Adventitious sprouting of *Pinus leiophylla* in response to salt stress

Marcos Jimenez-Casas, Janusz J. Zwiazek

► To cite this version:

Marcos Jimenez-Casas, Janusz J. Zwiazek. Adventitious sprouting of *Pinus leiophylla* in response to salt stress. *Annals of Forest Science*, 2014, 71 (7), pp.811-819. 10.1007/s13595-014-0379-z . hal-01102891

HAL Id: hal-01102891

<https://hal.science/hal-01102891>

Submitted on 13 Jan 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Adventitious sprouting of *Pinus leiophylla* in response to salt stress

Marcos Jimenez-Casas · Janusz J. Zwiazek

Received: 29 January 2014 / Accepted: 14 April 2014 / Published online: 1 May 2014
© INRA and Springer-Verlag France 2014

Abstract

• **Context** Although adventitious shoots are produced in many tree species in response to injury, little is known about the effects of salinity on sprouting.
• **Aims** The main objective was to examine the sprouting capacity of *Pinus leiophylla* seedlings in relation to injury and physiological changes induced by NaCl.
• **Methods** Seedlings were grown in controlled-environment growth rooms and treated with 0, 100, 150, and 200 mM NaCl. Numbers of adventitious shoots were recorded and growth and physiological parameters measured after 64 days of treatments and following a 30-day recovery period.
• **Results** NaCl treatments triggered sprouting of adventitious shoots, mainly in the lower parts of the stems. However, fewer sprouts were induced by 200 mM NaCl compared with the lower concentration treatments. These changes were accompanied by needle necrosis, decreased chlorophyll concentrations, seedling dry mass, and stem diameter. Stomatal conductance, net photosynthesis, and root hydraulic conductance decreased with increasing NaCl concentrations and did not return to the control levels after 30 days of stress relief.
• **Conclusions** *Pinus leiophylla* has the regeneration potential when exposed to salinity. However, very high salt

concentrations induce severe physiological impairments and, consequently, a decrease of this regeneration potential.

Keywords Adventitious shoots · Gas exchange · Root hydraulic conductance · Salt stress

1 Introduction

Conversion of forests to agricultural lands used for annual crop production has intensified in the recent years in Central Mexico (Masera 1996; Alix-García et al. 2005). Between 1970 and 1990, agricultural lands increased by 39 % and the area dedicated to pasture more than doubled, while forests declined by 13 % (Segura 2000). Land conversion has had a major impact on hydrological cycle and salinization. It also increased soil compaction and erosion, decreased soil fertility, diminished natural plant regeneration, and promoted the establishment of undesirable weedy plants (Bettenay 1986; Ezcurra 1991; Rengasamy 2006). Earlier reports estimated the salinization of agricultural soils in Mexico at about 1.5 million hectares (Ortiz 1993). These areas are considered unsuitable for intensive agriculture and are frequently abandoned. Intensive reclamation efforts which include selection of salt resistant species are required prior to their subsequent reforestation.

Pinus leiophylla Schl. et Cham. is a widely distributed tree species in Mexico with high potential for the reclamation of degraded land due to its adaptability to different environments and its tolerance of stress conditions (Eguiluz-Piedra 1978; Musálem and Martínez-García 2003). *P. leiophylla* has been used in experimental plantations for erosion control and soil conservation purposes in Asia, Africa, Caribbean, South America, and Oceania (Rodríguez-Franco 2002).

Unlike many other pine species, *P. leiophylla* develops sprouts from the root collar and along the trunk and branches in response to defoliation, fire, pruning, and other stress

Handling Editor: Erwin Dreyer

Contribution of the co-authors Jimenez-Casas carried out the PhD research project and drafted the manuscript. Zwiazek co-designed and supervised the research and prepared the manuscript for submission.

M. Jimenez-Casas · J. J. Zwiazek (✉)
Department of Renewable Resources, University of Alberta,
4-42 Earth Sciences Bldg., Edmonton, AB, Canada T6G 2E3
e-mail: janusz.zwiazek@ualberta.ca

Present Address:

M. Jimenez-Casas
Especialidad Forestal, Colegio de Postgraduados, Km. 36.5 Carretera
México-Texcoco Montecillo Edo, de México 56230, Mexico

factors (Lanner 2002; Rodríguez-Trejo and Fulé 2003). This attribute is considered an important survival strategy under conditions which cause injury to aerial parts of trees and this strategy may increase tree survival in disturbed areas (Del Tredici 2001).

In trees, sprouts can be derived from epicormic buds, which fail to flush during the growth season and remain dormant in the bark, or from adventitious buds, which may be formed from different living tissues as a result of injury (Quine 2004; Moreira et al. 2009). Although the origin of sprouts in *P. leiophylla* is not known, they have been reported to form mostly at the root collar and to decrease with tree height (Del Tredici 2001) suggesting that they are of adventitious origin, produced as a result of wounding and environmental stimuli. The formation of adventitious buds may depend on factors such as the extent of injury, hormonal status, and accumulation of carbohydrates in the parenchyma cells of sapwood (Lanner 2002; Del Tredici 2001; Climent et al. 2004). Mature parenchyma cells may resume division in response to these external factors and revert to an undifferentiated meristem which gives rise to adventitious shoots in various parts of the plant (Esau 1977; Bowes 1996).

We have earlier demonstrated that direct toxicity and needle injury were largely responsible for the reduction of photosynthetic rates in *P. leiophylla* subjected to salt stress (Jimenez-Casas and Zwiazek 2013). However, shoot apical meristems in *P. leiophylla* appeared to be protected from salt injury even in the highest studied NaCl concentrations. Therefore, it is plausible that *P. leiophylla* may have effective mechanisms allowing the trees to regenerate and survive potentially lethal exposures to salinity. Tree regeneration through adventitious bud flushing requires a functional root system and vascular tissues and is more common in trees suffering from mechanical injury and traumatic events which affect hormonal distribution in trees (Del Tredici 2001). Although salt injury is mostly manifested in foliage it is usually systemic and affects different tissues and organs (Hasegawa et al. 2000). Therefore, in addition to hormonal and nutritional balance, salt injury may affect sprouting potential by affecting the processes required to support the growth of adventitious buds. We expected that sprouting and growth of adventitious shoots would be reduced in plants severely injured by salt.

In the present study, we investigated the effects of NaCl treatment concentrations on growth, needle injury, and physiological parameters in *P. leiophylla* seedlings, including gas exchange and root hydraulic conductance in relation to sprouting. We examined the hypotheses that (1) NaCl-induced injury would promote the formation of adventitious shoots in *P. leiophylla* seedlings and (2) severe impact on physiological processes by high concentration NaCl treatments would diminish the sprouting capacity of seedlings.

2 Materials and methods

2.1 Plant material and growth conditions

Seeds of *Pinus leiophylla* Schl. et Cham. were collected from open-pollinated trees growing in Tlaxcala State-Mexico (19°19' N and 98°00' W). The seeds were soaked in running water for 24 h and placed at 4 °C for another 24 h to enhance germination. The seeds were germinated in the dark at 26 °C in Petri dishes containing moist sand. After radicle emergence from the seed coat, seedlings were transferred to Spencer-Lemaire root trainers (170 cm³ vol, Spencer-Lemaire Industries Ltd., Edmonton, AB, Canada) filled with a mixture of peat moss and sand (2:1 by volume). The seedlings were grown for 14 months in a controlled-environment growth room set to 75 % relative humidity, 24/18 °C (day/night) temperature, 18-h photoperiod, and photosynthetic photon flux (PPF) of approximately 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the seedling level. Seedlings were watered twice per week and fertilized every 2 weeks with modified Hoagland's solution (Epstein 1972), containing 224 mg L⁻¹ N, 235 mg L⁻¹ K, 160 mg L⁻¹ Ca, 62 mg L⁻¹ P, 32 mg L⁻¹ S, 24 mg L⁻¹ Mg, 1.77 mg L⁻¹ Cl, 0.27 mg L⁻¹ B, 0.11 mg L⁻¹ Mn, 0.131 mg L⁻¹ Zn, 0.032 mg L⁻¹ Cu, 0.05 mg L⁻¹ Mo, and 1.12 mg L⁻¹ Fe. The nutrients were supplied by placing the containers with seedlings in 25-L tubs and soaking the soil for 4 h. After 14 months, the plants were transferred to 4-L pots using the same substrate and growth conditions as described above.

2.2 NaCl treatments

Sixteen-month-old dormant *P. leiophylla* plants were subjected to 0 (control), 100, 150, and 200 mM NaCl treatments (0.71, 10.3, 15.1, 19.6 dS m⁻¹ electrical conductivity, respectively). All treatments were prepared in distilled water. The treatments were applied by soaking the pots for 24 h every 3 days. All plants were fertilized every 15 days with the modified Hoagland's solution as above. Leachate electrical conductivity of randomly selected pots was checked every 8 days, and the soil was flushed with distilled water every 10 days to prevent salt accumulation. The treatments were applied randomly among the blocks of four plants and were replicated three times resulting in 12 plants per treatment for the total of 48 plants. The treatments were carried out for 64 days followed by a recovery period of 30 days. At the start of the recovery period, the soil was flushed with distilled water and all plants were irrigated with water and supplied with modified Hoagland's solution without NaCl. During the recovery period, soil electrical conductivity varied between 0.80 and 1.03 dS m⁻¹.

2.3 Determinations of biomass, growth, and adventitious shoot sprouting

Stem diameter at the root collar and terminal shoot lengths were measured at the end of NaCl treatments and after the recovery period. Adventitious shoots greater than 2-cm in length were counted at the end of the recovery period. The plants were harvested at the end of the recovery period, their roots and shoots were separated, and new needles (grown after the onset of NaCl treatment) were removed from the stems. The tissues were then dried at 70 °C (24 h for roots and 48 h for stems and needles) for dry mass determinations.

2.4 Determinations of survival, needle injury, and chlorophyll content

Needle injury and seedling survival were monitored daily. At the end of the recovery period, necrotic parts of the needles were separated from the green, healthy, tissues. The necrotic and green needle parts were then dried at 70 °C for 24 h and weighed. The proportion of needle necrosis was calculated from the dry weights of green and necrotic needle tissues. Plants were considered dead when needle necrosis was above 70 %, shoot apical meristems were necrotic and there was no new needle growth.

Needle chlorophyll contents in all plants were determined at the end of NaCl treatments according to Hiscox and Israelstam (1979). Six needles were randomly sampled from each part of the shoot (top, middle, and bottom), cut into small pieces and mixed. Tissue samples, each containing 100 mg of needles, were extracted with 7 ml DMSO at 65 °C for 25 min. The extracts were diluted with 7 ml DMSO and the total chlorophyll concentrations were determined spectrophotometrically. Chlorophyll a and b were calculated using the Arnon's equation (Arnon 1949).

2.5 Measurements of gas exchange and root hydraulic conductance

Net photosynthesis (P_n) and stomatal conductance (g_s) were measured every 8 days during 64 days of NaCl treatments and after the recovery period of 30 days. For the measurements, six seedlings per treatment were randomly taken, and four fascicles per seedling were measured. Each fascicle with five fully grown needles was selected approximately 3 cm below the terminal shoot apex. Prior to the measurements, the pots with plants were soaked overnight in water to the field capacity. The measurements started two hours after the onset of photoperiod and were taken with a portable infrared gas analyzer equipped with the conifer sample chamber (LCA4, Analytical Development Company Limited, Hoddesdon, UK). During the measurements, leaf temperature in the

Table 1 Root, stem, needle, and total dry mass of *P. leiophylla* seedlings subjected to NaCl treatments for 64 days and control plants (0 mM NaCl). Means ($n=12\pm SE$) with different letters in the same column indicate significant differences at $\alpha=0.05$ level as determined by the Duncan's standardized range test

[NaCl] mM	Roots (g)	Stems (g)	Needles (g)	Total (g)
0	39.8 $\pm$ 11.3 a	14.8 $\pm$ 2.36 a	46.8 $\pm$ 4.82 a	101.4 $\pm$ 8.86 a
100	21.9 $\pm$ 3.14 b	9.64 $\pm$ 1.72 b	37.9 $\pm$ 6.36 b	69.6 $\pm$ 5.35 b
150	15.6 $\pm$ 3.35 c	6.25 $\pm$ 0.86 c	30.9 $\pm$ 3.56 c	53.0 $\pm$ 5.76 c
200	10.7 $\pm$ 1.95 c	5.96 $\pm$ 0.55 c	24.2 $\pm$ 4.37 d	40.9 $\pm$ 3.47 d

sample chamber was 24 ± 1 °C and PPF was $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Needle surface areas were obtained after computer scanning and calculated using Sigma Scan Pro 5.0 software (SPSS, Jandel Scientific, San Rafael, CA, USA).

After the recovery period, the pots with plants were soaked overnight in water to minimize the effects of possible soil moisture differences for the maximum hydraulic conductance and the next morning, they were moved to the laboratory for the measurements of root hydraulic conductance (K_r) (Tyree et al. 1995). For K_r measurements, the shoots were excised about 3 cm above the root collar, and the roots were connected through the cut stem to the high-pressure flow meter (HPFM) via a piece of flexible high-pressure tubing. The

Fig. 1 Effects of 0 (control), 100, 150, and 200 mM NaCl on stem diameters and terminal shoot lengths of seedlings after 64 days of treatments and after the recovery period. Bars are means ($n=12$) $\pm$ SE. Different letters indicate significant differences at $\alpha=0.05$ determined by the Duncan's test

Table 2 Numbers of adventitious shoots per plant and percentages of plants with adventitious shoots (in brackets) present at the root collar, main stem, and branches; and dry weights of new needles in seedlings

[NaCl] mM	Number of adventitious shoots			New needles	
	Root collar	Main stem	Branches	Total	(g)
0	1.58±1.13 b (75)	2.61±2.15 b (67)	0.333±0.651 b (25)	4.60	15.6±1.57 a
100	7.33±1.71 a (100)	11.6±2.11 a (100)	4.58±1.56 a (100)	23.5	9.66±1.14 b
150	7.83±2.12 a (100)	12.7±1.93 a (100)	4.33±1.49 a (100)	24.9	4.93±1.13 c
200	7.16±2.75 a (100)	3.63±5.31 b (33)	1.50±2.46 b (33)	12.2	0.969±0.42 d

subjected to 0 (control), 100, 150, and 200 mM NaCl. Means ($n=12\pm SE$) in each column followed by different letters are significantly different at $\alpha=0.05$ level as determined by the Duncan's standardized range test

measurements were carried out in the transient mode with the roots pressurized up to 0.5 MPa (Calvo-Polanco et al. 2009).

2.6 Statistical analysis

The data analysis was performed with SAS-GLM procedure, and means of treatments were compared by Duncan's range test. P_n and g_s were analyzed by repeated measurements ANOVA using the split-plot design with time (weeks) as a subplot effect. The analysis was conducted using the PROC MIXED-SAS/PC for Windows version 8.2 (SAS institute Inc. 2001).

3 Results

3.1 Biomass, growth, and adventitious shoot sprouting

After 64 days of treatments, shoot and root dry mass of plants treated with 100 mM, 150 mM, and 200 mM NaCl was significantly lower compared with the control, untreated plants (Table 1). Shoot and root dry mass of treated plants decreased with increasing NaCl treatment concentrations (Table 1).

Terminal shoot length and stem diameter growth of treated plants significantly decreased with increasing NaCl concentration compared with control plants (Fig. 1).

Plants treated with NaCl showed a significant increase in the adventitious shoot production (Table 2). Sprouting of adventitious shoots in plants was first observed 40 days after the start of NaCl treatments. The first adventitious shoots were produced at the base of the stem near the root collar (Fig. 2), while sprouting of adventitious shoots from branches occurred towards the end of NaCl treatments. About 33 % of plants treated with 200 mM NaCl produced adventitious shoots compared with 100 % of seedlings that were treated with 100 and 150 mM NaCl (Table 2). Sprouting density was higher in the upper parts of the terminal shoot compared with the base of the terminal shoot or branches (Table 2). The production of needles was significantly reduced by increasing NaCl concentrations. The total dry mass of

new needles was reduced by as much as 94 % in the 200 mM NaCl treatment (Table 2).

3.2 Survival, needle injury, and chlorophyll content

Seedlings which were subjected to 0, 100, and 150 mM NaCl for 64 days showed 100 % survival compared with 67 % survival for plants treated with 200 mM NaCl (Table 3). Injury symptoms started appearing on older needles after 24 days of treatment, first as needle tip chlorosis followed by the appearance of necrotic lesions about 10 days later. Although needle injury was observed in all NaCl-treated plants, the extent of injury was the greatest in plants exposed to 200 mM NaCl with 80 % of needle weight affected by necrosis (Table 3). The seedlings treated with 200 mM NaCl also had the lowest chlorophyll needle concentrations with chlorophyll a and b reduced to approximately 42 and 13 %, respectively, of the control level (Table 3). Plants treated with 100 mM NaCl had the chlorophyll a and b concentrations reduced to 71 and 81 %

Fig. 2 Adventitious shoots in the *Pinus leiophylla* seedling treated with 150 mM NaCl for 64 days

Table 3 Needle chlorophyll concentration ($n=6$), needle necrosis and survival ($n=12$) of seedlings subjected to NaCl treatments for 64 days and in control plants (0 mM NaCl). Means $\pm$ SE are shown. In each column,different letters indicate significant differences at $\alpha=0.05$ as determined by the Duncan's test

[NaCl] mM	Chlorophyll (mg g ⁻¹)			Needle necrosis (% needle weight)	Survival (%)
	a	b	Total		
0	0.720 $\pm$ 0.019 a	0.476 $\pm$ 0.013 a	1.19 $\pm$ 0.028 a	0	100
100	0.518 $\pm$ 0.022 a	0.387 $\pm$ 0.012 b	0.865 $\pm$ 0.025 b	39.9 $\pm$ 9.23 c	100
150	0.370 $\pm$ 0.046 c	0.180 $\pm$ 0.012 c	0.551 $\pm$ 0.055 c	63.2 $\pm$ 7.21 b	100
200	0.304 $\pm$ 0.051 d	0.062 $\pm$ 0.016 d	0.367 $\pm$ 0.059 d	80.4 $\pm$ 6.96 a	33.3

of the control level and with 40 % of needle necrosis (Table 3). Control plants had no needle necrosis (Table 3).

not measured due to low seedling survival and high extent of needle injury.

3.3 Gas exchange and root hydraulic conductance

Both P_n and g_s significantly decreased over time with increasing NaCl concentration treatments overtime (Table 4), and the most rapid decreases occurred in 150 and 200 mM NaCl compared with 100 mM NaCl (Fig. 3 a, b). At the end of the treatments, the g_s values of plants treated with 100 and 150 mM were approximately 70 and 90 %, respectively, lower than those of control plants (0 mM NaCl) (Fig. 3a). The g_s of plants treated with 200 mM NaCl treatment was completely inhibited at the end of treatment (Fig. 3a). At the end of the recovery period, the g_s values slightly increased in NaCl-treated plants, but were still several fold lower compared with untreated controls (Fig. 4a). The P_n values of treated plants followed similar patterns as those shown by g_s (Fig. 3b). At the end of the NaCl treatments, the P_n values of plants treated with 100, 150, and 200 mM were 42, 64, and 84 % lower, respectively, than those of control plants.

Similarly to g_s P_n , and K_r values did not return to the control levels after 30 days of stress relief (Fig. 4b, c). The recovery of P_n , g_s , and K_r in plants treated with 200 mM was

Table 4 F values in the analysis of variance of the effects of NaCl (Sal), block (B), and treatment duration (time) on net photosynthesis (P_n) and stomatal conductance (g_s) in plants treated for 64 days

Source	DF	Gas exchange	
		P_n	g_s
Sal	3	745.8**	901.7**
B	2	1.1	4.0
Sal X B	6	0.9	3.3
Time	7	209.9**	497.5**
Time X Sal	21	42.3**	43.6**
Time X B	14	1.0	0.8
Time X B X Sal	42	1.1	0.7

**Significance level, $P \leq 0.01$

4 Discussion

In our study, adventitious sprouting was observed at the stem base, main stem, and branches of all NaCl-treated *P. leiophylla* seedlings. This points to the ability of this species to sprout in response to salt injury similarly to trees damaged by fire (Perry 1991) and herbivory (Jasso-Mata and Jiménez-Casas 2001). Sprouting is considered as part of the plant regeneration strategy after damage caused by biotic and abiotic factors (Vest and Westoby 2004; Wan et al. 2007). Sprouting of adventitious shoots is thought to be related to apical dominance with auxins and cytokinins likely involved in the control of these processes (Cline 1991; Wan et al. 2006). On the other hand, the sprouting ability of sugar maple, red alder, giant sequoia, Canary Islands pine, and other tree species has been explained by the carbohydrate reserves present in sapwood parenchyma (Mooney and Hayes 1973; Harrington 1984; Climent et al. 2004; O'Hara et al. 2008).

Both 100 and 150 mM NaCl were more effective than 200 mM NaCl in stimulating sprouting of adventitious shoots from the lateral branches and from the upper parts of the main stem, but the number of adventitious branches that were produced from the stem base was similar for all NaCl treatments (Table 2). The sprouting from the stem base also took place in seedlings which suffered extensive damage to the shoots. Similar findings were reported for *Arbutus arizonica* where sprouting capacity decreased as a result of shoot injury severity that was caused by fire (Barton 2005). However, in *Sequoia gigantea*, sprouts were more frequent and larger on more severely pruned trees (O'Hara et al. 2008). These results indicate that the frequency and number of sprouts vary according to the severity and type of stress. The inhibition of photosynthesis by salt stress may severely reduce the stored carbohydrates. The declines in tree productivity may take a long time to recover as evidenced in the present study. The slow photosynthetic recovery is likely partly due to the effect

Fig. 3 Changes in net photosynthesis (P_n) and b stomatal conductance (g_s) in seedlings during 64 days of 0 (control), 100, 150, and 200 mM NaCl treatments. Each point represents mean ($n=6$) $\pm$ SE

of NaCl on needle chlorophyll concentrations which may need more time to recover compared with the stomatal responses. In our study, accumulation of Na and Cl in tissues and the injury level in plants treated with 200 mM NaCl likely affected the ability of plants to mobilize carbohydrates which, in turn, reduced the number of sprouts in stems and branches but not in the root collar section. Root collar is a storage place of starch reserves in many conifers (White 1981; Chomba et al. 1993). Therefore, buds close to the root collar had likely higher concentrations of available carbohydrate reserves.

The effects of salinity on plants are associated with ion toxicity and low osmotic potential which triggers stomatal closure and decreases photosynthetic rates (Allen et al. 1994; Hasegawa et al. 2000; Ashraf and Harris 2004). The reduction of P_n and g_s in NaCl-treated *P. leiophylla* plants increased with increasing NaCl concentrations and progressed over time. It is interesting that P_n values in plants treated with 200 mM NaCl for 56 and 64 days were very low, but still positive and g_s was near 0. Although this could be explained by minor instrument calibration issues, the changes in P_n and g_s showed a clear NaCl concentration-dependent pattern of decline in both parameters over time. Similar responses were observed in plants of *P. pinaster*, *Arbutus unedo*, and *Aster tripolium* subjected to a NaCl gradient where P_n and g_s also

was reduced progressively by NaCl in a concentration-dependent manner (Loustau et al. 1995; Robinson et al. 1997; and Navarro et al. 2007).

Compared with other studied conifers (Fostad and Pedersen 2000; Apostol and Zwiazek 2003; Franklin and Zwiazek 2004; Renault 2005), *P. leiophylla* seedlings showed a high degree of salt tolerance with no seedling mortality in NaCl concentrations as high as 150 mM after 64 days of treatment. Needle chlorosis and necrosis in *P. leiophylla* were likely related to Na and Cl tissue concentrations. In tamarack and jack pine treated with NaCl, the increase in needle concentrations of Na and Cl corresponded to the applied NaCl concentrations and were correlated with the extent of needle injury (Apostol et al. 2002; Renault 2005; Calvo Polanco et al. 2008).

The reduced levels of P_n , g_s , and K_r in NaCl-treated plants at the end of the recovery period indicate that seedlings may require a relatively long time to fully recover from the effects of salt and this may affect the ability of plants to produce new roots and needles to replace the biomass loss caused by salinity. The loss of root tissue was likely among the main factors responsible for the reduction of K_r . Contrary to drought, salinity effects on gas exchange and water transport may be more persistent due to Na uptake. The principal mechanism of salt tolerance in many woody plants is Na

Fig. 4 Stomatal conductance (g_s) (a), net photosynthesis (P_n) (b), and root hydraulic conductance (K_r) (c) in seedlings subjected for 64 days to 0 (control), 100, and 150 mM NaCl treatments followed by 30 days of recovery. Bars are means ($n=6$) $\pm$ SE. Different letters indicate significant differences at $\alpha=0.05$ determined by the Duncan's test

exclusion from the shoot tissues through its sequestration in roots (Renault et al. 2001; Muhsin and Zwiazek 2002). Therefore, in severely salt-stressed plants, the presence of Na in shoots hampers the gas exchange recovery processes. Although in plants subjected to 200 mM NaCl, the roots were too severely damaged to measure K_r , the injury patterns displayed in response to 100 and 150 mM NaCl indicate that the increasing NaCl concentrations reduced the ability of the root system to deliver water to the shoots. Therefore, the delivery of water and mineral nutrients could also be a restricting factor to growth of adventitious shoots in high NaCl concentrations. In less injured plants, the adventitious branch sprouting in response to NaCl may contribute to the production of new photosynthetic tissues and consequently

improve P_n levels to increase the photosynthate production needed for the re-growth of plants injured by salinity.

In conclusion, NaCl treatments stimulated adventitious sprouting in *P. leiophylla* seedlings and caused reductions in growth, P_n , g_s , and K_r . In 100 and 150 mM NaCl treatments, the increased adventitious sprouting was associated with an increase in the extent of needle injury. However, in the highest concentration, 200 mM NaCl treatment, the production of adventitious shoots decreased in all parts of the seedlings with the exception of root collar. The results indicate that *P. leiophylla* has the potential to regenerate even after exposure to relatively high NaCl concentrations. However, at high levels of NaCl injury, the regeneration potential is likely to be affected by the physiological effects of NaCl including

decreased photosynthetic productivity and the resulting depletion of stored energy.

Acknowledgments We thank the Natural Sciences and Engineering Research Council of Canada and the Mexican Consejo Nacional de Ciencia y Tecnología (CONACyT) and Colegio de Postgraduados for the financial support of this study.

References

- Alix-García J, De Janvry A, Sadoulet E (2005) A Tale of two communities: explaining deforestation in Mexico. *World Dev* 33:219–235
- Allen JA, Chambers JL, Stine M (1994) Prospects for increasing the salt tolerance of forest trees: a review. *Tree Physiol* 14:843–853
- Apostol GK, Zwiazek JJ (2003) Hypoxia affects root sodium and chloride concentrations and alter water conductance in salt-treated jack pine (*Pinus banksiana*) seedlings. *Trees* 17:251–257
- Apostol GK, Zwiazek JJ, MacKinnon DM (2002) NaCl and Na₂SO₄ alter responses of jack pine (*Pinus banksiana*) seedlings to boron. *Plant Soil* 240:321–329
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15
- Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Sci* 166:3–16
- Barton AM (2005) Response of *Arbutus arizonica* (Arizona madrone) to fire in Southeastern Arizona. *Southwest Nat* 50:7–11
- Bettenay E (1986) Salt affected soils in Australia. *Reclam Reveget Res* 5: 167–179
- Bowes BG (1996) A color atlas of plant structure. Manson Publishing Ltd, London
- Calvo Polanco M, Zwiazek JJ, Jones MD, MacKinnon MD (2008) Responses of mycorrhizal jack pine (*Pinus banksiana*) seedlings to NaCl and boron. *Trees* 22:825–834
- Calvo-Polanco M, Jones MD, Zwiazek JJ (2009) Effects of pH on NaCl resistance of American elm (*Ulmus americana*) seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. *Acta Physiol Plant* 31:515–522
- Chomba BM, Guy RD, Weger HG (1993) Carbohydrate reserve accumulation and depletion in Engelmann spruce (*Picea engelmannii* Parry): effects of cold storage and pre-storage CO₂ enrichment. *Tree Physiol* 13:351–364
- Climent J, Tapias R, Pardos JA, Gil L (2004) Fire adaptations in the Canary Islands pine (*Pinus canariensis*). *Plant Ecol* 171:185–196
- Cline MG (1991) Apical dominance. *Bot Rev* 57:318–358
- Del Tredici P (2001) Sprouting in temperate trees: a morphological and ecological review. *Bot Rev* 67:121–140
- Eguiluz-Piedra T (1978) Ensayo de integración de los conocimientos sobre el género *Pinus* en México. Dissertation Universidad Chapingo, México
- Epstein E (1972) Mineral nutrition of plants: principle and perspectives. Wiley, New York
- Esau K (1977) Anatomy of seed plants. Wiley, New York
- Ezcurra E (1991) The basin of Mexico. In: Turner BL (ed) The earth as transformed by human action: global and regional changes in the biosphere over the past 300 years. Cambridge, New York, pp 577–588
- Fostad O, Pedersen PA (2000) Container-grown tree seedling responses to sodium chloride applications in different substrates. *Environ Pollut* 109:203–210
- Franklin JA, Zwiazek JJ (2004) Ion uptake in *Pinus banksiana* treated with sodium chloride and sodium sulfate. *Physiol Plant* 120:482–490
- Harrington CA (1984) Factors influencing sprouting of red alder. *Can J For Res* 14:357–361
- Hasegawa PM, Bressan RA, Zhu J, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annu Rev Plant Physiol Plant Mol Biol* 51:463–499
- Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57:1332–1334
- Jasso-Mata J, Jiménez-Casas M (2001) Resistencia y control de una plaga en un huerto semillero sexual de *Pinus leiophylla* Schl. et Cham. In Resúmenes: V Congreso Mexicano de Recursos Forestales. 7-9 Nov. Guadalajara, Jal. México 237 pp
- Jimenez-Casas M, Zwiazek JJ (2013) Effects of branch pruning and seedling size on total transpiration and tissue Na and Cl accumulation in *Pinus leiophylla* seedlings exposed to salinity. *Forest Sci* 59: 407–415
- Lanner RM (2002) Why do trees live so long? *Ageing Res Rev* 1:653–671
- Loustau D, Crepeau S, Guye GM, Sartore M, Saur E (1995) Growth and water relations of three geographically separate origins of maritime pine (*Pinus pinaster*) under saline conditions. *Tree Physiol* 15:569–576
- Masera O (1996) Desforestación y Degradación Forestal en México. Grupo Interdisciplinario de Tecnología Rural Apropiada GIRA AC. Michoacán, México
- Mooney JA, Hayes RI (1973) Carbohydrate storage cycles in two California Mediterranean-climate trees. *Flora*, Jena 162:295–304
- Moreira F, Catry F, Duarte I, Acácio V, Silva JS (2009) A conceptual model of sprouting responses in relation to fire damage: an example with cork oak (*Quercus suber* L) trees in Southern Portugal. *Plant Ecol* 201:77–85
- Muhsin T, Zwiazek JJ (2002) Ectomycorrhizae increase water conductance and protect white spruce (*Picea glauca*) seedlings against salt stress. *Plant Soil* 238:217–225
- Musálem MA and Martínez-García S (2003) Monografía de *Pinus leiophylla* Schl. et Cham. Proyecto de investigación manejo sustentable y conservación de la biodiversidad de los bosques de clima templado y frío de México. INIFAP. Chapingo, México
- Navarro A, Bañón S, Olmos E, Sánchez-Blanco MJ (2007) Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. *Plant Sci* 172:473–480
- O'Hara KL, York RA, Heald RC (2008) Effect of pruning severity and timing of treatment on epicormic sprout development in giant sequoia. *Forestry* 81:103–110
- Ortiz O M (1993) Distribución y extensión de los suelos afectados por sales en México y en el mundo. Publicaciones del departamento de suelos, UACh, Chapingo, México
- Perry JP (1991) The pines of Mexico and Central America. Timber, Portland Oregon
- Quine CP (2004) Development of epicormic sprouts on Sitka spruce stems in response to windthrown gap formation. *Forestry* 3:225–233
- Renault S (2005) Tamarack response to salinity: effects of sodium chloride on growth and ion, pigment, and soluble carbohydrate levels. *Can J For Res* 35:2806–2812
- Renault S, Croser C, Franklin JA, Zwiazek JJ (2001) Effects of NaCl and Na₂SO₄ on red-osier dogwood (*Cornus stolonifera* Michx.) seedlings. *Plant Soil* 233:261–268
- Rengasamy P (2006) World salinization with emphasis on Australia. *J Exp Bot* 57:1017–1023
- Robinson MF, Anne-Aliénor V, Sanders D, Mansfield TA (1997) How can stomata contribute to salt tolerance? *Ann Bot* 80:387–393
- Rodríguez-Franco C (2002) *Pinus leiophylla* Schtdl. & Cham. In: CAB international (ed) Pines of silvicultural importance. CABI publishing, Wallingford, pp 202–pp 205
- Rodríguez-Trejo DA, Fulé PZ (2003) Fire ecology of Mexican pines and fire management proposal. *Int J Wildland Fire* 12:23–37

- SAS institute Inc. (2001) SAS/PC system for windows. Version 8.2. SAS Institute, Cary, NC, USA
- Segura G (2000) Mexico's forest sector and policies: a general perspective. In: Constituting the commons: crafting sustainable commons in the New Millennium, the eighth conference of the international association for the study of common property, Bloomington, IN, pp 27
- Tyree MT, Patiño S, Bennink J, Alexander J (1995) Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *J Exp Bot* 46:83–94
- Vest PA, Westoby M (2004) Sprouting ability across diverse disturbances and vegetation types worldwide. *J Ecol* 92:310–320
- Wan X, Landhäusser SM, Lieffers VJ, Zwiazek JJ (2006) Signals controlling root suckering and adventitious shoot formation in aspen (*Populus tremuloides*). *Tree Physiol* 26:681–687
- Wan XH, Kent M, Fang XF (2007) Evergreen broad-leaved forest in Eastern China: its ecology and conservation and the importance of resprouting in forest restoration. *For Ecol Manag* 245:76–87
- White JB (1981) The influence of seedling size and length of storage on longleaf pine survival. *Tree Planter's Notes* 1:3–4