before moving laterally downslope as unsaturated flow (Holm and Henry 1982; Henry et al. 1987). As the groundwater follows the local hydraulic gradient downslope, it dissolves and carries salts until concentrating them at or near the soil surface through capillary action and evaporation, particularly during the drier mid-summer months. Consequently, there is a distinct gradient of increasing soil salinity moving downslope, often with the formation of a white salt crust in the depressional area where the salt concentration is the highest.

Soils were intensively sampled along a 300-m transect, from the top of the knoll to the depression, and their electrical conductivities measured using a Accumet AP85 pH/EC meter (Fisher Scientific, Pittsburgh, PA). Four slope positions were selected to represent the range of soil salinity encountered along the hillslope. Soil was collected from the Ap horizon (approximately 0–20 cm) at each location, air-dried, and then blended to to achieve the desired salinity levels for the pot study – determined using electrical conductivity values derived from 1:2 (soil:water) extractions ($EC_{1:2}$). The four target salinity levels ($EC_{1:2}$; dS m^{-1}), classified according to Henry et al. (1987), were: non-saline (0.1); slightly saline (1.0); moderately saline (2.0); and severely saline (4.0). Logistically, the use of 1:2 extractions supported the quickest and most precise blending of the soil into the desired salinity levels; however, the salinity of the saturated paste extract (EC_e) was also determined for each soil and will be referred to henceforth. Additionally, subsamples of each soil type were collected and submitted to a local soil testing lab (ALS Laboratory Group, Saskatoon, SK) for detailed salinity and nutrient availability assessment (Table 1).

Experimental Design, Willow Material, Growing Conditions, and Sampling Protocol

The experimental setup was a completely randomized design with four replicates. A total of 592 pots were used (37 willow varieties × four saline soils × four replicates). Plant material of 37 different willow varieties was collected from 1-yr-old stools in the spring of 2009 from clonal trial plots located in Saskatoon (Table 2) and sectioned into 15-cm cuttings. Cutting diameter varied considerably among the willow varieties, which

Table 1. Selected properties of saline soils used to screen for salt tolerance among different native and exotic willow (*Salix* spp.) varieties

	Nutrients[z]												
	NO_3-N	P	K	SO_4-S	Ca	Mg	Na	Cl	$EC_{1:2}^{y}$	EC_e^{x}			
Soil type	(mg kg^{-1})				(mg L^{-1})				(dS m^{-1})		SAR[w]	ESP[v]	pH
Non-saline	7	19	423	13	74	41	44	8	0.1	0.8	1.4	0.8	7.1
Slightly saline	8	51	649	295	410	219	239	22	1.0	3.6	3.4	3.6	7.4
Moderately saline	17	54	657	708	494	334	404	36	2.0	5.0	4.9	5.6	7.6
Severely saline	16	40	674	1610	486	462	900	72	4.0	8.0	9.9	11.7	7.9

[z]Extractable nutrients.
[y]$EC_{1:2}$, electrical conductivity of a 1:2 (soil:water) extract.
[x]EC_e, electrical conductivity of a saturated paste extract.
[w]SAR, sodium adsorption ratio derived using equation from Henry et al. (1987): $SAR = ([Na])\,((0.5 \times ([Ca]+[Mg]))^{-1/2}$; where [ ] is in mmol L^{-1}.
[v]ESP, exchangeable sodium percentage derived using equation from Henry et al. (1987): $ESP = ((1.47 \times SAR) - 1.26)\,((0.0147 \times SAR) + 0.99)^{-1}$.

can influence establishment success (Burgess et al. 1990), so initial cutting diameters were measured and subsequently related to the willow growth variables at the end of the experiment for each saline soil. One-litre pots were filled with 1.3 kg of selected saline soil (bulk density approximately 1.3 g cm^{-3}) and watered to field capacity (28%, vol vol^{-1}), before inserting a single willow cutting. Pots were maintained at field capacity by watering every 2 d for the first 2 wk and then daily for the remainder of the experiment. The surface of each pot was covered with white plastic beads to reduce evaporative losses. All pots were placed randomly in a Conviron® controlled environment chamber (Conviron Ltd., Winnipeg, MB). The willow were grown under an 18:6 h (light:dark) photoperiod, with air temperatures of 22:18°C (day:night). Relative humidity was approximately 70%. Lighting was provided using Cool White VHO fluorescent and incandescent lamps (Sylvania, Drummondville, ON). Photon flux density was approximately 400 μmol m^{-2} s^{-1} at canopy level and was measured using a LI-COR quantum light meter (model LI-189; LI-COR Inc., Lincoln, NE). After 60 d, plant heights (tallest shoot) were measured before each pot was harvested and separated into leaves, shoot(s), cutting, and roots. The roots were collected by washing the potted soil over a fine (0.5 mm) mesh sieve. All plant material was dried to a constant weight and the above- and below-ground plant biomass was determined. Prior to drying the leaves, the total leaf area for each plant was determined using a leaf surface area meter (LI–3100; LI-COR Inc., Lincoln, NE). Root mass fraction was calculated as root biomass:total biomass (Coyle and Coleman 2005). Given the large variation in cutting size among the willow varieties, cutting biomass was not included in the total biomass value. In order to facilitate the comparison of salt tolerance among the different varieties, relative biomass assessments were made by normalizing the willow growth response to increased salinity relative to its growth under non-saline conditions (Steppuhn et al. 2008).

Table 2. Thirty-seven selected native and exotic willow (*Salix* spp.) varieties screened for salt tolerance

Variety	Species	Sex	Variety	Species	Sex
(1) Allegany	*S. purpurea*	F	(20) Saratoga	*S. purpurea × S. miyabeana*	F
(2) Alpha	*S. viminalis*	F	(21) Saskatoon D3	*S. discolor*	?
(3) Canastota	*S. sachalinensis × S. miyabeana*	M	(22) Saskatoon E3	*S. eriocephala*	?
(4) Charlie	*S. alba × S. glatfelteri*	?	(23) Sherburne	*S. sachalinensis × S. miyabeana*	F
(5) Cicero	*S. sachalinensis × S. miyabeana*	F	(24) SV1	*S. dasyclados*	F
(6) Fabius	*S. viminalis × S. miyabeana*	F	(25) SX-61	*S. sachalinensis*	F
(7) Fish Creek	*S. purpurea*	M	(26) SX-64	*S. miyabeana*	M
(8) Hotel	*S. purpurea*	?	(27) Taberg	*S. viminalis × S. miyabeana*	F
(9) India	*S. dasyclados × ?*	M	(28) Truxton	*S. viminalis × S. miyabeana*	F
(10) Juliet	*S. eriocephala*	?	(29) Tully Champion	*S. viminalis × S miyabeana*	F
(11) Marcy	*S. sachalinensis × S. miyabeana*	F	(30) Verona	*S. viminalis × S. miyabeana*	F
(12) Millbrook	*S. purpurea × S. miyabeana*	F	(31) 94001	*S. purpurea*	M
(13) Oneida	*S. purpurea × S. miyabeana*	M	(32) 00X-026-082	*S.eriocephala*	M
(14) Oneonta	*S. purpurea × S. miyabeana*	M	(33) 00X-032-094	*S. eriocephala*	?
(15) Onondaga	*S. purpurea*	M	(34) 01X-268-015	*S. viminalis × (S. sachalinensis × S. miyabeana)*	?
(16) Otisco	*S. viminalis × S. miyabeana*	F	(35) 9837-77	*S. eriocephala*	F
(17) Owasco	*S. viminalis × S. miyabeana*	F	(36) 9882-041	*S. purpurea*	F
(18) S25	*S. eriocephala*	F	(37) 99208-038	*S. viminalis × S. miyabeana*	F
(19) S365	*S. caprea*	F			

Statistical Analyses

Measurement variables were analysed using PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC). Means comparisons were performed using least significant differences (LSD) at a significance level of 0.05. The LSD option was used to carry out pairwise *t* tests (equivalent to Fisher's protected LSD) of the different means, with groupings obtained using the pdmix800 SAS macro (Saxton 1998). Homogeneity of variances and normality of distributions of all data sets were checked prior to the analysis. No data transformations were necessary.

RESULTS AND DISCUSSION

Willow Establishment and Growth Response to Salinity

Although there was a marked delay in bud flush observed for all varieties as salinity increased, there were no differences in plant mortality among the four saline soil types, with 97% survival overall at the end of the experiment. The native variety Saskatoon D3 (*Salix discolor*) was the only willow variety unable to survive the highest salinity level in this study ($EC_e \leq 8.0$ dS m^{-1}). The ease of willow establishment on a variety of soil types, from non-rooted cuttings, is well established (Volk et al. 2006) and is advantageous for supporting its widespread use as a purpose-grown biomass energy crop. The apparent sensitivity of willow to increased salt level was dependent on the growth variable assessed, in that only the severely saline conditions ($EC_e \leq 8.0$ dS m^{-1}) significantly impacted plant height, shoot biomass, leaf biomass, and leaf surface area (Fig. 1). Conversely, the number of shoots per plant, root biomass, and total plant biomass were more sensitive to increasing soil salinity. The largest root mass fraction (root biomass:total biomass) occurred with the highest salinity level (Fig. 1) and is indicative of increased plant stress (Coyle and Coleman 2005). There was no correlation between initial planted cutting diameter and any measured willow growth variable among the saline soil types (data not shown), which highlights the superseding importance of cutting quality, as opposed to size, that is key for successful willow establishment and growth.

While the majority of native and exotic willow varieties tested were sensitive to increasing soil salinity, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with severe salinity ($EC_e \leq 8.0$ dS m^{-1}; Figs. 2, 3, and 4). Additionally, unlike the others, these five varieties had no change in the measured root mass fraction with increasing substrate salinity (data not shown) and, therefore, presumably were less-stressed and this was reflected in their sustained growth under increasingly saline conditions. Conversely, the occurrence of chlorotic leaves, necrotic patches, and premature leaf senescence for the remainder of willow varieties growing on the severely saline soil, are common nutrient deficiency/ion toxicity symptoms in woody plants due to high tissue concentrations of Na^{+} and Cl^{-} (Kozlowski 1997; Chen et al. 2002). Moreover, the five relatively salt-tolerant varieties had a noticeably more lush appearance (i.e., greener and larger shoots) with increasing salinity after the 60-d growth period (Fig. 4d) and this improved growth is probably attributable to the presence of residual fertilizer nitrogen and phosphorus in these highly saline soils. Specifically, past management practices at the site where the soils were collected involved uniform application of a consistent rate of fertilizer across all regions of the hillslope over several years. However, given the historically poorer crop growth in the salt-affected areas, reduced plant uptake and removal have resulted in higher extractable soil nutrient levels in these saline lower slope soils (i.e., Soils 3 and 4; Table 1). For this reason, saline areas can often be the most nutrient-rich locations within a field and, in this study, provided a growth advantage for the salt-tolerant varieties.

Relative Salt Tolerance Among Willow Varieties

Anecdotally, willow is generally believed to have moderate tolerance to soil salinity (Kuzovkina and Quigley 2005), which is confirmed by this study; however, a clear trend in effect of parentage was apparent among the five salt-tolerant willow varieties. Relatively greater salt tolerance was observed with the presence of *S. viminalis* or the hybridization of *S. viminalis* with *S. miyabeana* (Table 2). The only exception may be the variety India, because its parentage is unknown, although some believe that it is a hybridized *S. viminalis* species (Cheryl Hendrickson, personal communication), which is realistic given its measured salt tolerance in this study. However, recent DNA fingerprinting work indicates that India is related to *S. dasyclados* (Ngantcha 2010). Nevertheless, it is reasonable to hypothesize that a possible introgression of the *S. viminalis species* genome into the *S. dasyclados* genome occurred, by the back-crossing of an interspecific *S. viminalis* × *S. dasyclados* hybrid with its *S. dasyclados* parent, to produce the variety India (Alain Ngantcha, personal communication). Such an introgression would explain the salt tolerance observed in India compared with the relatively salt-intolerant *S. dasyclados* willow variety SV1 tested (Fig. 3). There was still considerable variabiltity in salt tolerance among the pure or hybrid *S. viminalis* varieties tested, and this genotypic variation in salt tolerance among willow has been reported elsewhere (Stolarska et al. 2008; Aronsson et al. 2010; Mirck and Volk 2010). Identifying the specific physiological mechanism(s) responsible for the measured differences in salt tolerance among willow varieties is clearly beyond the scope of this screening trial; however, speculation regarding potential mechanisms based on the available literature is warranted.

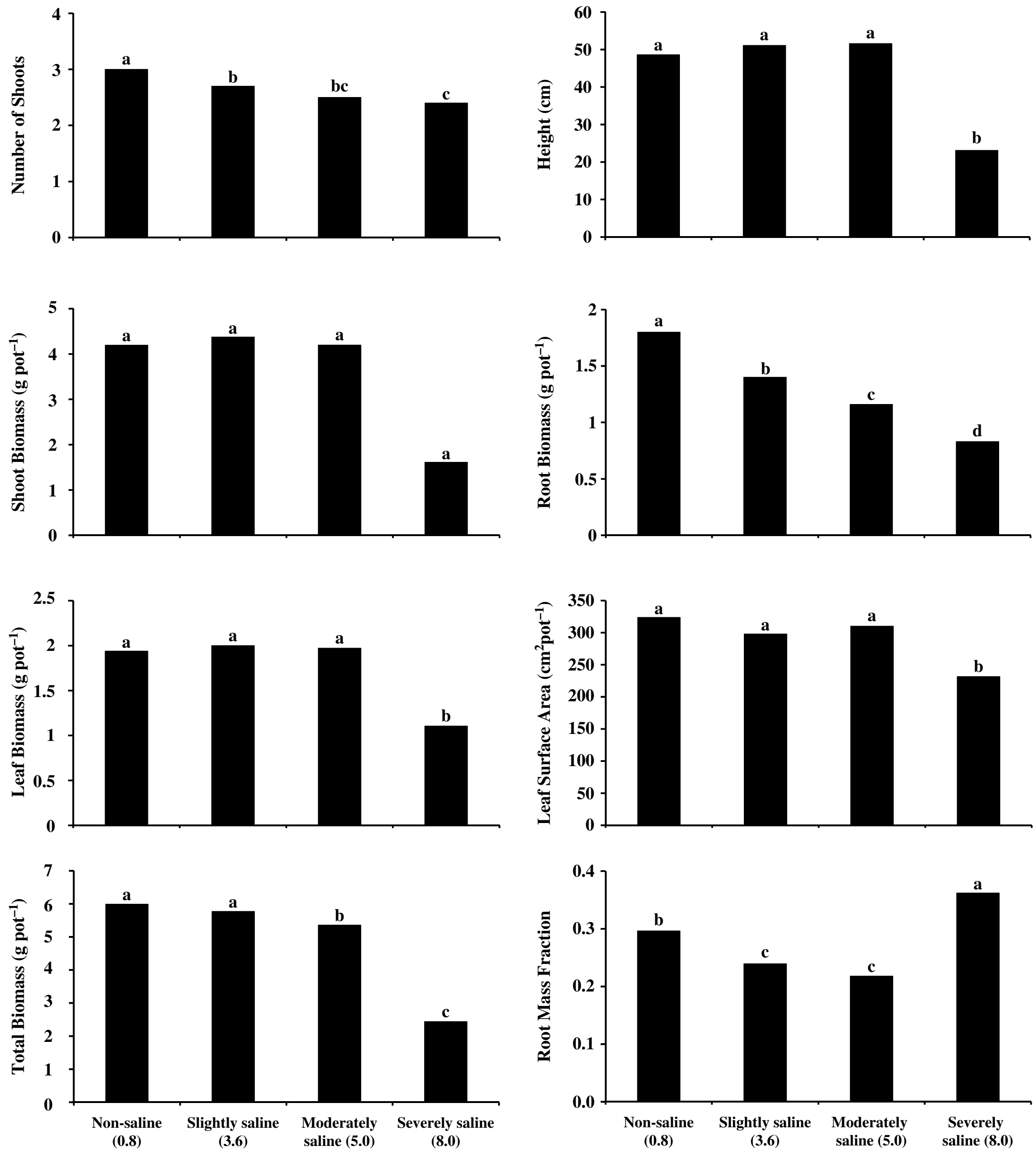

Fig. 1. Mean ($n \geq 144$) number of shoots, shoot height, shoot (including leaves) and root biomass, leaf biomass, leaf surface area, total biomass (shoot + root), and root mass fraction (root biomass:total biomass) of 37 different native and exotic willow varieties grown for 60 d in soils with increasing salinity (EC_e; dS m^{-1}). Bars having the same letter are not significantly different ($P < 0.05$) using LSD. Note: due to plant mortality, the number of replicates was not equal (i.e., 148) among soil salinity levels.

Possible Physiological Adaptations of Willow to Salinity

Woody plants are known to synthesize and accumulate compatible organic solutes, such as glycine betaine, proline, and soluble carbohydrates, in the cytoplasm to regulate osmotic potential (Kozlowski 1997). For example, proline content in leaves of *S. viminalis* was found to be an excellent indicator of salt tolerance among different varieties (Stolarska et al. 2008). Accumulating the endogenous phytohormone abscisic acid, which increases water-use efficiency by supporting plant morphological and physiological responses to saline stress, is another commonly known plant adaptation to salt-induced water deficit also utilized by willow (Liu et al. 2001). Measuring the response of gas exchange variables to increasing salinity, such as reduced stomatal conductance, is also an indicator of the concomitant water stress associated with salt stress in willow (Liu et al. 2001; Wrobel et al. 2006; Mirck and Volk 2010). As a reduction in stomatal conductance is a common

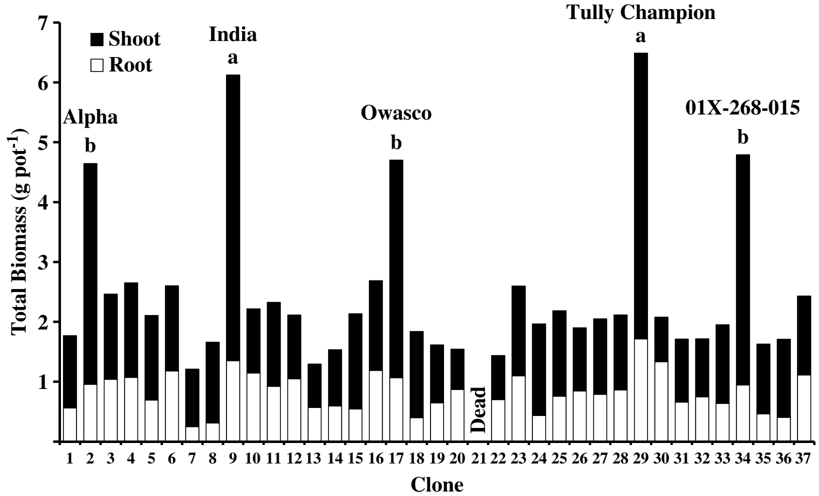

Fig. 2. Total biomass (i.e., shoot + root; $n = 4$) of different native and exotic willow varieties grown for 60 d in severely saline ($EC_e \leq 8.0$ dS m^{-1}) soil. See Table 2 for variety identification. Bars having the same letter are not significantly different ($P < 0.05$) using LSD. Note: shoot biomass includes leaf biomass.

plant response to osmotic stress in an effort to mitigate water deficiency, it was thought that the relative differences in adaptation to salinity among the willow varieties tested would be mirrored by their adaptation to water stress (Munns 2002). An increasingly popular surrogate measure of water-use efficiency is the use of measured $^{13}C/^{12}C$ carbon isotope ratios within sampled plant leaves and the relationship between water-use efficiency and $\Delta^{13}C$ has been confirmed with willow (Weih and Nordh 2002). In a separate clonal field trial, from which the plant material for this study was collected, $\Delta^{13}C$ value-based water-use efficiency rankings of the 37 clones were determined. The assumption is that the five relatively salt-tolerant varieties in this study would have been ranked within the top 10; however, their water-use efficiency rankings were scattered, with no discernible trend among the 37 willow varieties (data not shown). The lack of correlation between water-use efficiency and salt tolerance supports the concept that root exclusion mechanisms, which minimize root uptake of antagonistic cations and anions (e.g., Na^+ and Cl^-), are the principal response to soil salinity for woody plants (Allen et al. 1994; Munns 2002).

Additionally, high soil calcium concentrations have been reported to mitigate salt stress experienced by woody plants (Rengel 1992; Maas 1993) and evidence

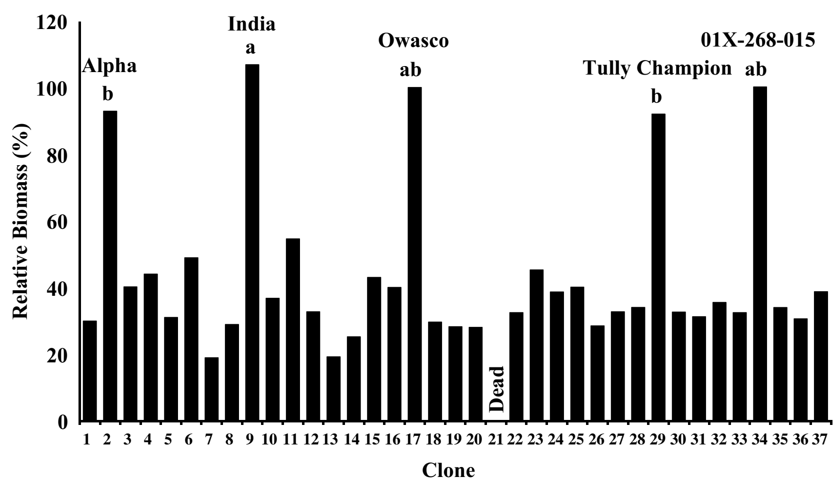

Fig. 3. Relative total biomass (i.e., shoot + root; $n = 4$) of different native and exotic willow varieties grown for 60 d in severely saline ($EC_e \leq 8.0$ dS m^{-1}) soil. Relative biomass was determined by normalizing the willow growth response to increased salinity relative to its growth on non-saline soil. See Table 2 for variety identification. Bars having the same letter are not significantly different ($P < 0.05$) using LSD. Note: shoot biomass includes leaf biomass.

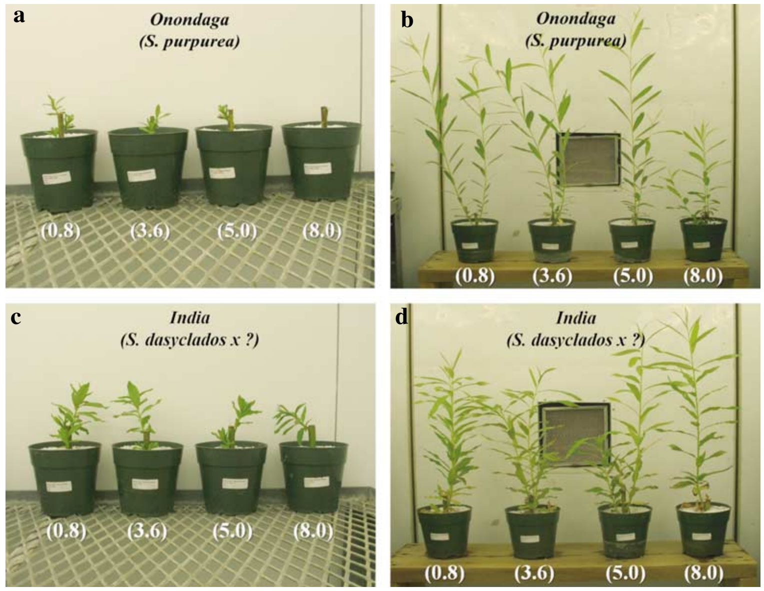

Fig. 4. The effect of increasing soil salinity (EC_e; dS m^{-1}) on growth of relatively salt intolerant (Onondaga, above) and tolerant (India, below) willow varieties after 10 d (a, c) and 60 d (b, d).

suggests a buffering ability of cytosolic Ca^{2+} to maintain cell wall membrane integrity and serve as an important intracellular secondary messenger controlling plant water relations (Suhayda et al. 1992; Anil et al. 2005; Cousson 2007). Consequently, willow varieties possessing high Ca^{2+} root uptake capacities and ability to maintain high Ca^{2+} tissue levels may be ideal for planting on these highly calcareous soils typical within the western Canadian prairies (Table 1).

Reclaiming Salt-affected Marginal lands Using Salt-tolerant Willow

From a land management point of view, the degree of saline seep expression is controlled by local climatic, hydrogeological, and agricultural factors. The potential to mitigate the aggravating effects of adverse climate and hydrogeological processes is limited. Consequently, implementing agricultural practices aimed at managing hillslope water dynamics is the only practical option available to help prevent, control, or reverse saline seep development. Specifically, cropping systems that adopt the use of deep-rooted, phreatophytic, and perennial species, such as willow, would greatly reduce the accumulation and deep percolation of available soil water lost below the rooting zone in the recharge area, (Miller et al. 1981; Henry et al. 1987; Wiebe et al. 2007). Furthermore, establishing salt-tolerant willow within seepage areas also would support the amelioration of this saline soil, by lowering of the water table in these shallow groundwater flow system discharge areas, thereby supporting leaching of the salts from the profile over time (Daniels 1987; Henry 2003). The opposite becomes apparent when willow rings around sloughs are removed, which often hastens slough-ring salinity problems by trapping less snow (i.e., reduced leaching potential) and increasing evapotranspiration-driven capillary rise and accumulation of root-zone salts (Prairie Farm Rehabilitation Administration 2000).

Future Work and Practical Considerations with Identifying Salt-tolerant Willow

Willow has a very broad genetic base, with an estimated 450 species within the genus *Salix* (Argus 1997), of which 125 species are currently being investigated for use in short-rotation intensive culture plantations (Keoleian and Volk 2005). Given that the willow varieties examined in this study were primarily hybrids among only 10 different willow species (Table 2), the apparent differences in salt tolerance observed among the relatively small number of varieties tested is promising, considering the enormous amount of untested willow genotypes available. However, the logistics involved in screening large numbers of willow genotypes for salt tolerance using pot studies is impractical. Instead, the use of in vitro screening techniques (i.e., tissue culture) need to be developed, which should be a more cost-effective and rapid method to facilitate the selection process, until genes controlling salt tolerance are identified and their associated screening tools developed. After narrowing down the number of potential candidates, further refinement in selection prior to field validation should be done using Canada's Salt Tolerance Testing Facility, which has been successfully used to assess salt tolerance among hybrid poplar varieties (Steppuhn et al. 2008). Such a facility could also determine whether the salinity tolerance responses of willow observed in this study, using sulphate-dominated soils typically found in western Canada (Table 1), are similar to chloride-dominated saline soils that are more common globally (Szabolcs 1989).

CONCLUSION

Establishing purpose-grown willow plantations with salt-tolerant varieties on salt-affected soil provides utility for otherwise non-productive land, thereby avoiding the displacement of arable land from food production. The identification of salt-tolerant willow varieties is important when considering options for reclaiming salt-affected marginal lands within western Canada, such as toe-slope areas within prairie landscapes, which preclude the growth of many annual crops. Most of the willow varieties tested in this study were able to tolerate moderately saline soils ($EC_e \leq 5$ dS m^{-1}). Furthermore, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with a severe salinity level ($EC_e \leq 8$ dS m^{-1}). However, field trials ultimately are required to validate the differences in salt tolerance among willow varieties observed in this controlled environment study.

ACKNOWLEDGEMENTS

Thanks to the Saskatchewan Ministry of Agriculture and NSERC for funding; B. Schroeder (PFRA) and T. Volk (SUNY-ESF) for the willow material; Y. Suprayogi for assisting with the statistical analyses; R. Brewster, C. Fatteicher, M. Goertz, B. Goetz, L. & R. & R. Hangs, L. Henry, T. King, S. Konecsni, M. Paré, C. Stadnyk, M. St. Arnaud, and K. Strobbe for their logistical support; and, two anonymous reviewers for their critical comments and suggestions that improved the manuscript.

Acton, D. F. and Gregorich, L. J. (eds). 1995. The health of our soils – towards sustainable agriculture in Canada. Centre for Land and Biological Resources Research, Research Branch, Agriculture and Agri-Food Canada, Ottawa, ON. 138 pp.

Allen, J. A., Chambers, J. L. and Stine, M. 1994. Prospects for increasing the salt tolerance of forest trees: a review. Tree Physiol. **14**: 843–853.

Anil, V. S., Krishnamurthy, P., Kuruvilla, S., Sucharitha, K., Thomas, G. and Mathew, M. K. 2005. Regulation of the uptake and distribution of Na^+ in shoots of rice (*Oryza sativa*) variety Pokkali: role of Ca^{2+} in salt tolerance response. Physiol. Plant. **124**: 451–464.

Argus, G. W. 1997. Infrageneric classification of Salix (*Salicaceae*) in the New World. Syst. Bot. Monogr. **52**: 1–121.

Aronsson, P., Dahlin, T. and Dimitriou, I. 2010. Treatment of landfill leachate by irrigation of willow coppice – Plant response and treatment efficiency. Environ. Pollut. **158**: 795–804.

Berndes, G., Hoogwijk, M. and van den Broek, R. 2003. The contribution of biomass in the future global energy supply: a review of 17 studies. Biomass Bioenerg. **25**: 1–28.

Burgess, D., Hendricksson, O. Q. and Roy, L. 1990. The importance of initial cutting size for improving the growth performance of *Salix alba* L. Scand. J. For. Res. **5**: 215–224.

Chen, S. J., Jinke, L., Fritz, E., Wang, S. and Huttermann, A. 2002. Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. Forest Ecol. Manage. **168**: 217–230.

Cousson, A. 2007. Two calcium mobilizing pathways implicated within abscisic acid-induced stomatal closing in *Arabidopsis thaliana*. Biol. Plant. **51**: 285–291.

Coyle, D. R. and Coleman, M. D. 2005. Forest production responses to irrigation and fertilization are not explained by shifts in allocation. Forest Ecol. Manage. **208**: 137–152.

Cuddihy, J., Kennedy, C. and Byer, P. 2005. Energy use in Canada: environmental impacts and opportunities in relationship to infrastructure systems. Can. J. Civ. Eng. **32**: 1–15.

Daniels, R. B. 1987. Saline seeps in the Northern Great Plains of the USA and the southern prairies of Canada. Pages 381–404 *in* M. G. Wolman and F. G. A. Fournier, eds. Land transformation in agriculture. John Wiley & Sons Ltd., Chichester, UK. 531 pp.

Dumanski, J., Coote, D., Lucerek, G. and Lok, C. 1986. Soil conservation in Canada. J. Soil Water Conserv. **41**: 204–210.

Eilers, R. G., Eilers, W. D., Pettapiece, W. W. and Lelyk, G. 1995. Salinization of soil. Pages 77–86 *in* D. F. Acton and L. J. Gregorich, eds. The health of our soils – towards sustainable agriculture in Canada. Centre for Land and Biological Resources Research, Research Branch, Agriculture and Agri-Food Canada, Ottawa, ON. 138 pp.

Henry, J. L. 2003. Henry's handbook of soil and water. Henry Perspectives, Saskatoon, SK. 218 pp.

Henry, J. L., Harron, B. and Flaten, D. 1987. The nature and management of salt-affected land in Saskatchewan. Saskatchewan Agriculture. Agdex 518. 23 pp.

Henry, J. L., Bullock, P. R., Hogg, T. J. and Luba, L. D. 1985. Groundwater discharge from glacial and bedrock aquifers as a soil salinization factor in Saskatchewan. Can. J. Soil Sci. **65**: 749–768.

Hogg, T. J. and Henry, J. L. 1984. Comparison of 1:1 and 1:2 suspensions and extracts with the saturation extract in estimating salinity in Saskatchewan soils. Can. J. Soil Sci. **64**: 699–704.

Holm, H. M. and Henry, J. L. 1982. Understanding salt-affected soils. Plant Industry Branch, Saskatchewan Agriculture, Regina, SK. 14 pp.

Keller, C. K. and Van der Kamp, G. 1988. Hydrogeology of two Saskatchewan tills. II. Occurrence of sulfate and implications for soil salinity. J. Hydrol. **101**: 123–144.

Keoleian, G. A. and Volk, T. A. 2005. Renewable energy from willow biomass crops: life cycle energy, environmental and economic performance. Crit. Rev. Plant Sci. **24**: 385–406.

Kozlowski, T. T. 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monograph No. 1. 29 pp.

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

Liu, L., McDonald, A. J. S., Stadenberg, I. and Davies, W. J. 2001. Abscisic acid in leaves and roots of willow: significance for stomatal conductance. Tree Physiol. **21**: 759–764.

Maas, E. V. 1993. Salinity and citriculture. Tree Physiol. **12**: 195–216.

Miller, M. R., Brown, P. L. and Donovan, J. J. 1981. Saline seep development and control in the North American Great Plains – hydrogeological aspects. Agric. Water Manage. **4**: 115–141.

Mirck, J. and Volk, T. A. 2010. Response of three shrub willow varieties (*Salix* spp.) to stormwater treatments with different concentrations of salts. Bioresource Technol. **101**: 3484–3492.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. **25**: 239–250.

Ngantcha, A. C. 2010. DNA fingerprinting and genetic relationships among willow (*Salix* spp.). M.Sc. thesis, Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK. 87 pp.

Prairie Farm Rehabilitation Administration. 2000. Prairie agricultural landscapes: A land resource review. PFRA, Regina, SK. 196 pp.

Rengel, Z. 1992. The role of calcium in salt toxicity. Plant Cell Environ. **15**: 625–632.

Rennie, D. A. and Ellis, J. G. 1978. The shape of Saskatchewan. Saskatchewan Institute of Pedolgy, Publication M41, University of Saskatchewan, Saskatoon, SK. 62 pp.

Saskatchewan Centre for Soil Research. 1985. The soils of the Swift Current map area, Number 72J. SCSR-Soil Survey Staff, University of Saskatchewan, Saskatoon, SK.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243–1246 *in* Proc. 23rd SAS Users Group Intl. SAS Institute, Cary, NC.

Steppuhn, H., Kort, J. and Wall, K. G. 2008. First year growth response of selected hybrid poplar cuttings to root-zone salinity. Can. J. Plant Sci. **88**: 473–483.

Stolarska, A., Wróbel, J. and Przybulewska, K. 2008. Free proline content in leaves of Salix *viminalis* as an indicator of their resistance to substrate salinity. Ecol. Chem. Eng. **15**: 139–146.

Suhayda, C. G., Redmann, R. E., Harvey, B. L. and Cipywnyk, A. L. 1992. Comparative response of cultivated and wild barley species to salinity stress and calcium supply. Crop Sci. **32**: 154–163.

Szabolcs, I. 1989. Salt-affected soils. CRC Press, Boca Raton, FL. 274 pp.

Van Rees, K. C. J. 2008. Developing a national agroforestry and afforestation network for Canada. Policy Options **29**: 54–57.

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

Wiebe, B. H., Eilers, R. G., Eilers, W. D. and Brierley, J. A. 2007. Application of a risk indicator for assessing trends in dryland salinization risk on the Canadian Prairies. Can. J. Soil Sci. **87**: 213–224.

Weih, M. and Nordh, N. E. 2002. Characterising willows for biomass and phytoremediation: growth, nitrogen and water use of 14 willow clones under different irrigation and fertilisation regimes. Biomass Bioenergy **23**: 397–413.

Wrobel, J., Mikiciuk, M. and Stolarska, A. 2006. Effect of salt soil stress on gas exchange in three forms of basket willow (*Salix viminalis* L.) Adv. Agric. Sci. Prob. **509**: 269–281.