SALINE PRE-TREATMENT OF JACK PINE SEEDLINGS FOR PLANTING ON RECLAMATION SITES IMPACTED BY SALINE TAILINGS[1]

by

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Abstract. The Athabasca oil sands in northern Alberta, Canada are saturated with bitumen, which is recovered by surface mining. Current tailings management includes the production of a non-segregating clay/sand material, which is deposited in holding cells as slurry. When the deposit is trafficable, plans are to reclaim the land to a productive forest. The tailings and associated waters are relatively saline, with the main ions being sodium, chloride and sulfate. The objective of this study was to increase survival rates of jack pine planted on tailings deposits by selecting tolerant seedlings prior to planting. Jack pine seedlings were treated with saline solutions containing NaCl, Na_2SO_4 or tailings water at 30-days-of-age. Surviving seedlings were planted on a test tailings deposit, or in a controlled environment chamber where plants were treated with tailings water or salts alone. Results show that salt treatments increased survival of field-planted seedlings, likely by increasing their drought tolerance.

Additional Key Words: sodium, sulfate, chloride, acclimation

Introduction

The Athabasca oil sands in northern Alberta, Canada, cover a total land area of 40,000 square kilometers, and are saturated with bitumen, which is recovered by surface mining. Two large-scale commercial mining operations (Suncor Energy Inc. and Syncrude Canada Ltd.) are currently in production, and further development is underway. Current tailings management includes the containment of tailings within settling basins, and the production of a material known as consolidated (Suncor) or composite (Syncrude) tailings (CT) (Matthews et al. 2000). This involves the mixing of a sand fraction with mature fine tailings, and addition of gypsum at a rate of 750-1200g/ton as a chemical coagulant. The resulting non-segregating material is deposited in holding cells as slurry. This material is expected to de-water relatively rapidly, but pore water will continue to be expressed over a number of years as the deposit settles further. When the deposit becomes trafficable, plans are to reclaim the land to a mosaic of self-sustaining ecosystems, a large portion of which will be productive native forest. The tailings and associated waters that are currently being produced are relatively saline, with the main ions being sodium and chloride from the ore, and sulfate from the added gypsum. The relative proportions of these ions vary with variations in ore and processing, and salinity is expected to increase over time due to recycling of the process water. In addition to salinity, other chemical properties of CT that may affect plant growth include alkalinity and elevated levels of boron.

Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia. Jack pine is an early-successional species that is typically found on nutrient-poor sandy soils (Cayford et al. 1967), and may therefore be a suitable reclamation species for these sites. Because jack pine is a dominant tree of mesic to xeric sites in the pre-disturbance ecosystem, and has potential commercial value, the re-establishment of this species is highly desired. Once established, reclamation sites may appear similar to natural stands as these tend to be even-aged and low in diversity. Previous studies of conifer species have found that while lodgepole pine (*Pinus contorta* var. *latifolia*), white spruce (*Picea glauca*) and black spruce (*Picea mariana*) are only moderately tolerant of CT

[1] Paper presented at the 2001 National Meeting of the American Society for Surface Mining and Reclamation, Albuquerque, New Mexico, June 3-7, 2001. Pub. by ASSMR, 3134 Montavesta Rd., Lexington KY 40502

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Proceedings America Society of Mining and Reclamation, 2001 pp 97-102
DOI: 10.21000/JASMR01010097

https://doi.org/10.21000/JASMR01010097

water, there was a large amount of individual variation in response (Renault et al. 1998). Much of the reclamation of these mine sites will likely involve the planting of nursery-grown seedlings, however previous results suggest that high mortality rates will result in high reclamation costs.

Selection of salt tolerant seedlings prior to planting should result in increased survival rates of seedlings planted on reclamation sites. In order to test this hypothesis, jack pine seedlings that had survived a previous treatment with saline solutions were planted on a test deposit of composite tailings at Syncrude Canada Ltd., and monitored over two years. A parallel experiment was conducted in a controlled environment chamber to allow the effects of water chemistry to be distinguished from the effects of other environmental variables.

Materials and Methods

Plant material

Seeds were collected in October of 1997 from three jack pine stands. One stand is located on the Syncrude site (56°56.06N 111°31.95W), another near Suncor (57°05.95N 111°38.90W), and the third near Smokey Lake, Alberta (54°06.88N 112°10.38W). Seedlings were grown in foam seedling blocks (Beaver Plastics, Edmonton, Alberta: 160/60 ml) filled with quartz/feldspar sand (porosity of 28%) that had been washed with deionized water to eliminate silt and ions. Plants were grown in a controlled environment chamber at 70% relative humidity, 24°/18° C (day/night) temperature and 18 hour photoperiod, and watered daily with a nutrient solution (80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, and 0.10 mg L^{-1} Mo) recommended for pine seedling production (Wood 1995).

Pre-treatment

At 30 days of age, seedling blocks were placed in trays of distilled water with nutrients (control), CT release water with nutrients, or distilled water with nutrients and various concentrations of salt (30mM NaCl, 60mM NaCl, 30mM Na_2SO_4, or 60mM Na_2SO_4) for a period of two weeks. The chemical composition of CT water was analyzed by Syncrude, and the major ions are reported in Table 1. The Na^+ level is approximately equivalent to 39mM, and Cl^- is approximately 15 mM. Boron and fluoride were present in concentrations of 3.5 mg L^{-1} and 2.1 mg L^{-1}, respectively, and the pH was 9.05.

Table 1. Major ions present in CT release water from pond 5 of Syncrude's 1995 NST Field Test that was based on gypsum treatment.

Cations	Concentration (mg L^{-1})	Anions	Concentration (mg L^{-1})
Na^+	904	F^-	2.1
K^+	25.6	Cl^-	490
Mg^{2+}	23.8	SO_4^{2}	1300
Ca^{2+}	64.3	HCO_3^-	350

Three replicate trays were used for each treatment, containing between 150 and 216 plants per tray. The greater numbers of seedlings were used for the highest salt pre-treatments, where high mortality was anticipated. Mortality was monitored over the treatment period, and over the following 30 days. A sample of 24 seedlings per treatment combination (seed source x pre-treatment) was harvested, and freeze-dried prior to measurement of dry weight and ion analysis. Four seedlings from each treatment combination were randomly selected for ion analysis. Shoot and root sodium and potassium contents of freeze-dried tissue were determined by atomic absorption after digestion with sulfuric acid and hydrogen peroxide at 350° C. The sand was then flushed with deionized water, and returned to the previous daily watering schedule. Dormancy was induced, and from 20 to 28 weeks of age, seedlings were stored at 3° C in darkness.

Field experiment

In June 1998, seedlings were removed from cold storage, and one third of these from each pre-treatment group were randomly assigned to the field experiment. Three 10m x 10m plots were randomly located on the test CT deposit. These were rotor-tilled to break the surface crust, then lightly compacted prior to planting. Irrigation was provided six times during the first month after planting. In each plot, between 145 and 150 seedlings were planted in rows 1.5 m apart, with a spacing of 3 plants m^{-1} within the rows. Survival and the flushing of apical buds were recorded in July and August of 1998, one and two months after planting, and in June and August of 1999. Seedling height was measured prior to removal from cold storage, and again in August of 1999.

Growth chamber experiment

A second experiment was performed in a controlled environment chamber to separate the effects of CT water chemistry from other environmental variables. Seedlings were removed from cold storage and placed in trays of treatment solution containing

Table 2. Tissue sodium and potassium content (mg g^{-1} dry weight) of jack pine seedlings treated at 30 days of age (n = 12). A 14-day treatment with 60 mM Na_2SO_4 or 60 mM NaCl was followed by a 30 day recovery period in nutrient solution. Standard errors are shown in parentheses. Different letters indicate a significant difference between treatments at $\alpha = 0.05$.

		Control	60 mM Na_2SO_4	60 mM NaCl
Na^+	Shoot	0.20 (0.06) a	2.51 (0.42) ab	4.53 (1.17) b
	Root	0.47 (0.11) a	4.11 (0.46) bc	5.69 (1.62) c
K^+	Shoot	16.25 (1.00)	16.01 (1.35)	14.93 (1.23)
	Root	23.33 (0.89)	18.62 (1.84)	19.27 (1.47)

nutrients and 60 mM NaCl, 60 mM Na_2SO_4, CT water, or de-ionized water (control). Plants were removed from treatment trays for 24 of every 48 hours to avoid flooding stress. The sand was flushed with deionized water weekly, at which time treatment solutions were replaced. Environmental conditions within the chamber were as described above. A total of 1704 seedlings were used in a completely randomized full factorial experiment with three replicate trays per treatment. Factors considered were seed source (3), pre-treatment (6) and treatment (4), for a total of 72 treatment combinations. The number of seedlings per treatment combination varied due to differences in survival of the pre-treatment groups, but was a minimum of 8 plants. Survival and terminal bud flushing were monitored every 4 days for a period of 10 weeks, then all shoots were weighed and measured.

Data analysis

Data were analyzed using a general linear model (GLM). Pre-treatment survival was analyzed using a repeated measures design. The means were compared using the Duncan's multiple range test, and were considered significantly different at $\alpha = 0.05$. All data were analyzed using "SPSS 8.0" statistical software package (SPSS Inc., Chicago, IL).

Results

Pre-treatment

Seedling mortality was approximately 40% in the 60 mM Na_2SO_4 treatment, 12% in the 30 mM Na_2SO_4 treatment, and less than 5% in all other treatments. Seedlings from different seed sources did not differ significantly with respect to any of the measured parameters, and were combined for further analysis. Biomass of surviving was similarly reduced by all saline treatments with the exception of 30 mM Na_2SO_4 (Fig. 1). Sodium accumulation was greater in both roots and shoots of plants treated with 60 mM NaCl compared with those treated with 60 mM Na_2SO_4 (Table 2). Shoot and root potassium levels were slightly reduced by both of these treatments.

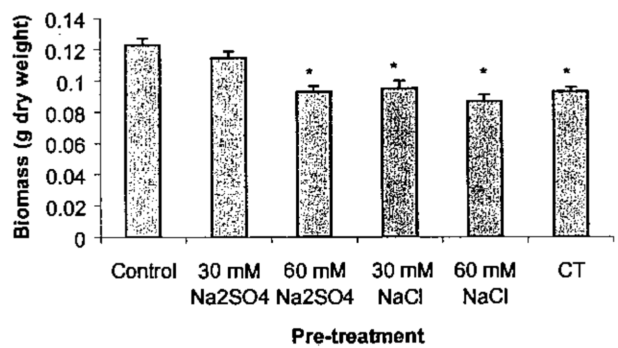

Fig. 1. Total biomass of jack pine seedlings treated for 2 weeks with saline solution. Bars represent standard error. * indicates a significant difference from control at $\alpha = 0.05$.

Field experiment

Seedlings from different seed sources did not differ significantly with respect to any of the measured parameters, and were combined for further analysis. Therefore, only pre-treatment was considered as a factor in the analysis. Seedling survival over the first growing season was greater than 90%, however some needle injury was evident. Winter mortality was greater than 50%, and survival at the end of the second growing season (Aug. 1999) averaged 6.4%. Burial by blowing sand was a frequent cause of seedling mortality, and affected all pre-treatment groups equally. In a small number of seedlings (> 2%) mortality could be attributed to other mechanical injury, and no damage due to insects or rodents was apparent. A main cause of mortality within the growing seasons appeared as a drying and browning of the tissues. Seedling survival increased with increasing concentration of the treatment solution (Fig. 2), and was significantly greater in seedlings that had been treated with 60 mM NaCl than in controls.

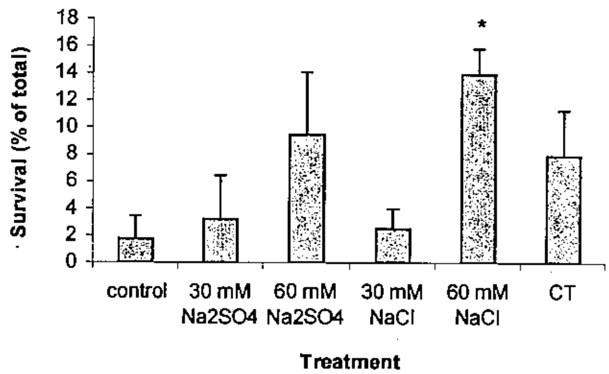

Fig. 2 Survival, after two growing seasons of jack pine seedlings treated with saline solutions prior to planting. * indicates a significant difference from control at $\alpha = 0.05$.

Approximately 35% of all seedlings exhibited flushing of the terminal bud during the first growing season (Fig. 3). No significant differences were found between the flushing of control and treated seedlings, however there was a clear trend of earlier flushing in salt-treated seedlings. Height growth at the end of the second growing season followed the same trend as survival, with seedlings receiving CT water and 60 mM NaCl pre-treatments showing heights of 7.4 cm and 7.3 cm, respectively, compared to 4.7 cm in control plants, however the difference was not statistically significant.

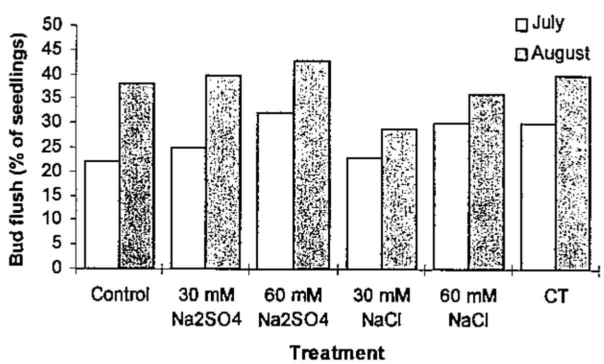

Fig. 3 Flushing of the terminal bud over the first growing in jack pine seedlings treated with saline solutions at 30-days-of-age.

Growth chamber experiment

Pre-treated seedlings did not differ from control seedlings with respect to survival, height, or bud flushing. Survival was greater than 95% in all treatments. Fresh weight was significantly reduced in two treatment combinations; 2.78 ± 0.12g in CT water/ CT water, and 2.36 ± 0.08g in 60 mM NaCl/ 60 mM Na_2SO_4 (pre-treatment/treatment) as compared to 4.77 ± 0.14g in controls. Although seed sources differed significantly with respect to some of the variables measured, they did not differ in response to treatment (treatment*seed source interaction $p<0.05$).

Discussion

In the field experiment, survival by the end of the growing season was extremely low. Winter mortality was high, and was followed by high mortality over the summer of 1999. Because plants in the parallel growth chamber experiment had very high survival rates when exposed to CT water for 10 weeks, water chemistry alone was not the main cause of mortality in the field. Plants exposed to CT water in the greenhouse did show injury over the growing season, suggesting that water chemistry contributed to plant stress in the field. The site received very little summer rainfall, and temperatures on the bare surface of the deposit were sometimes as high as 45°C. A moderate to strong wind frequently blew fine sands and silts across the surface of the deposit, abrading the surface of needles, and contributing to water deficit stress. Jack pine on adjacent plots showed low transpiration rates and high diffusive resistance, indicating stomatal closure, while species that were able to maintain transpiration showed high survival rates (Renault et al. 1998b). Drought stress was therefore a likely cause of mortality, and irrigation in the month following planting may have accounted for the high survival rate during the first growing season.

Reclamation plans for CT deposits call for the placement of a capping material of tailings sand or overburden (minimum 1m thick), plus a thinner layer of reclamation soil prior to planting. The direct planting of seedlings on the deposit was used to allow a rapid assessment of effects of CT on jack pine establishment. While direct seeding is probably not a likely reclamation procedure on these sites, successful seed reproduction will be required to maintain a self-sustainable forest. Although jack pine suffered high mortality on this site, other species such as willow and poplar have thrived after direct planting on this deposit (Croser et al. 2000, Franklin et al. 2001). The relative tolerance of jack pine to salts and CT water in the controlled environment chamber suggests that this species could perform well in the field. In previous studies, planting failure has been linked to drought, wind, and heat (Cayford et al. 1967), and early growth and establishment is aided by light shading (Rudolph 1958). The establishment of a nurse crop could reduce the effects of wind and provide some shade, thereby decreasing water deficit stress in planted jack pine seedlings. A clear trend toward increased survival of plants with increasing salt content of the pre-treatment solution was observed in the field experiment. This trend follows the results predicted by the hypothesis: that saline treatment had resulted in more salt tolerant seedlings, with higher concentrations of salt treatment acting as a stronger selective force than lower treatment concentrations. However, results of the growth chamber experiment show that salt treatment did not convey any advantage to seedlings subsequently exposed to sub-lethal concentrations of salts alone. Saline treatment therefore appears to have increased the frequency of traits conveying drought tolerance, rather than salt tolerance. Furthermore, the greatest survival rates were found in seedlings that had been treated with NaCl and CT water, where little mortality (and therefore selection) had occurred. These results suggest that salt treatment induced acclimation in the seedlings. Acclimation to salt has been previously reported in *Sorghum bicolor* L. (Amzallag 1990). Our results show that concentrations of sodium in roots and shoots were greater in plants treated with NaCl than in those treated with isomolar Na_2SO_4. It is possible that higher solute contents of pre-treated plants allowed the uptake of water under lower soil water potentials.

Injury, including needle necrosis and chlorosis was observed in seedlings treated with salts and CT water in the growth room. Previous studies have shown conifers to be more affected by CT water than many broadleaf species, but less affected than raspberry and strawberry (Renault et al. 1998a). Injury resulting from NaCl treatment was greater than from that from isomolar Na_2SO_4, indicating that ion toxicity was more important than osmotic stress in salt-treated jack pine and in birch (*Betula papyrifera*) (Franklin et al., 2001). The reduced fresh weight of plants exposed twice to CT water in this growth room experiment suggests that some properties of CT, in addition to the major ions, may have a negative impact on plant growth, as similar weight reductions were not observed in treatments containing similar salt levels. In order to predict long-term effects of salts on jack pine growth, further work must investigate the mechanisms of ion toxicity, and the uptake and translocation rates of salt ions and mineral nutrients under saline conditions.

Growth chamber results suggest that jack pine is relatively tolerant of CT water, and so has potential as a reclamation species on CT-affected sites. Its establishment would be valuable ecologically as well as commercially. Once established, the young trees may aid in reducing salt levels in the upper layers of soil by increasing infiltration, and lowering of the water level (Tomar 1997). The poor survival rates seen in this study suggest that attention must be paid to microsite conditions when planting nursery-grown seedlings. We conclude that while pre-treatment of seedlings did not appear to select for salt tolerance, it may have induced acclimation. The use of saline treatment to increase survival rates of seedlings planted in arid conditions is worthy of further study.

Acknowledgements

This study was funded by the Environmental Science and Technology Alliance Canada, Natural Science and Engineering Research Council of Canada, Suncor Energy, Inc. and Syncrude Canada Ltd. We would like to thank Steve Tuttle and Don Sheeran (Suncor Energy Inc.), Michael MacKinnon and Martin Fung (Syncrude Canada Ltd.), Sylvie Renault (University of Manitoba), Clare Croser and Edmund Redfield (University of Alberta), and the anonymous reviewers for their valuable advice and assistance.

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