Growth and Physiological Responses of Conifer Seedlings to Salt Stress and Nutrient Deficiency

by

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Abstract

Oil sands extraction and refining can cause many negative environmental impacts and because these activities usually take place in the forest region, the Government of Alberta requires that, after mine sites are closed, the companies perform reclamation. The properties of reclaimed oil sands soils may include a poor nutrient regime and high concentration of dissolved Na and Cl, affecting soil quality and plant development. The present research examined the relationship between salt uptake, tissue concentration, and plant injury in black spruce (*Picea mariana*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*) subjected to different treatment concentrations of NaCl (30, 60, and 90 mM), and how other environmental factors, including deficiency of N, P, and K, affect plant growth and development in white spruce and jack pine. The studies were conducted through a series of controlled-environment experiments in sand culture and hydroponic system. Compared with control seedlings, stem diameter and heights of black spruce, white spruce, and jack pine were significantly reduced by all NaCl treatments, and white spruce and jack pine also exhibited reduced biomass. Needle chlorophyll concentrations decreased under NaCl treatments in all species. Visible symptoms of salt injury were observed in young and old needles of seedlings treated with NaCl and significant necrosis was present in most of the plants under 90 mM NaCl treatment. The greatest effect was observed in white spruce, with an average of 58% of the needles injured. However, jack pine showed a slightly higher accumulation of Na and Cl in the needles than white spruce. The critical Na concentration differed between species. In black spruce, a concentration of 2.67 mg g^{-1} of Na in needles of seedlings treated with 30 mM NaCl caused up to 10% of necrosis while 17.72 mg g^{-1} of Na in needles of seedlings treated with 90 mM NaCl had 34% needle necrosis. In white spruce, with 11.62 mg g^{-1} of Na in needles of seedling

treated with 30 mM NaCl caused 10% necrosis. Similarly, jack pine with10.91 mg g^{-1} of Na in needles of seedling treated with 30 mM NaCl, had 18% needle necrosis, suggesting that level of salt injury can be correlated, and therefore, predicted from shoot Na concentrations. The effects of combined NaCl and nutrient concentrations treatments varied between the species studied, but, in general, the effects of NaCl on stem diameters, heights, total dry weights, chlorophyll concentrations, net photosynthesis, and transpiration rates were aggravated by P deficiency, with greater impacts on jack pine compared with white spruce. Therefore, P fertilization should be considered as an important factor in revegetation areas.

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List of Abbreviations

AE	Alberta Environment
Al	aluminium chemical element
ANOVA	analysis of variance
ATP	adenosine triphosphate
Ca	calcium chemical element
CH_4	methane
chl	total chlorophyll
Cl	chloride chemical element
cm	centimetre
CO_2	carbon dioxide
CO_3^{-2}	carbonate ion
Cu	copper chemical element
DMSO	dimethyl sulfoxide
dS m^{-1}	deciSiemens per metre
E	transpiration rate
EC	electric conductivity
Fe	iron chemical element
GHGs	greenhouse gases
ha	hectare
HCO^{-3}	bicarbonate ion
HNO_3	nitric acid
H_2PO_4	dihydrogen phosphate ion
K	potassium chemical element
km	kilometre
km^2	square kilometre
KOH	potassium hydroxide
m	meter
m^3	cubic meter

mg	milligram
mg g^{-1}	milligram per gram
mg kg^{-1}	milligram per kilogram
Mg	magnesium chemical element
µmol m^{-2} s^{-1}	micromole per square meter per second
mg L^{-1}	milligram per liter
mL	milliliter
mM	millimolar
Mn	manganese chemical elements
Mo	molybdenum chemical element
N	nitrogen chemical element
Na	sodium chemical element
NaCl	sodium chloride
NaOH	caustic soda
NH_3	ammonia
nm	nanometer
NO_2	nitrogen dioxide
NO_3^-	nitrate ion
O_3	ozone
P	phosphorus chemical element
pH	power of hydrogen
Pn	net photosynthesis rate
PO_4^{-3}	phosphate ion
PPFD	photosynthetic photon lux density
SO_2	sulfur dioxide
SO_4^{-2}	sulphate ion
^{232}Th	thorium isotope
^{238}U	uranium isotope
Zn	zinc chemical element
w/v	weight/volume

Chapter 1

Introduction and literature review

1.1 Introduction

Salinity is a worldwide problem frequently associated with agricultural activities. Forest soils rarely contain an excess an excess of salt because of the natural drainage systems. However, elevated levels of salts, such as sodium chloride (NaCl), can be also present in some boreal forest, as a result of industrial activities, such as oil sands mining. The concentrations of NaCl in reclaimed areas succeeding oil sands mining vary depending on the source and management of tailings (Renault et al. 1998). In general, plant growth and development become affected when the electrical conductivity (EC) of the soil exceeds 4 dS m^{-1}, which is equivalent to about 40 mM NaCl (Munns and Tester 2008). Nevertheless, plant species used to revegetate these sites have different levels of tolerance to NaCl, but salt tolerance in trees has not been extensively studied. The main plant species considered for productivity and commercial value include black spruce (*Picea mariana*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*), and they were therefore selected for the present studies.

Soil salinity interferes with plant water and nutrient transport mechanisms, reducing germination and growth, and affecting the productivity of the ecosystem (Grattan and Grieve 1999). The osmotic impact of salinity can be observed just after salt exposure and it continues for the duration of contact, resulting in cell division and expansion inhibition, as well as stomatal closure (Munns 2002, Flowers 2004). Long-term exposure to salt leads to ionic stress, which can cause the premature senescence of adult leaves, and consequent reduction in the photosynthetic area available to sustain plant growth (Cramer and Nowak 1992). If the rate at what the leaves die

is greater than new leaves growth rate, the plant's photosynthetic capacity will no longer be capable of supplying the energy requirements of the young leaves, reducing their growth potential (Munns and Tester 2008).

Since most of the research concerning salt impact on plants has been carried out with short-lived, agricultural crop plants, the relationship between the rate of salt uptake, tissue concentration, and plant injury has not been thoroughly studied in woody perennials where it is of greatest importance. So, understanding this relationship and how selected conifer species respond to salinity in the presence of other unfavorable soil factors such as limited nutrient supply, is critical to comprehending salt tolerance mechanisms in plants and to successfully revegetating oil sands mining areas.

To gain a better understanding of the effects of salinity on plant growth and physiology in the presence of other confounding factors like mineral nutrition, and the damaging effects caused by salt accumulation, controlled-environment studies on conifer species recommended for reclamation were carried. These studies were designed to generate the fundamental knowledge required to develop protocols for vegetation monitoring in areas affected by salinity accompanied by other soil factors that are unfavourable to plants.

The objectives of the studies were to:

1) Examine if the rate of salt uptake and tissue concentration are related to plant injury in black spruce, white spruce, and jack pine.

2) Determine how environmental factors, like mineral nutrition, affect the plant responses to tissue Na accumulation.

The following hypotheses were tested:

1) Level of salt injury can be correlated, and therefore, predicted from shoot Na and Cl concentrations.
2) Macroelements (N, K, and P) deficiencies aggravate the effects of salt stress in white spruce and jack pine.

1.2 Literature review

1.2.1 Oil sands mining and reclamation

The Canadian oil sands deposit, located in the Athabasca, Cold Lake, and Peace River regions, in Northern Alberta, is the largest type of mining in Canada and the third-largest oil reserve in the world. The crude oil production has increased by more than 2.4 million barrels/day over the last 35 years. In Alberta, bitumen can be extracted using two different methods, depending on how deep the deposits are beneath the surface. About 20% of the oil sands resources are near enough to the surface (up to 75 m) to be recoverable by open pit methods like truck-and-shovel mining. The remaining 80% are too deep to mine using traditional methods, thus the oil is extracted with drilling technologies (Carrigy 1973).

The oil sands extraction and refining are an important part of the Canadian economy, and essential to meet the world's energy requirements (Attanasi and Meyer 2010), but they cause many negative environmental impacts such as high carbon and greenhouse gas emissions, release of toxic materials to the soil, water, and air, loss of biodiversity, water pollution, toxicity and leakage from

tailings ponds, and forest destruction. The surface mining extraction removes the vegetation and overburden (i.e. rocks, soil, and earth layers above the bitumen) and creates large open-pit mines, causing considerable disturbance and land degradation, affecting the region and also the planet (Gosselin et al. 2010).

The extraction of bitumen from sand using hot or warm water processes produces a slurry waste that is transported and stored within surface tailings ponds. There, the heaviest material, typically sand, settles to the bottom, separating from the top layer of water which can be recycled. The intermediate layer, known as mature fine tailings, contains about 30% fine clay and 70% water at a pH 8-9 and high levels of Na and Cl due to the addition of NaOH and other chemicals in the extraction process.The residual water that contains concentrations of Na and Cl of 10-1000 mg kg^{-1}. The Na and Cl concentrations have shown a 200-300% increase from 1979 to 1993 (Mikula et al. 1996).

Reclamation material, including upland surface soil (coarse and fine), transitional soil, peat, peat-mineral mix, subsoil (coarse and fine), and suitable overburden are salvaged and storage in stockpiles to reconstruct the soil on the reclamation process and transfer the benefits from pre-mined areas back onto reclaimed landscapes. The upland surface soil is the most valuable reclamation material available for use as coversoil. It provides an unique and crucial source of organic matter, plant nutrients and woody debris. Because of the limited amount of upland versus wetland soils prior to disturbance, most of the post-disturbance area will be reclaimed using peat-mineral mix as coversoil, but it has poorer nutrient regimes and drier moisture regimes than undisturbed areas (MacKenzie 2011). Also, releases of salt also might occur in association with oil and gas production, specially in the tailings storage ponds, causing adverse environmental impacts on soil, surface water, and groundwater (Government of Alberta 2001).

To reduce the environmental and social impacts of the oil sands industry, land use and reclamation became part of the oil sands company's projects. Before mining operations begin, complete environmental assessments identify potential impacts that can affect land, air, water, and biodiversity. Steps are then taken during the project life, to minimize any negative effects. Companies are required to file a Conservation and Reclamation Plan as part of their preliminary project (Government of Alberta 2013). According to the *Environmental Protection and Enhancement Act* (Government of Alberta 2000), applied to energy resource activities, reclamation includes any or all the following: (a) removing equipment or buildings and other structures; (b) decontaminating buildings, other structures, land or water; (c) stabilizing, contouring, maintaining, conditioning, or reconstructing the surface of the land to a state of equivalent land capability.

In Canada, land reclamation is frequently focused on areas disturbed during mining, oil, and gas operations. Because these activities usually take place in the forest region, recreating healthy, resilient ecosystems is an important part of reclamation. Reclamation certificates are not delivered until monitoring determines that the requirements meet the criteria established on the reclamation plan (Government of Alberta 2016).

1.2.2 Boreal forest

The estimated current forested area in Canada is 3.47 million km^2 or 35% of the country's land (NFI 2013). Alberta is the fourth largest province in Canada with an area of 661,845 km^2, of which 381,000 km^2 is boreal forest, but only 35% intact forest. The Canadian oil sands deposits in Northern Alberta covers approximately 142,000 km^2 of land (Government of Alberta 2016).

The forest performs many significant ecological and social roles, like atmospheric carbon sequestration, control storms and melt-water runoff, filter and purify water, offer habitat for a large variety of wildlife, and have cultural, economic, recreational, and spiritual value to people (Pembina Institute 2013). The diversity of forest trees and shrubs contribute to soil and water conservation and the animals are an essential component on forest ecology, including pollination, seed dispersal and germination, and feed on potential pest species. Forest diversity is an important element of the concept of *Sustainable Forest Management*, approved by the General Assembly of the United Nations in 2007, and of the *Non-Legally Binding Instruments on All Types of Forests*, chapter 11 of Agenda 21, Combating Deforestation (Lindquist et al. 2012). According to Parks Canada (2001), the boreal forest biodiversity in Canada include 4,521 native vascular plant species, with 109 of them at risk, and 1,221 exotic vascular plant species.

The most common boreal forest trees are the conifer species, including black spruce (*Picea mariana*), white spruce *(Picea glauca*), jack pine (*Pinus banksiana*), balsam fir (*Abies balsamea*), and tamarack (*Larix laricina*). Many species are resilient and tolerant to cold weather and drought, acidic or poor soils, wildfires, and other natural disturbances (Waring 2002). Most of these are distributed in limited geographic locations, but black spruce and white spruce extend from coastal British Columbia to the Atlantic Ocean.

Conifer trees are typically evergreen and usually have needle-like leaves, retained year-round, allowing the trees to photosynthesize, at a reduced rate, on the occasional sunny, warm winter days (Landhäusser et al. 1997). They have simple anatomy, with most of the wood formed by longitudinal thin cells called tracheids, being the tree's support system and allowing water conduction to the leaves. The remaining cells are called ray parenchyma cells, responsible for storage and radial transport of materials (Wheeler 1997). Conifers wood is soft, white or pale

yellow, with low density, long fibers, straight grained, resistant to shrinking and swelling, and typically less expensive compared to hardwood. They have a wide range of applications such as building components (windows and doors), furniture, medium-density fiberboard, pulpwood, and Christmas decoration (Wood Work Basics 2010).

Previous researches on boreal forest plant species and salinity has studied the response of woody plants on tailings substrates (Renault et al. 1998, 2000, 2003, 2004), targeting species such as black spruce, white spruce, jack pine, and trembling aspen (*Populus tremuloides*) (Burgers 2005). These species are also the main plants considered to evaluate soil productivity and capability on reclaimed areas (Leskiw 1998). Forest vegetation can be established on saline soils, as long as the salts are beneath the rooting zone (Purdy et al. 2005). However, many studies do not take into consideration the variances in soil properties, including pH and nutritional levels, which are known to change plant responses to salt.

Black spruce is a wide-ranging, abundant conifer found across the Northern range of North America. It can be associated with balsam fir, lodgepole pine (*Pinus contorta*), paper birch (*Betula papyrifera*), trembling aspen, and white spruce. It also can be found in association with jack pine on more xeric sites, or tamarack on poorly-drained soils. However, black spruce apparently grows slowly and have lower wood volume than many associated trees (Nesom 2004). The main use of black spruce wood is for pulp for paper products (Amiri et al. 2004). Germination is successful on moist sphagnum mosses or feathermosses during wet years. Moist mineral soils also can offer a good seedbed, but exposed mineral soil may be too waterlogged or subject to frost in some areas. Seedlings can develop in as little as 10% of full light intensity. They usually grow on wet dark brown and blackish peat, with high organic matter content, but also on clay, coarse till, loam, sand, and shallow soils. It is a pioneer species that regenerates naturally following fire disturbance. The

fire generally results in fast reestablishment and subsequent dominance of black spruce (Viereck and Johnston 1990, Nesom 2004).

White spruce has a widespread geographic range and abundance across Canada and co-occurs with black spruce, exhibiting a distinctive competitive relationship relating to water deficit tolerance, vegetative reproduction, flowering periods, and growth rate. It is important for food and shelter for many species, maintaining soil stability, watershed, and recreation. It also has historical importance for American Indians, providing food, shelter, medicine, fuel, and other uses (Nesom 2003). Germination commonly occurs on mineral soil after windthrow and flood. It is frequently found growing on well-drained, but moist, silty soil, or establish immediately following disturbance (Nesom 2003). The species can survive and growth under many environmental conditions including low nutrition levels, different moisture conditions, low light levels, and over a large range of pH (Sutton 1969, Brand et al. 1986). It is tolerant to flooding, permafrost, and high soil acidity. It is considered a climax species of the boreal forest and is commonly used for reclamation (Purdy et al. 2002).

Jack pine is a moderately hard and heavy pine, characteristic of Western North America and the most extensively distributed conifer species in Canada. Usually it grows in mono-specific stands or is associated with black spruce, it is less common in mixed woods or with other species (Moore 2006). Germination is more efficient on mineral soils, soil with less than 0.2-inch-deep, or organic matter. Shade may be good for germination, but young seedlings need full sun to establish. It is highly shade-intolerant compared to associated species such as balsam fir, black spruce, and white spruce. However, because of abundant seed production, few mature trees are necessary to regenerate a stand (Moore 2006). Typically grows in dry, sandy, acidic soils (lower pH limit of 4.0), but also can develop on loamy soils, on thin soils over granites and metamorphosed rocks,

over limestone, on peat, and on soil over permafrost (Cayford et al. 1967, Moore 2006). It does not grow naturally in moderately alkaline soil, but can survive in calcareous soils (up to pH 8.2) if associated with mycorrhizal fungi (Rudolf 1965). Jack pine is the best fire-adapted conifer on boreal forest, easily invading areas where mineral soil has been exposed by fire, becoming dominate as a pioneer species (Moore 2006).

1.2.3 Soil salinity and plant growth

Soil salinity denotes the occurrence of high concentration of soluble salts on the plants root-zone. When dissolved, these salts release cations (e.g. Ca^{+2}, K^{+}, Mg^{+2}, Na^{+}) and anions (e.g. Cl^{-}, CO_3^{-2}, HCO^{-3}, SO_4^{-2}). The concentrations of salts and their high osmotic pressures affect soil, surface water, groundwater, and vegetation. A salinity problem develops if the concentration of salts affects plant survival and growth, since they can reduce or prevent the plants uptake of water and absorption of essential nutrients, because of the increased osmotic pressure (Government of Alberta 2001, Tester and Devenport 2003).

Optimal pH values for most plants are 6.0 - 7.5, but reclaimed soils have higher pH levels (> 8.5), and are likely to have high salinity, with EC ranging between 0.5 - 4.0 dS m^{-1} in the root zone (Howat 2000). Very salt-sensitive species may be affected at less than 2.0 dS m^{-1}. Na and Cl also can be used as indicators of degree of contamination, as surface soils in Alberta are generally low in these ions (Government of Alberta 2001).

Areas where a peat-mineral mix was applied tend to have a poor nutrient regime, low organic material, high erosion potential, low water storage, low cation exchange capacity, and the absence of microbial activity (Fung and Macyk 2000). The high concentration of dissolved Na and

Cl can affect soil quality, leading to dispersion of clay particles, degradation of soil structure, and surface crusting. When clay particles come apart, the water movement in soils is obstructed, decreasing hydraulic conductivity, resulting in low drainage, excess moisture, and insufficient aeration, detrimental conditions for germination, seedling emergence, and roots penetration and elongation (Government of Alberta 2001). It also may displace other mineral nutrients, causing the plants to absorb Na and Cl instead of essential nutrients such as K and P (Beckerman and Lerner 2009).

Many plants can suffer from Ca deficiency when the Na^{+}:Ca^{+2} ratio in soil exceed a certain level (Maas 1996). Na movement into the plants cells is believed to be an active process, with Na influx being regulated by active efflux through Na^{+}/H^{+} antiports (Blumwald et al. 2000). The antiports help to maintain cellular ion homeostasis and mediate the transport of Na out of the cytosol and into the vacuole, therefore it is essential for salt tolerance in plants (Xu et al. 2010).

Sodium also has an antagonistic effect on Ca and Mg uptake. It is able to compete with K for allosteric sites of enzymes and interact with ion channels. Plasma membrane K channels also may facilitate Na influx, when trying to maintain K equilibrium potential (Cerana and Colombo 1993, Schachtman 2000). Also, the decrease in Mg might be part of the leaf chlorosis condition, as Mg is a key element on the chlorophyll molecule, essential for plant photosynthesis (Khasa et al. 2002).

Chloride, on the other hand, is an essential micronutrient for plants. Its influx is an active process, with energy catalyzed by $Cl^{-}/2H^{+}$ symporters (Sanders 1980, Felle 1994). It is absorbed by roots in substantial amounts and accumulated in leaves, where is evenly distributed in the cytoplasm and vacuoles (Eshel and Waisel 1979). Most species tolerate tissue concentrations as

high as 400 mM Cl, and even the most sensitive species can tolerate up to 250 mM Cl (Munns and Tester 2008).

Whit a direct role in photosynthesis, Cl regulates the function of several enzymes, structural component of some polypeptides (Coleman et al. 1987), essential for osmotic adjustment, stomatal regulation, supresses a number of physiological disorders and diseases, and it is involved on the transport of Ca, K, and Mg (Engel et al. 1997). Together with K, Cl helps to maintain xylem volume flow and root pressure. However, several plants are known to be sensitive to Cl toxicity and can experience a decrease in growth even when water is not a limiting factor (Marschner 1995).

Elevated Cl levels can cause plant growth reduction, inhibit protein synthesis and enzyme action (Greenway and Munns 1980), modify plasma membrane permeability and membrane lipid composition (Kuiper 1968, Franklin and Zwiazek 2004), leaf necrosis and abscission, first expressed in older foliage (Farnham et al. 1995). In conifers, the initial symptom is yellow discoloration of the needles, followed by their death (TETRA 2004).

Salinity stress has a major influence on vegetation establishment as many plant species show sensitivity to higher EC and Na (Maas 1986, Allen et al. 1994). Salt accumulation in the reclaimed areas come from the recycling process-affected waters and leaching of overburden deposits, and can reach levels that cause high plant mortality (Leung et al. 2003). Therefore, general in-situ remediation of salt contaminated soils may include replacement of Na on the soil particles with Ca^{+2} and removal of salts by leaching with natural precipitation or irrigation. For both options, there must be no adverse effects and the potential movement of salts to receptors must be considered (Government of Alberta 2001).

Movement of dissolved mineral salts in soil is dependent on water flow speed and direction. Highly soluble ions like NO_3^-, Cl^-, and Na^+ can move freely downwards with the soil water,

leaching salt towards the water table. Salts can also move by diffusion and hydrodynamic dispersion, usually from higher to lower concentrations, or be carried upwards with capillary movement and, when the water evaporates, become concentrated at the subsurface layer (Government of Alberta 2001).

Plants are most affected by salinity in the initial stages of growth, from germination through the four-leaf stage. Generally, survival and development decrease with increasing salinity (McKenzie et al. 1994, Maynard et al. 1996), affecting various physiological and metabolic processes such as protein synthesis, gas exchange, and energy and lipid metabolism (Parida and Das 2005), making the plants more susceptible to pests and abiotic stresses (Khasa et al. 2002). Initially, salinity represses plant growth in the form of osmotic stress, followed by ion toxicity (James et al. 2011).

Another important response to high salinity is a reduction in leaf surface expansion and decrease in stomatal aperture, followed by a total interruption of expansion. Since water absorption from roots decreases and water loss from leaves intensifies due to osmotic stress, salinity stress is also considered hyperosmotic stress (Munns 2002). The chemical potential of the saline solution can cause an imbalance in water potential between the apoplast and symplast that leads to turgor reduction (Bohnert et al. 1995). Cellular dehydration starts when the difference in water potential is greater than can be compensated by turgor loss. The response to turgor decrease is osmotic adjustment through synthesis and accumulation of proline and other amino acids, and decrease in the net CO_2 assimilation rate (Ashraf and Harris 2004, Taiz and Zeiger 2006, Marcińska et al. 2012).

Most halophytes and glycophytes tolerate salinity with relatively similar strategies, typically compartmentalizing Na and Cl into the vacuole, using them as osmotic solutes (Niu et al.

1995, Blumwald et al. 2000). Many plants develop mechanisms to bear high salinity that include: (a) selective accumulation or exclusion of salt; (b) regulation of ion uptake by roots; (c) synthesis of compatible osmotic solutes; (d) change in photosynthetic electron transport pathways; (e) change in cell membrane structures; (f) induction of the activities of antioxidative enzymes; and (g) induction of phytohormones (Parida and Das 2005). Salt excluders, which have selective permeability, are capable of uptaking K over Na, consequently having low Na and Cl content. On the other hand, salt accumulators manage uptake of high salt concentrations through resistant cell membranes able to tolerate high levels of intercellular salts, a characteristic common of halophytes; or through removal of additional salts entering the plant, where the roots can take up salt ions but prevent their injurious effects (Badr and Shafei 2002).

In vitro research demonstrated that, for many enzymes, Na inhibition starts at concentrations approaching 100 mM (Greenway and Osmond 1972), while other enzymes are sensitive to lower salt levels. The concentration at which Cl becomes toxic in plants is not well defined, but is probably similar to Na levels (Flowers and Dalmond 1992). Nevertheless, Skerrett and Tyerman (1994) suggested that the cytosolic Cl level is probably around 10-20 mM, but can be higher in saline conditions. Most boreal forest trees are susceptible to salt damage, however, some boreal mixedwood forest communities can be found on saline areas, typically where the soil EC is below 4 dS m^{-1} (Lilles et al. 2010).

In general, salinity is a major challenge to the reclamation process. Despite extensive research on effects of salinity on boreal forest species (Renault et al. 1998, 1999, 2001, Calvo-Polanco et al. 2009, Calvo-Polanco and Zwiazek 2011), studies on plant responses to Na accumulation under different environmental factors correlated, such as different salt levels and

mineral nutrition deficiencies are required to develop methods for the effective reclamation of oil sand areas.

Chapter 2

Tissue sodium and chloride concentrations in relation to needle injury

2.1 Introduction

Boreal forests in northern Alberta has been disturbed by the rapidly growing oil sands mining industry. However, the existing knowledge is insufficient to predict the impact of disturbance and reclamation on the boreal forest tree species.

Soil salinity and sodicity in oil sands reclamation areas have been listed among the most challenging revegetation concerns (Howat 2000). The synergistic effect between Na and Cl can cause greater injury when both ions are present (Martin and Koebner 1995) and Na uptake and translocation increase in the presence of Cl (Muralitharan et al. 1992, Renault et al. 2001).

Responses of plants to elevated NaCl levels are complex and include changes in their morphology, physiology, and metabolism (Hilal et al. 1998). The rate of leaf expansion is reduced, new leaves emerge slowly, and lateral buds develop tardily or remain quiescent, and, therefore, fewer new branches and lateral shoots are formed (Munns and Tester 2008). However, plants can develop strategies to survive under salt stress such as selective accumulation or exclusion of ions, and regulation of ion uptake (Parida and Das 2005) by retention of Na and Cl in roots to delay accumulation in shoots (Franklin and Zwiazek 2004). The capacity to limit Na and Cl uptake by shoots seems to play an important role in salt tolerance on many woody plants (Allen et al. 1994). In contrast, salt-sensitive plants can accumulate Na and Cl in the leaf tissues, instead of keeping the ions in roots and stems (Boursier et al. 1987).

Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis and necrosis) in mature leaves due to high Na which affects plants by disrupting protein

synthesis and interfering with enzyme activity (Munns and Termaat 1986, Hasegawa et al. 2000, Munns 2002). It is commonly known that, for many species, shoot growth is more sensitive to salt stress than root growth (Läuchli and Grattan 2007), since a reduction in leaf area relative to root growth would decrease the water uptake, allowing the plant to preserve soil moisture under salinity stress conditions (Munns and Tester 2008).

Sodium accumulation also affects photosynthetic enzymes and pigments (Davenport et al. 2005). The ionic stress caused by high Na also results in premature senescence, as well as leaf chlorosis and necrosis (Munns and Termaat 1986, Hasegawa et al. 2000, Munns 2002), whereas Cl is actively transported to the leaves and interfere with photosynthesis and chlorophyll production (Bayer and Njue 2016).

Little research has been conducted concerning the relationship between salt uptake, tissue concentration, and plant injury in woody perennials. In the present study, I examined if the extent of injury and mortality caused by salt stress can be correlated, and therefore predicted from shoot and root Na and Cl concentrations, in seedlings of three conifer species that are commonly used in oil sands reclamation. This knowledge is important to predict revegetation success in salt-impacted areas.

2.2 Materials and methods

2.2.1 Plant material and growth conditions

One-year-old, container-grown, black spruce (*Picea mariana* (Miller) B.S.P.), white spruce (*Picea glauca* (Moench) Voss), and jack pine (*Pinus banksiana* Lamb.) dormant seedlings were

obtained from Tree Time Services Inc, Edmonton, AB, Canada. Seedlings were stored for two weeks at 4^{o}C in the dark prior to the experiments.

The uniform plants were selected and transplanted to 700 mL Styroblocks (Beaver Plastics Ltd, Edmonton, AB, Canada) filled with forestry filter sand (Target Products Ltd, Abbotsford, BC, Canada), saturated with deionized water, and placed in a controlled-environment growth room at 65 ± 10% relative humidity, 22/18°C (day/night) temperature, 16-h photoperiod, and approximately 300 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the seedling level, provided by cool white fluorescent lights. Before the start of treatments, the seedlings were fertilized with half-strength modified Hoagland's nutrient solution (Epstein 1972) until buds flushed, new shoots elongated, and new roots were found.

2.2.2 Treatments

The Styroblocks containers with seedlings were placed in 38.8 L plastic tubs (Fig. 2.1) filled with half-strength modified Hoagland's nutrient solution (Epstein 1972) (control) or nutrient solution with different NaCl levels, 30 mM NaCl, 60 mM NaCl or 90 mM NaCl, at pH 5.0-5.5. Every two weeks the tubs were drained and washed, and solutions were replaced, to avoid salt build-up.

To avoid osmotic shock, the plants were subjected to increasing NaCl concentrations by 15 mM NaCl at each subsequent immersion, every five days, until each group reached the desired concentration (Jimenez-Casas and Zwiazek 2013). Over the entire experiment, the EC of the solutions was measured using a portable conductivity meter (Traceable, Thermo Fisher Scientific Inc, Waltham, MA, USA).

The experimental design was a randomized complete block with four replications per treatment (Fig. 2.2). The experimental unit was a container with eight seedlings per species. The treatments lasted for 14 weeks.

2.2.3 Plant growth and dry biomass measurements

Immediately before and after 14 weeks of treatments, all seedling (n = 32) heights were measured from the root collar to the shoot tip, and stem diameter measured at the root collar. The relative shoot height growth and relative stem diameter growth were calculated by dividing the difference in the initial and final values by the initial value.

For dry weight determinations, roots were separated from shoots in eight randomly-selected seedlings per treatment (n = 8), and their dry weights obtained after drying in an oven at 65ºC for 72-h.

2.2.4 Needle injury

At the end of the experiment, six seedlings per treatment (n = 6) were randomly selected and green, healthy needles were separated from the chlorotic (light green to yellow) and/or necrotic (brown) needles. The needles were dried in an oven at 65ºC for 72-h, and weighed. The proportion of necrosis was calculated from the dry weight percentages of healthy and unhealthy needles.

2.2.5 Chlorophyll determination

Needle chlorophyll concentrations were determined according to Hiscox and Israelstam (1979). Four seedlings from each treatment (n = 4) were randomly sampled, freeze-dried for 72-h, ground with a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA), and weighed.

Chlorophyll was extracted from pulverized needles samples (100 mg dry weight) with 8 mL of dimethyl sulfoxide (DMSO) at 65°C for 24-h. Chlorophyll absorbance was determined with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Upps., Sweden) at 648 nm for Chl-*a* and 665 nm for Chl-*b*. Total chlorophyll concentration was calculated from the absorbance values using Arnon's equation (Arnon 1949).

2.2.6 Sodium and chloride tissue concentrations

Concentrations of Na and Cl in the needles and roots of six seedlings per species per treatment (n = 6) were determined in the Natural Resources Analytical Laboratory of the University of Alberta, Edmonton, AB, Canada. Na concentrations were measured by the Inductively Coupled Plasma - Optical Emission Spectrometry (iCap 6000, Thermo Fisher Scientific Inc, Waltham, MA, USA) (Renault et al. 2001, Calvo-Polanco et al. 2009) in needle and root samples containing 250 mg of ground tissue, digested with acid (5% HNO3) according to Mars Xpress Digestion System (CEM Corp, Matthews, NC, USA). Concentrations of Cl in the needles and roots were analyzed using ion chromatography (DI 300, Dionex Corp, Sunnyvale, CA, USA) (Tabatabai and Frankenberger 1996) in filtered hot water extracts (Apostol et al. 2002).

2.2.7 Statistical analysis

All data were analyzed by R Software for Statistical Analysis (3.2.4, R Development Core Team, Vienna, VA, Austria) to determine statistically significant ($p \leq 0.05$) differences between species and treatments. After analyzing the differences among group means with one-way analysis of variance test (ANOVA), comparisons between different treatments and species were conducted using Tukey's HSD Post-hoc test. A representative table of the ANOVA results is shown in Appendix 1.

Linear regressions were used to investigate the relationship between plant injury (dependent variable) and concentrations of Na and Cl in needles and roots (independent variable) and to predict the value of the dependent variable based on the value of the independent variables according the equation:

$$y = \alpha + \beta x + \varepsilon$$

where $\alpha + \beta x$ is the deterministic portion of the model and ε is the random error.

Scatter diagrams to represent the data were created using Minitab Statistical Software (18, Minitab, State College, PA, USA).

2.3 Results

2.3.1 Plant growth and chlorophyll concentrations

Relative stem diameter growth (RSDG) and relative shoot height growth (RSHG) of black spruce, white spruce, and jack pine were significantly reduced by the 30 mM and higher NaCl concentration treatment (Table 2.1). For RSDG, significant differences were found between control and NaCl-treated seedlings and between 30 mM NaCl treatment and the other NaCl treatments, while for RSHG, significant differences were found only between 90 mM NaCl and all the other treatments.

Significant differences were found on RSDG and RSHG between black spruce, white spruce, and jack pine. Jack pine RSDG and RSHG were markedly higher than black spruce and white spruce. In 30 mM NaCl treatment, white spruce RSDG was 13.6% lower compared to black spruce. No significant difference in RSDG between white spruce and black spruce was found in the 60 mM NaCl and 90 mM NaCl treatments. There was a significant effect of NaCl treatments in RSHG, specially in black spruce. In the 90 mM NaCl treatment, black spruce had average RSHG 22.15% lower than white spruce, and 36.34% lower than jack pine.

Plants treated with NaCl showed a significant decrease in total dry weight compared with the control treatment (Fig. 2.3), with exception of black spruce which didn't have a significantly difference on total dry weight between treatments.

The effects of NaCl on growth parameters varied within tree species. The total dry weight of jack pine seedlings was significantly superior than in black spruce and white spruce. Under NaCl treatments, white spruce and jack pine showed a similar behaviour, with total dry weight

significantly lower on the NaCl treated seedlings compared to control. In the 90 mM NaCl treatment, the total dry weight of black spruce and white spruce were about 35% lower than jack pine on the same treatment.

No significant differences were found between treatments for shoot:root partitioning of biomass on black spruce and white spruce. In jack pine, the shoot:root ratio was higher in the control treatment compared to 90 mM NaCl (Fig. 2.4).

There were differences between all species within treatments. In white spruce, dry mass distribution between above- and belowground portions of the plant was clearly toward shoot growth while in black spruce and jack pine the preferential allocation was for roots. In the 90 mM NaCl treatment, root contributions to total biomass were generally 64% in black spruce, 36% in white spruce, and 56% in jack pine.

Plants treated with NaCl showed a significant decrease in needle chlorophyll concentrations compared to control treatment, with exception for white spruce on 30 mM NaCl treatment, with no significant difference on chlorophyll concentrations compared to the other treatments (Fig 2.5).

No significantly difference was found for needle chlorophyll concentrations between the species studied. In the 90 mM NaCl treatment, white spruce and jack pine chlorophyll concentrations were about 24% lower than black spruce on the same treatment.

2.3.2 Sodium and chloride tissue concentrations

Increasing treatment concentration of NaCl resulted in a higher accumulation of Na and Cl in needles and roots and the concentrations of these elements were higher in jack pine than in black spruce and white spruce.

In black spruce, the average needle Na concentration ranged from 0.06 mg g^{-1} in the control to 16.59 mg g^{-1} in seedlings treated with 90 mM NaCl, and significant differences between control and 60 mM AND 90 mM NaCl treatments were found (Fig 2.6). In white spruce, the concentration of Na in needles also increased with increasing NaCl, ranging from 0.11 mg g^{-1} (control) to 23.75 mg g^{-1} (90 mM NaCl), with significantly difference between all treatments. Similar results were found in jack pine, with the concentrations ranging from 0.07 mg g^{-1} (control) to 25.04 mg g^{-1} (90 mM NaCl). However, the magnitude of the accumulation of Na in needles didn't varied within the plant species.

Roots Na concentrations also increased when NaCl was added (Fig 2.7), with statistically significant differences between all the treatments tested, except for black spruce that had no differences between 30 mM NaCl and the other NaCl treatments. The average concentrations of root Na was similar in all species. Black spruce had concentrations ranging from 0.17 mg g-1 (control) to 11.42 mg g-^{1} (90 mM NaCl), white spruce from 0.38 mg g^{-1} (control) to 11.47 mg g^{-1} (90 mM NaCl), and jack pine from 1.06 mg g^{-1} (control) to 11.75 mg g^{-1} (90 mM NaCl). No overall differences were found between the species studied.

High accumulation of Cl occurred in needles of seedlings exposed to NaCl, with statistically significant differences between control and NaCl-treated plants. Nevertheless, white spruce had differences in Cl needle concentrations also between 30 mM and 90 mM NaCl, and jack between 30 mM and 60 mM NaCl compared to 90 mM NaCl treatment (Fig. 2.8).

Black spruce needle Cl concentrations ranged from 2.86 mg g^{-1} (control) to 34.32 mg g^{-1} (90 mM NaCl), white spruce from 2.76 mg g^{-1} (control) to 40.85 mg g^{-1} (90 mM NaCl), and jack pine from 1.50 mg g^{-1} (control) to 46.58 mg g^{-1} (90 mM NaCl).

The Cl concentrations in roots showed different trends to those in needles, with significant differences between control and NaCl treatments and between 90 mM NaCl and the other salt treatments. Distinctively, in white spruce the concentrations of Cl in roots showed differences between all treatments (Fig. 2.9).

Increasing NaCl treatment concentration led to an increase of Cl in roots, but the changes were less pronounced compared with those observed in needles. No significant differences were found between species within treatments. Black spruce roots had Cl concentrations varying between 0.66 mg g-^{1} (control) to 18.73 mg g^{-1} (90 mM NaCl), white spruce from 0.78 mg g^{-1} (control) to 16.35 mg g^{-1} (90 mM NaCl), and jack pine from 0.80 mg g^{-1} (control) to 18.61 mg g^{-1} (90 mM NaCl).

The pattern of Cl tissue concentration was similar to Na, although Cl concentrations tended to be higher, reaching a maximum of 46.58 mg g^{-1} in needles and 18.61 mg g^{-1} in roots of jack pine seedlings treated with 90 mM NaCl. Needle and root concentrations of Na and Cl corresponded with the severity of plant necrosis.

2.3.3 Needle injury

Control seedlings from all species appeared healthy, green, and turgid. Symptoms of salt injury (chlorosis and necrosis) progressed from the needle tips towards the base, both in young and old needles of the plants treated with NaCl. All species showed statistically significant differences between the treatments for the percentage of necrotic needles. Most of the seedlings under 90 mM NaCl treatment showed substantial needle necrosis and needle loss (Fig. 2.10).

Although significant differences were not found between species within treatments, treatments, the higher negative effect, observed at the end of the experiment, was in white spruce under 90 mM NaCl treatment, with an average of 58% of the needles injured (chlorosis and necrosis).

Regression analysis indicated that there is a strong positive relationship between needle necrosis and Na concentration and also among needle necrosis and Cl concentration, demonstrating that high needle necrosis is closely associated to high concentrations of Na or Cl in the studied plant species, as shown in the scatter plots from the linear regressions (Fig. 2.11).

Regression analysis indicated that there is also a positive relationship between necrosis and root Na concentration and among necrosis and root Cl concentration (Fig. 2.12), as shown in the scatter plots from the linear regressions.

The regression coefficients are useful to predict necrosis, showing that needle Na and Cl concentrations have a strongest linear relationship with plant necrosis than root Na and Cl, giving a better summary of the responses of black spruce, white spruce, and jack pine under NaCl treatments.

The equations show that the coefficient for necrosis from Na needle is 1.98 for black spruce and 2.26 for white spruce, and 2.03 jack pine, indicating that for every additional milligram of Na in the needles can expect to increase necrosis by an average of 1.98% for black spruce and 2.26% for white spruce, and 2.03% for jack pine (Table 2.2).

The analysis demonstrated that to cause complete necrosis and plant death would be necessary an accumulation of 48.9 mg g^{-1} of Na in needles of black spruce, 45.6 mg g^{-1} of Na in white spruce, and 49.6 mg g^{-1} of Na in jack pine. The slope of the regression line demonstrated that black spruce needles accumulated Na faster than the other studied species.

The regression coefficients for necrosis from Cl needle concentrations are 1.16 for black spruce, 1.33 for white spruce, and 1.14 for jack pine, demonstrating that for every additional milligram of Cl in the needles can expect to increase necrosis by an average of 1.14% in black spruce, 1.33% in white spruce, and 1.14% in jack pine (Table 2.3).

The regression coefficients demonstrated that to cause complete necrosis and plant death would be necessary an accumulation of 88.2 mg g^{-1} of Cl in needles of black spruce, 79 mg g^{-1} of Cl in white spruce, and 89.1 mg g^{-1} of Cl in jack pine. For this parameter, the slope of the regression line demonstrated that black spruce and jack pine needles accumulated Cl faster than white spruce.

2.4 Discussion

To understand the physiological mechanisms of salt tolerance in plants, it is important to distinguish between the adverse impacts caused by the osmotic effects of salt in the soil solution and the direct ionic effect of salt within the plant (Munns and Tester 2008). The results of my study showed a strong correlation between Na and Cl concentrations in the needles and roots and the extent of injury in seedlings of the three studied conifers. Similarly to other studies (Beltagi et al. 2006, Mustard and Renault 2006, Gama et al. 2007, Jamil et al. 2007a, 2007b, Houimli et al. 2008, Rui et al. 2009, Memon et al. 2010) the decrease in RSDG and RSHG was associated with the increase in NaCl concentration. Significant differences were found also between the three studied conifer species. Jack pine RSDG and RSHG were markedly higher than black spruce and white spruce, in agreement with Renault et al. (1998) study that considered white spruce to be the most sensitive to NaCl among the conifer species.

Several studies have shown that the fresh and dry weights of plants may be either negatively (Saffan 2008, Rui et al. 2009, Taffouo et al. 2010) or positively (Jamil et al. 2005, Niaz et al. 2005, Memon et al. 2010) affected by salt depending on the type of salt and plant species. A few studies have found a positive effect from salt on fresh and dry weights with NaCl concentrations between 10 mM to 200 mM, in different plant species including lettuce (*Lactuca sativa*) (Andriolo et al. 2005), cowpea (*Vigna unguiculata*) (Dantus et al. 2005), fodderbeet (*Beta vulgaris* subsp. vulgaris) and sea beet (*Beta vulgaris* subsp. maritima) (Niaz et al. 2005), and bean (*Vicia faba*) (Qados 2011). However, most of the studies with tree seedlings have demonstrated that salt treatments reduce their total dry weights (Franklin and Zwiazek 2004, Nguyen et al. 2006, Jimenez-Casas and Zwiazek 2013, Calvo-Polanco et al. 2014). In my study, even at the lowest salt level of 30 mM NaCl reduced the total dry weight in white spruce and jack pine, but no significant impact on black spruce. The decrease in shoot growth can occur in two stages: a quick response to the increased osmotic pressure, and a slower response to the accumulation of Na in leaves (Towfique et al. 2013). The accumulation of large amounts of salt in the tissues can also contribute to the total dry weights.

The osmotic stress is similar to drought stress and certain species can resist this stress by a number of different mechanisms including a development of a larger root system can exploit a greater volume of soil, thus increasing potential for water absorption (Gowda et al. 2011). Moreover, increases in root-to-shoot ratio proportionally decrease the evaporation area of shoots relative to the absorptive area of roots. (Silva et al, 2012).

The osmotic stress has greater and immediate influence on plant growth compared with ionic stress, which impacts plants later with a less pronounced effect (Munns and Tester 2008). However, under high concentration of certain salts sensitive species that are not capable to control Na influx, ionic effect suppresses the osmotic effect. In the present study, all species were affected

by salt treatments with significant differences between 30 mM NaCl and 90 mM NaCl. The maximum biomass under salt treatment was recorded in jack pine, with 20.08 mg g^{-1} dry weight in the 30 mM NaCl treatment, about 40% higher than in black spruce and white spruce subjected to the same treatment.

Many plant species have been reported to respond to water stress with an increase in root-to-shoot ratio, usually attributed to a decrease in shoot growth (Sharp and Davies 1979, Bachelard, 1986; Steinberg et al., 1990). The shoot:root dry weight ratios in white spruce were higher than in black spruce and jack pine, specially in the NaCl treatments. Many studies have reported that increasing salinity levels interfere with the partitioning of photosynthates and affect dry matter distribution (Cakmak et al. 1994).

Significant different effects in needle chlorophyll concentrations were observed between control and NaCl-treated seedlings. The total chlorophyll concentrations in control plants had the maximum values of 13.3 mg g^{-1} in black spruce, 12.9 mg g^{-1} in jack pine and 10.5 mg g^{-1} in white spruce. Similarly to the present results, the adverse effect of NaCl on leaf chlorophyll concentrations have been reported for other plant species including rice (*Oryza sativa*) (Yeo et al. 1990), barley (*Hordeum vulgare*) (Belkhodja et al. 1994), spinach (*Spinacia oleracea*) (Kaya et al. 2001), citrus (*Citrus sinensis*) (Iglesias et al. 2004), and yellow pine (*Pinus leiophylla*) (Jimenez-Casas and Zwiazek 2014). The loss of chlorophyll under salt stress could be related to photoinhibition or formation of reactive oxygen species (ROS) (Kato and Shimizu 1985). ROS causes chlorophyll degradation and membrane lipid peroxidation, decreasing membrane fluidity and selectivity (Sevengor et al. 2011).

An excess of Na in the plant tissue can cause enzyme inactivation, inhibition of nucleic acids and protein synthesis, and lead to cell membrane damage (Bañuls et al. 1997, Renault et al.

1998, 2000). Sodium may accumulate in the apoplast and result in the dehydration of cytoplasm and subsequent inhibition of enzymes involved in carbohydrate assimilation, or in the chloroplasts and inhibit photosynthetic processes (Munns and Tester 2008).

High NaCl concentrations can also lead to premature senescence, chlorosis, and necrosis of leaves (Munns 2002). Leaf necrosis is a typical symptom of Na (Chen et al. 1991, Maas 1992, Muralitharan et al. 1992) and Cl (Jimenez-Casas and Zwiazek 2013) toxicity, and was observed in many species including citrus (*Citrus* spp.) (Maas 1992), tobacco (*Nicotiana tabacum*) (Sergeeva and Martynenko 1992), Eucalyptus (*Eucalyptus* spp.) (Prat and Fathi-Ettai 1995), dogwood (*Cornus stolonifera*) and hybrid poplar (Renault et al. 1998), black spruce, jack pine, and white spruce (Nguyen et al. 2006). Leaf necrosis, an irreversible condition, followed by leaf abscission, could be a response to general stress or due to the fast accumulation of toxic levels of ions (Renault et al. 1998).

Leaves are usually more susceptible to NaCl than roots because Na and Cl accumulate in higher concentrations in shoots than roots (Tester and Davenport 2003, Nguyen et al. 2006), since the reduction in the leaf area relative to root growth would decrease the water use and allow the plant to save soil moisture and prevent salt accumulation in the root zone. Therefore, immediately after salt concentration around the roots reaches a certain level, shoot growth rate drop sharply (Munns and Tester 2008). In the present study, the highest Na accumulation of 25.04 mg g^{-1} occurred in needles of jack pine seedlings followed by white spruce, and black spruce, treated with 90 mM NaCl.

The higher levels of Na in shoots in all studied species suggest a limited root exclusion of salt (Renault et al. 1998). In addition, many species do not seem to be able to regulate Cl entry and accumulation in shoots (Jacoby 1994). In the present study, the accumulation of Na was lower than

Cl in needles and roots, in agreement with Lumis et al. (1976), Townsend (1980), Fostad and Pedersen (2000), and Nguyen et al. (2006). In some situations, Cl may be more injurious than Na (Chavan and Karadge 1980, Marcar and Termaat 1990) due to its ability to relatively readily pass through the cell membrane (Munns and Tester 2008). In general, Na accumulation in white spruce and jack pine had significant differences between all treatments while Cl in needles and roots didn't showed differences between 30 mM NaCl and 60 mM NaCl.

Salt toxicity is frequently associated with Na injury, but it may also be caused by other common anions associated with Na (Martin and Koebner 1995, Franklin and Zwiazek 2004). Indeed, Na and Cl together have greater effects than either ion alone, since Na moves faster with the highly mobile Cl (Martin and Koebner 1995). Therefore, the morphological appearance shown by plants under stress may not be sufficient to determine the injury causes (Qados 2011). Chloride is also an important contributor to salt injury in conifers needles (Franklin et al. 2002, Franklin and Zwiazek 2004). Therefore, needle Na and Cl concentrations provide a more precise estimation than root Na and Cl concentrations, concerning the injurious effects of salinity (Zekri 1993, Bañuls et al. 1990).

Basically, the salt injury occurs because of the plants' inability to restrict the uptake and storage of toxic ions in less sensitive areas within the plant (Greenway and Munns 1980). The ability of a specific species to exclude Cl is independent of its ability to exclude Na (Sykes 1993). When Na or Cl reach toxic concentrations, leaves become necrotic and die, probably because the salt level exceeded the plant's capacity to compartmentalize salt in the vacuoles, leading to their accumulation in the cytoplasm (Munns and Termaat 1986, Munns 2002, 2005, Munns et al. 2006). If the rate of necrosis is greater than new leaf production, the plant's photosynthetic capacity is not able to supply the necessary energy for plant survival.

For most plants, the main site of Na toxicity is the leaf blade, where Na accumulates after being deposited in the transpiration stream (Munns 2002). The pattern of accumulation of Na in leaves and roots may be used as an indicator of salt tolerance (Prat and Fathi-Ettai 1995). Woody plants vary in salt tolerance. Conifer species are considered to have a relatively low degree of salt tolerance (Fostad and Pedersen 2000, Apostol and Zwiazek 2003, Franklin and Zwiazek 2004, Renault 2005). In the present study, higher needles necrosis occurred in white spruce seedlings and needle concentrations of Na and Cl correlated significantly with the severity of plant necrosis, in agreement with the results reported by Apostol et al. (2002), Renault (2005), and Calvo-Polanco et al. (2008). Also, higher concentrations are associated with stronger responses.

The correlation analysis of the variables helped to understand the impacts caused by NaCl stress on the studied plants since needle concentrations of both Na and Cl corresponded with the severity of plant necrosis (Fostad and Pedersen 2000). Even if strong correlation doesn't imply causation, the results of regression analysis on this study show that the Na and Cl needle concentrations constitute a risk factor for plant necrosis

The critical Na and Cl needle concentrations differed between species. Every additional milligram of Na in the needles increase necrosis by an average of 1.98% in black spruce, 2.26% for white spruce, and 2.03% for jack pine. However, the slope of the regression line demonstrated that black spruce needles accumulated Na faster than the other studied species.

In black spruce, a concentration of 2.67 mg g^{-1} of Na in needles of seedlings treated with 30 mM NaCl was responsible for up to 10% of needle necrosis while 17.72 mg g^{-1} of Na in seedlings treated with 90 mM NaCl caused 34% overall needle necrosis. In white spruce and jack pine, on the other hand, 11.62 mg g^{-1} and 10.92 mg g^{-1} of Na in needles, respectively, in seedling treated with 30 mM NaCl, caused 10% and 19% needle necrosis. The same species, when treated

with 90 mM NaCl, had 21.8 mg g^{-1} and 22.6 mg g^{-1} of Na in needles, causing 45% and 47% necrosis.

For Cl needle concentration, every additional milligram increases necrosis by an average of 1.16% in black spruce, 1.33% in white spruce, and 1.14% for jack pine showing that the species studied are less sensitive to Cl needle concentrations than Na needle concentrations.

Therefore, the correlation between needles necrosis and Na levels is an important estimation method that does not require damaging the overall plant structure and helps predict imminent plant mortality. This parameter could be used as an effective monitoring tool to assist with preventive remediation steps and avoid plant mortality and revegetation failure.

2.5 Tables

Table 2.1 Effects of NaCl treatments on relative stem diameter growth (RSDG) and relative shoot height growth (RSHG) in black spruce, white spruce, and jack pine seedlings. Different letters beside the values indicate significant differences ($\alpha = 0.05$), between treatments (capital letters) and between plant species (lower case letters)

Species	RSDG (%)				RSHG (%)			
	Control	30 mM	60 mM	90 mM	Control	30 mM	60 mM	90 mM
Black spruce	4.36Ab	4.47Bb	4.19BCb	3.53Cb	29.08Ac	29.08Ac	29.08ABc	27.77Bc
White spruce	4.57Ab	3.86Bc	3.76BCb	3.72Cb	36.55Ab	35.67Ab	35.63ABb	33.55Bb
Jack pine	5.73Aa	5.73Ba	5.23BCa	5.08Ca	44.17Aa	43.62Aa	43.47ABa	39.55Ba

Table 2.2 Linear regression equations and predicted necrosis (ŷ) according to measurements of the observed necrosis (y) and Na needle concentrations (x) in black spruce, white spruce, and jack pine seedlings

Species	**Na needle (x) (mg g^{-1})**	**Necrosis (y) (%)**	**Necrosis (ŷ) (%)**
Black spruce	2.667	10	9
	9.882	22	28
	48.898	-	100
ŷ =3.497445+1.973576*Na needle			
White spruce	11.623	10	23
	13.911	28	28
	45.575	-	100
ŷ=-2.84936+2.256767*Na needle			
Jack pine	10.919	19	21
	17.064	35	33
	49.714	-	100
ŷ=-1.59964+2.043706*Na needle			

Table 2.3 Linear regression equations and predicted necrosis (ŷ) according to measurements of the observed necrosis (y) and Cl needle concentrations (x) in black spruce, white spruce, and jack pine seedlings

Species	**Cl needle (x) (mg g^{-1})**	**Necrosis (y) (%)**	**Necrosis (ŷ) (%)**
Black spruce	12.915	10	12
	26.365	28	28
	88.189	-	100
ŷ=-2.26953+1.159671*Cl needle			
White spruce	18.877	10	19
	25.273	28	28
	78.992	-	100
ŷ=-5.88493+1.340533*Cl needle			
Jack pine	19.453	19	20
	29.686	27	29
	89.081	-	100
ŷ=-2.47176+1.150326*Cl needle			

2.6 Figures

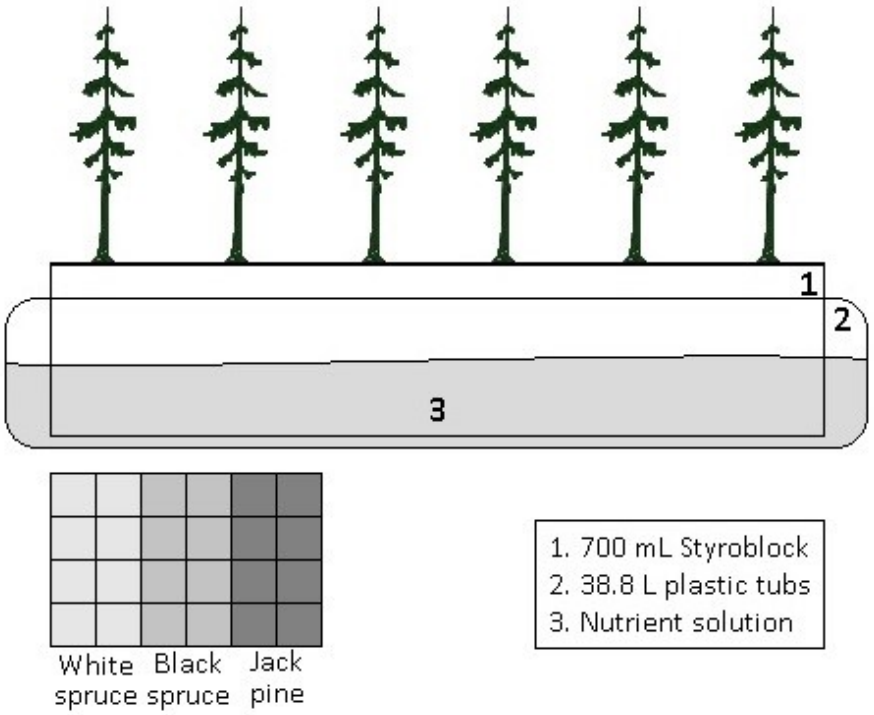

Figure 2.1 Experiment set up, container and species distribution

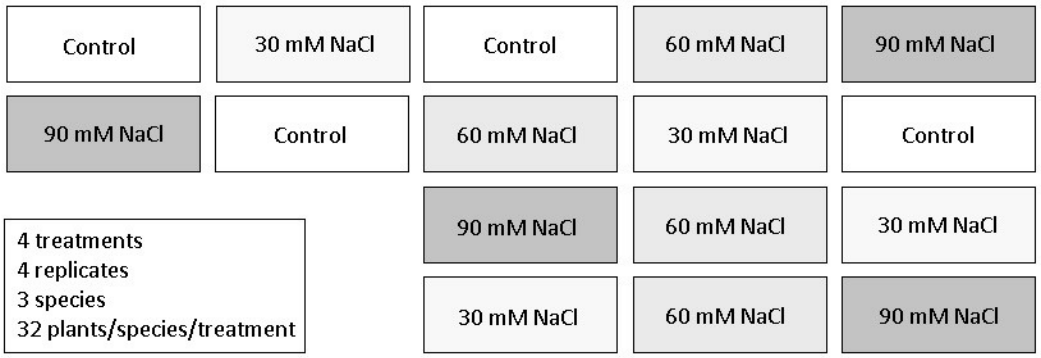

Figure 2.2 Randomized complete block design arrangement in the growth room

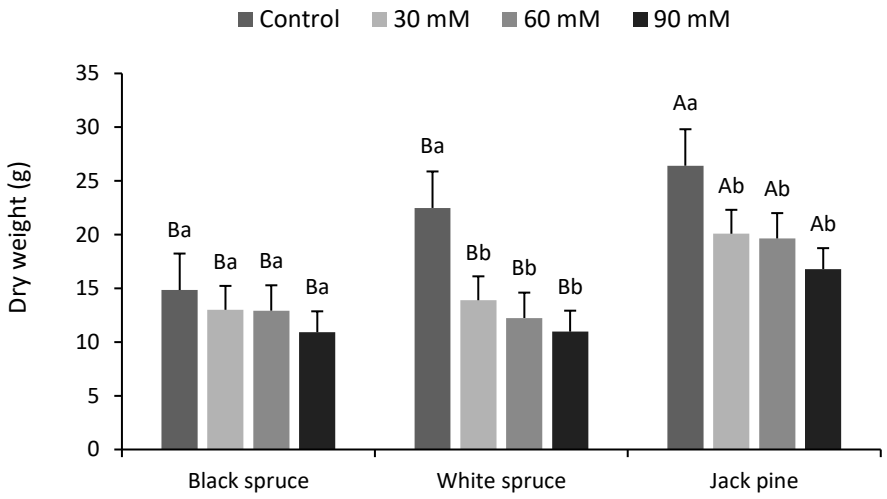

Figure 2.3 Effects of NaCl treatments on total dry weights in black spruce, white spruce, and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

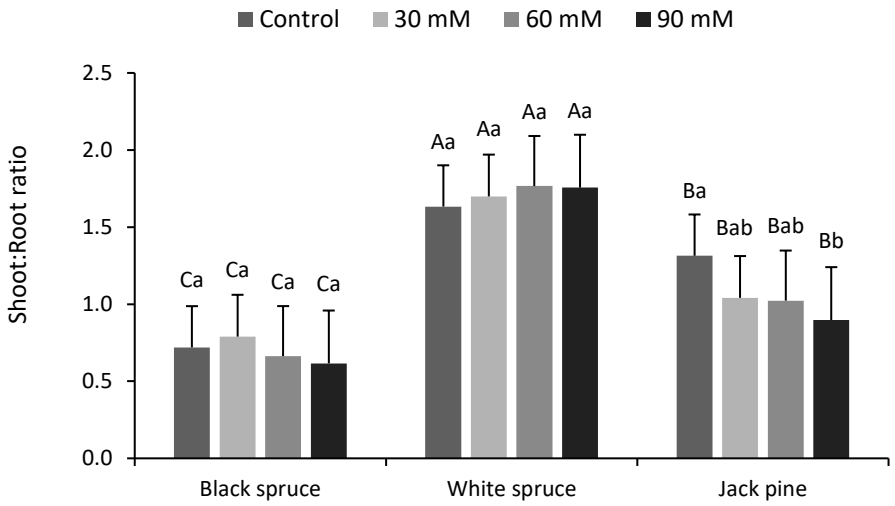

Figure 2.4 Effects of NaCl treatments on shoot:root dry weight ratios in black spruce, white spruce, and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

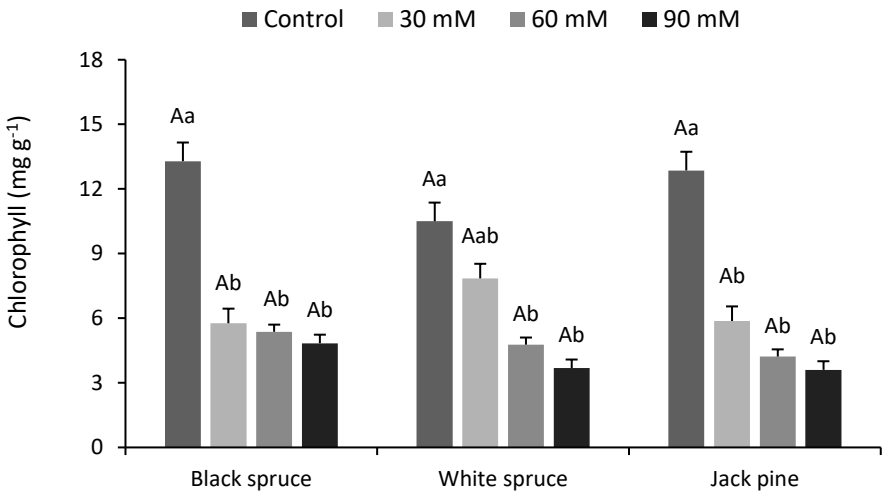

Figure 2.5 Effects of NaCl treatments on chlorophyll concentrations in needles of black spruce, white spruce, and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

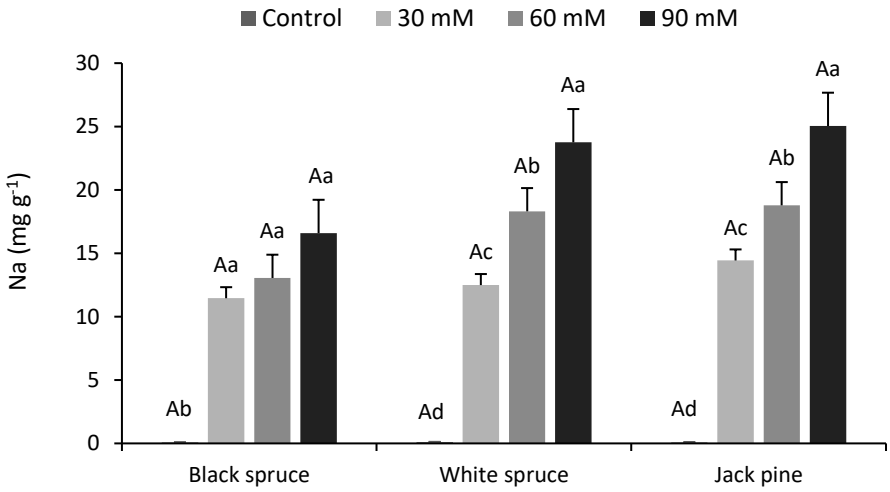

Figure 2.6 Effects of NaCl treatments on Na concentration in needles of black spruce, white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

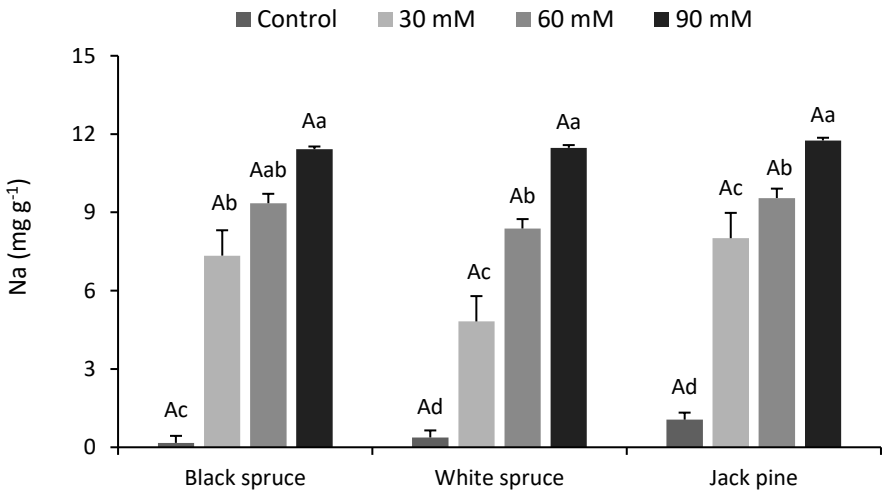

Figure 2.7 Effects of NaCl treatments on Na concentration in roots of black spruce, white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

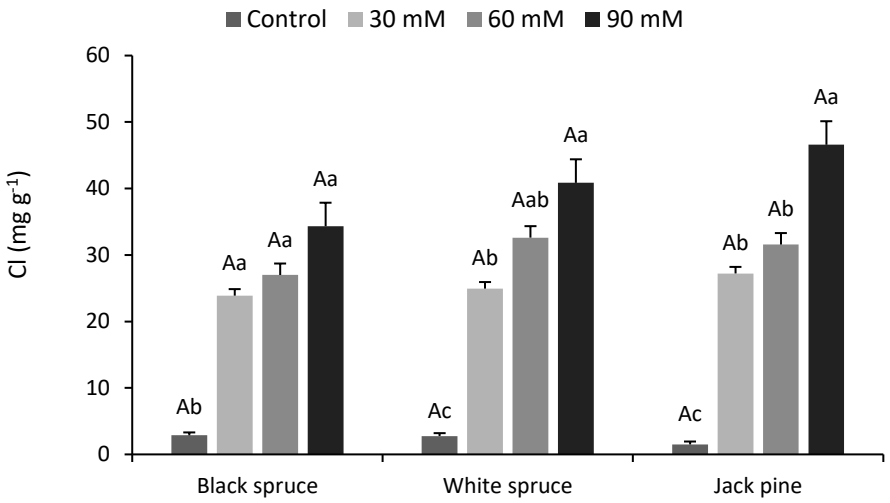

Figure 2.8 Effects of NaCl treatments on Cl concentration in needles of black spruce, white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$), between plant species (capital letters) and between treatments (lower case letters). Means ($n = 8$) ± SE are shown

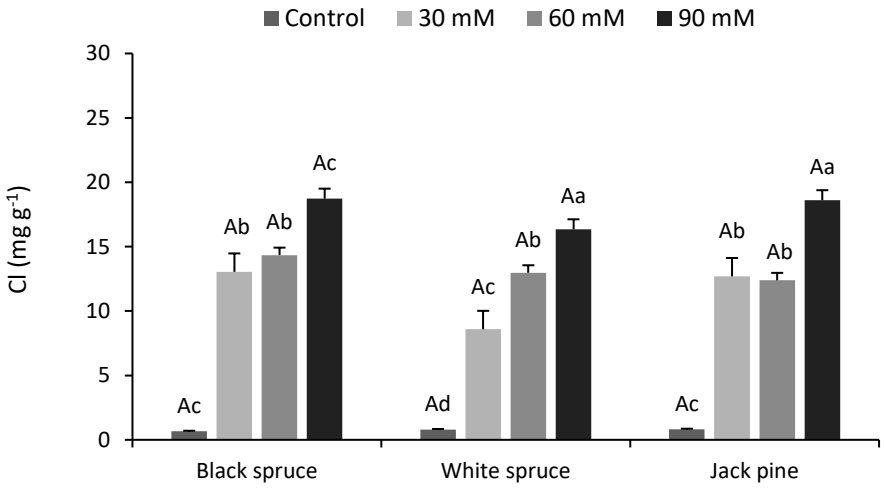

Figure 2.9 Effects of NaCl treatments on Cl concentration in roots of black spruce, white spruce and jack pine seedlings. Different letters beside the values indicate significant differences (α = 0.05), between treatments (capital letters) and between plant species (lower case letters). Means (n = 6) ± SE are shown

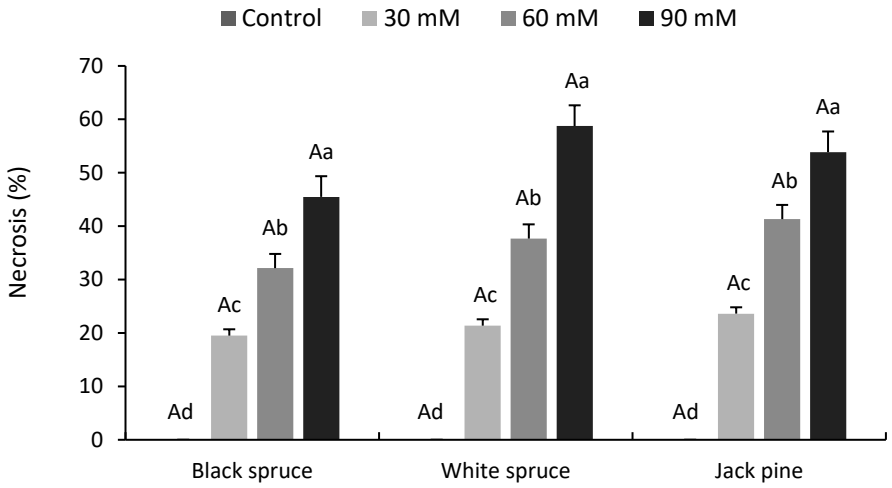

Figure 2.10 Needle necrosis caused by different treatments in black spruce, white spruce, and jack pine seedlings. Different letters beside the values indicate significant differences ($\alpha = 0.05$), between treatments (capital letters) and between plant species (lower case letters). Means ($n = 6$) ± SE are shown

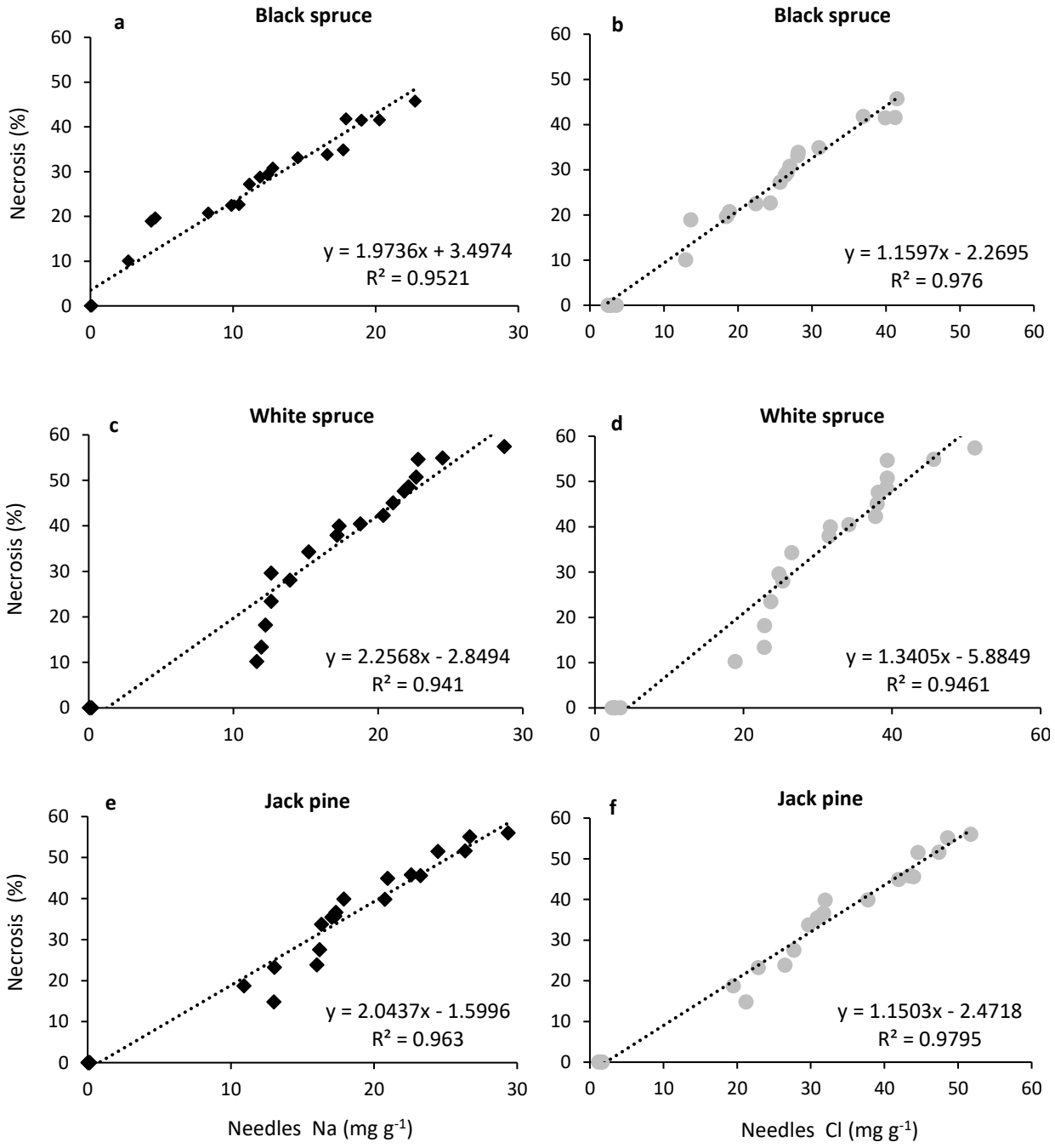

Figure 2.11 Scatter plot showing the relationship between needle Na concentrations (a, c, e) and needle Cl concentrations (b, d, f) on plant necrosis in black spruce, white spruce, and jack pine seedlings

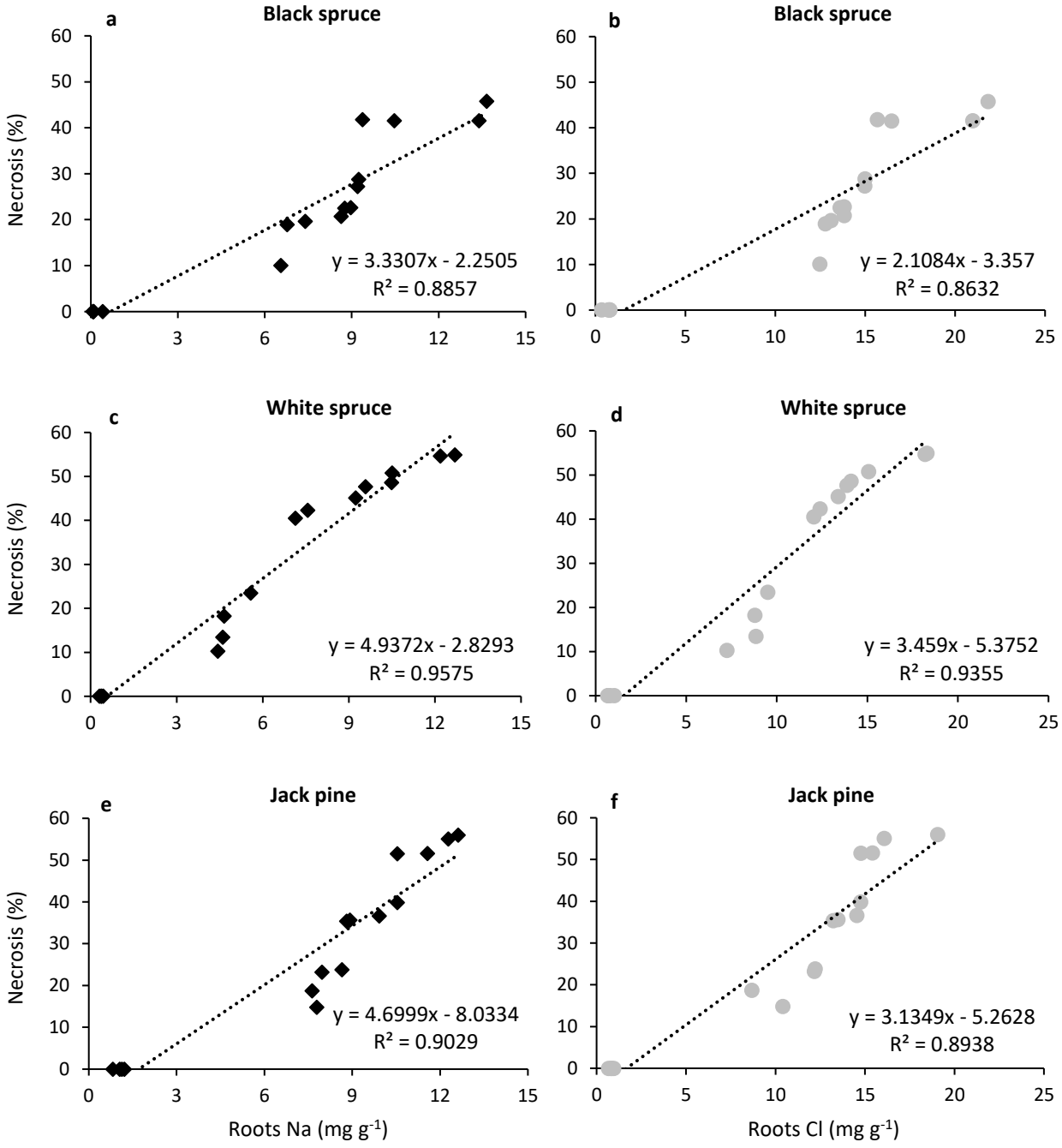

Figure 2.12 Scatter plot showing the relationship between root Na concentrations (a, c, e) and root Cl concentrations (b, d, f) on plant necrosis in black spruce, white spruce, and jack pine seedlings

Chapter 3

Effects of mineral deficiencies on conifer seedlings exposed to salt stress

3.1 Introduction

Salinity is an important environmental factor that causes reductions in plant growth and productivity. Na and Cl in the soil solution and cation exchange sites may interfere with the nutrient uptake. In addition to salt ionic and osmotic effects (Shukla and Mukhi 1979), the adverse effects of Na are associated with its antagonistic effect on Ca, K, and Zn uptake by plants (Marschner 1971). The availability of essential nutrients can also be affected by soil pH (IPNI 2010).

Plant species vary in their ability to tolerate salt stress, and this ability depends on numerous interacting variables such as the growth phase, salt concentration, and the duration of stress (Munns 2002, Zhu 2002, Vallejo et al. 2010). Overall, high salt concentration causes a decrease in osmotic potential, leading to the disruption of water uptake by root cells and making it difficult for the plant to get both water and nutrients (Talei et al. 2012). An increase in NaCl concentration was found to induce reductions in N, P, K, Ca, and Mg levels in fennel (*Foeniculum vulgare*) (Abd El-Wahab 2006), ajowan (*Trachyspermum ammi*) (Ashraf and Orooj 2006), peppermint (*Menhta piperita*), lemon verbena (*Lipia citriodora*) (Tabatabaie and Nazari 2007), chamomile (*Matricaria recutita*) (Baghalian et al. 2008), and yarrow (*Achillea fragrantissima*) (Abd EL-Azim and Ahmed 2009).

Fertilizer management can affect the productivity of plants under salinity conditions. Thus, the addition of nutrients can either improve or decrease plant resistance to salinity or have no effect at all, depending on the level of salt stress (Hu and Schmidhalter 2005).

Under salt conditions, the shoot N uptake (mg N/plant) usually decreases and the N tissue concentration (mg N/kg dry weight) increases or remains stable (Munns and Termaat 1986, Hu and

Schmidhalter 1998). The slower growth may help to avoid the dilution effect of nutrient elements and, therefore, no change in tissue N concentration may not indicate that there is no effect on N uptake. Studies have shown that salinity directly reduces the NO_3^- concentration in trees and vine plants (Hu and Schmidhalter 1998), causing a decrease in Cl uptake and accumulation due to NO_3^- /Cl^- antagonism (Bernstein et al. 1974, Hu and Schmidhalter 1997).

Phosphorus, next to N, is the most commonly limiting macronutrient to plant growth (Schachtman et al. 1998). P enters the plant through root tips, root hairs, and the outside layers of root cells. Absorption can also be increased by mycorrhizal fungi that grow in association with roots (IPNI 1999). P is highly mobile in plants and relatively immobile in soil, making the concentration in the soil solution also low. The only form of P that plants can readily absorb is inorganic phosphate, but its concentration rarely exceeds 10 mM in soil solutions (Bieleski 1973). It is most available for plant uptake between pH 5.0 and 6.0, where $H_2PO_4^{-2}$ dominates (Ullrich-Eberius et al. 1984, Furihata et al. 1992). Generally, P reacts when mixed with soil particles or by combining with elements like Ca, Fe, or Mg, to form insoluble compounds (Busman et al. 2002).

Any environmental factor that inhibits root growth, including salinity, can aggravate P deficiency symptoms (NRCS 2015). Under salinity conditions, P limitation may result in a reduction of plant water use and dry matter production, as well as induce leaf senescence, mostly because of the decrease of chlorophyll content (Kaya et al. 2007). An increase in the supply of P in a saline environment reduced the concentration of Na in shoots and grains (Khan et al. 2013), minimized the deleterious effects of salinity on plant growth and development (Shibli et al. 2001, Kaya et al. 2003), and increased salt tolerance in plants (Nieman and Clark 1976, Cerda et al. 1977, Garg al. 2005).

Chabra et al. (1976) reported competition between P and Cl uptake in tomato and Zhukovskaya (1973) stated that Cl and SO_4^{-2} reduced P uptake in barley and sunflower. However, the information in the literature about the mechanisms involved and the interaction between P and Cl in plants under salinity stress is frequently contradictory (Navarro et al. 2001).

Potassium, another mobile essential plant nutrient that is required in large amounts, is also considered to be the most abundant cation in the cells of nonhalophytic plants (Maathuis et al. 1997). About 90-95% of the total K existing in the cell is accumulated in vacuoles, contributing to cell turgidity (Subbarao et al. 2003). The uptake of K is influenced by its concentration in the plant tissues (Siddiqi and Glass 1982) and the concentration of competing ions (e.g. Mg^{+2}) in the soil solution (Cramer et al. 1991). Potassium also has a significant effect on protein synthesis, enzyme activation, photosynthesis, stomatal control, and water-relations in plants (Marschner 1995). When it is scarce, the stomata cannot function correctly, and water losses may reach harmful levels (Gething 1990).

There is abundant evidence about the link between K and Na absorption and plant development under salinity (Qadar 1988, Pandey and Srivastava 1991, Bohra and Dörffling 1993, Ghosh et al. 2016). Shabala (2009) proposed that Na induces K efflux, leading to a severe decrease in cytosolic K and rise in cytosolic Ca concentrations, which may increase reactive oxygen species and cause cell death. A reduced K:Na ratio is often a consequence of NaCl stress in glycophytic plants (Helal and Mengel 1979).

High levels of Na can relocate Ca from root membranes, altering their integrity and affecting the selectivity for K uptake (Cramer et al. 1985, 1987). This shows that salinity, in addition to decreasing the K uptake, also affects K translocation from roots to shoots, resulting in a lower K shoot content (Kant and Kafkafi 2002).

Most salinity-nutrient interaction studies were conducted with agricultural crop plants (Grattan and Grieve 1999), and found that, when the degree of salinity is not severe, the addition of mineral nutrients improves growth and yield in plants (Papadopoulos and Rendig 1983, Soliman et al. 1994).

In the present study, I investigated if deficiencies of the three major macroelements (N, K, and P) can aggravate the effects of salinity in jack pine and white spruce growth and physiology. This information will help to understand if salt stress is aggravated by the plant's poor nutritional status, especially in nutrients commonly deficient in boreal forest soils and contribute to mitigation and management of salinity-affected sites.

3.2 Materials and methods

3.2.1 Plant material and growth conditions

One-year-old, container-grown, white spruce (*Picea glauca* (Moench) Voss) and jack pine (*Pinus banksiana* Lamb.) dormant seedlings were obtained from Tree Time Services Inc, Edmonton, AB, Canada. Seedlings were stored for two weeks at 4ºC in the dark prior to the experiments.

The uniform plants were selected, and the roots were gently washed with running tap water to remove the potting soil. The seedlings were placed in aerated mineral solution culture in a controlled environment growth room at 65 ± 10% relative humidity, 22/18°C (day/night) temperature, 16-h photoperiod, and approximately 300 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the seedling level, provided by cool white fluorescent lights. Before the start of

treatments, the seedlings were fertilized with modified Hoagland's nutrient solution (Epstein 1972) until buds flushed, new shoots elongated, and new roots were formed.

3.2.2 Hydroponic set-up

The solution culture set-up consisted of 45 L opaque plastic containers protected with a Styrofoam cover containing holes through which seedlings were held with foam plugs (Fig 3.1) (Zhang et al. 2013). Each container had 10 seedlings per species with three replicates for each treatment (30 seedlings per species per treatment). Nutrient solution was circulated from 120 L plastic bins through the smaller containers with plants, within low-density polyethylene tubs through a PVC tubing connected to a water pump (model 9.5 950GPH, Danner MFG Inc, New York, NY, USA), to provide continuous aeration (dissolved O_2 of 6 mg L^{-1}).

An automatic pH control system (PHCN-70, Omega Engineering Inc, Laval, QC, Canada) connected to a pH electrode (Orion 9106 BNWP, Thermo Scientific, Rochester, NY, USA) was continuously measuring the solution pH. An electronic valve (model 8260G071 120/60 ASCO Valve, Inc, Florham Park, NJ, USA) was controlled by the pH controller and connected to a 5 % (w/v) KOH solution container to adjust the pH to 5.5 ± 0.1 range (Zhang et al. 2013).

3.2.3 Treatments

Two weeks after placing the seedlings in solution culture, the mineral solution was replaced with one of eight treatments including adequate concentrations of all macro- and micronutrients supplied by Hoagland's nutrient solution (control) or with one of the following elements supplied

in 10% concentration of the Hoagland's solution: N, P, and K. The plants were divided into two groups. One group was subjected to 60 mM NaCl treatment and the second group received no NaCl (Table 3.1). The amounts of N, P, and K of the treatment solutions were based on half-strength modified Hoagland's solution (Epstein 1972). To achieve the desired nutrient concentrations, substitutions were made as shown in Appendix 2.

To avoid osmotic shock, the plants were subjected to increasing NaCl concentrations by 15 mM NaCl at each subsequent immersion, every five days, until the final concentration of 60 mM NaCl was reached (Jimenez-Casas and Zwiazek 2013). Solution culture tubs, tubing, and pumps were cleaned once per week to prevent algal and bacterial buildup. Over the entire experiment the EC of the solutions was measured using a portable conductivity meter (Traceable, Thermo Fisher Scientific Inc, Waltham, MA, USA) to control changes in total salinity.

The experimental design was a randomized complete block with three replications per treatment (Fig. 3.2). The experimental unit was a container with 10 seedlings per species. The treatments lasted for 12 weeks.

3.2.4 Plant growth and dry biomass measurements

Immediately before and after 12 weeks of treatments, all seedling (n = 30) heights were measured from the root collar to the shoot tip, and stem diameter measured at the root collar. The relative shoot height growth and relative stem diameter growth were calculated by dividing the difference in the initial and final values by the initial value.

For dry weight determinations, roots were separated from shoots in six randomly-selected seedlings per treatment (n = 6), and their dry weights obtained after drying in an oven at 65ºC for 72-h.

3.2.5 Chlorophyll determination

Needle chlorophyll concentrations were determined according to Hiscox and Israelstam (1979). Six seedlings from each treatment (n = 6) were randomly sampled, freeze-dried for 72-h, ground with a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA), and weighed.

Chlorophyll was extracted from pulverized needles samples (100 mg dry weight) with 8 mL of dimethyl sulfoxide (DMSO) at 65°C for 24-h. Chlorophyll absorbance was determined with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Upps., Sweden) at 648 nm for Chl-*a* and 665 nm for Chl-*b*. Total chlorophyll concentration was calculated from the absorbance values using Arnon's equation (Arnon 1949).

3.2.6 Chemical analysis

Concentrations of macronutrients, Na, and Cl in the needles of four seedlings per species per treatment (n = 4) were determined in the Natural Resources Analytical Laboratory of the University of Alberta, Edmonton, AB, Canada. Dissolved metals P, K, Ca, Mg, S, and Na were measured by the Inductively Coupled Plasma - Optical Emission Spectrometry (iCap 6000, Thermo Fisher Scientific Inc, Waltham, MA, USA) (Renault et al. 2001, Calvo-Polanco et al. 2009) in needle samples containing 250 mg of ground tissue, digested with acid (5% HNO_3) according to

Mars Xpress Digestion System (CEM Corp, Matthews, NC, USA). Concentrations of Cl in the needles were analyzed using ion chromatography (DI 300, Dionex Corp, Sunnyvale, CA, USA) (Tabatabai and Frankenberger 1996) in filtered hot water extracts (Apostol et al. 2002).

3.2.7 Gas exchange

A week before the end of the experiments, six seedlings were randomly designated from each treatment (n = 6) and net photosynthesis and transpiration rates were determined with an infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE, USA) at 400 μmol m^{-2} s^{-1} photosynthetic photon flux density, at ambient humidity and CO_2 concentration, starting at 4 hours following the onset of the photoperiod. Needles were inserted about 3 cm in the leaf chamber, and to determine leaf area, the same needles were cut and scanned. Needle surface areas were calculated with Sigma Scan Pro 5.0 scanning software (Jandel Scientific, San Jose, CA, USA).

3.2.8 Statistical analysis

All data were analyzed by R Software for Statistical Analysis (3.2.4, R Development Core Team, Vienna, VA, Austria) to determine statistically significant ($p \leq 0.05$) differences between species and treatments. After analyzing the differences among group means with one-way analysis of variance test (ANOVA), comparisons between different species and treatments were conducted using Tukey's HSD Post-hoc test. A representative table of the ANOVA results is shown in the Appendix 2.

3.3 Results

3.3.1 Plant growth and chlorophyll concentrations

Plants treated with 30 mM NaCl had smaller relative stem diameter growth (RSDG) and relative shoot height growth (RSHG) compared with the control and nutrients treatments (Table 3.1).

Jack pine seedlings treated with 10% P + NaCl had about 52% smaller RSDG and approximately 34% lower RSHG compared with control. White spruce had about 50% smaller RSDG and about 24% lower RSHG on the same treatment.

Total dry weights were sharply reduced by the NaCl treatments (Fig. 3.3). When compared to control, the most impacting treatment was 10% P + NaCl, with about 53.5% lower dry weights in white spruce, and 58.4% in jack pine. Significant differences were found between 10% P + NaCl and the other treatments for shoot:root partitioning of biomass in white spruce, with higher ratio in the 10% P + NaCl treatment. In jack pine, on the other hand, the shoot:root ratio was higher in the 10% K treatment compared to 10% N + NaCl. The other treatments didn't show any significant differences (Fig. 3.4).

Chlorophyll concentrations were drastically reduced by the NaCl and nutrient treatments in both species, with the exception of white spruce subjected to 10% P treatment. The chlorophyll concentrations in white spruce ranged from 5.74 mg g^{-1} to15.18 mg g^{-1} and the treatments which had the greatest impact on chlorophyll concentrations were 10% N + NaCl and 10% P + NaCl. For jack pine, with chlorophyll concentrations ranging between 2.28 mg g^{-1} to 11.14 mg g^{-1}, the

treatments which had the greatest impact on chlorophyll concentrations were also 10% N + NaCl and 10% P + NaCl (Fig. 3.5).

3.3.2 Net photosynthesis and transpiration rates

In white spruce, the highest net photosynthetic rate was measured in the control, and significant reductions in this parameter were measured in all treatments, especially in the presence of NaCl. The treatment that had the greatest effect on net photosynthetic rate in white spruce was 10% N + NaCl, followed by 10% P + NaCl. Jack pine showed a similar photosynthetic response, under P deficiency combined with 60 mM NaCl having the greatest impact, followed by 10% N + NaCl, and 10% K + NaCl (Fig. 3.6).

The transpiration rates, in both species, decreased when NaCl was present. Overall, the reduction was greater in white spruce compared with jack pine. Similarly to net photosynthesis, the lack of P alone caused a sharp reduction in transpiration rates, especially in white spruce seedlings. For both species, the 10% N treatment had less effect on transpiration rates compared with P and K reduction treatments (Fig. 3.7).

3.3.3 Elemental analysis

The concentrations of N in white spruce and jack pine needles showed significant reductions in the treated plants, especially in the presence of NaCl. White spruce exhibited an important decrease in needle N concentrations in the 10% N and 10% N + NaCl treatments, with about 25 and 47%, respectively, lower N concentrations than the control treatment (Fig 3.8). For

jack pine, the reduction of N was found only in NaCl-treated plants, particularly when combined with low N and K concentrations in nutrient solution (Fig. 3.9).

Concentrations of P in needles varied between treatments in both species when compared to the control. In white spruce (Fig 3.8), the plants tended to have lower P concentrations when N or K were also lower, with no significant difference between 10% P + NaCl and 10% N + NaCl. In jack pine needles, there were little treatment effects in needle P concentrations, even in the presence of NaCl (Fig. 3.9).

There was a significant decrease in the concentrations of K in needles of white spruce (Fig 3.8) and jack pine (Fig 3.9) under NaCl and nutrient deficiency treatments. The most impacting treatments were 10% K + NaCl and 10% K for both species followed by 10% P + NaCl. When compared to the control treatment, white spruce treated with 10% K + NaCl had approximately 28% less K and jack pine had about 45% less K.

Calcium needle concentrations also decreased by the applied treatments, with the exception of white spruce treated with 10% K + NaCl. The treatment with greatest impact on Ca needle concentrations was 10% P + NaCl for white spruce and control + NaCl for jack pine (Fig. 3.10 and 3.11).

The needle concentrations of Mg in white spruce decreased as a result of all nutrient deficiency and NaCl treatments compared to the control (Fig. 3.10). However, in jack pine, needle Mg concentrations decreased in the N, P, and K deficiency treatments only in the presence of NaCl (Fig. 3.11). In the 10% K + NaCl treatment, the Mg needle concentration was approximately 37% lower compared with the control.

Needle S concentrations in white spruce were reduced by the nutrient deficiency and NaCl treatments, compared to the control. However, in jack pine, S needle concentrations decreased only

in N, P or K deficiency treatments combined with NaCl. The 10% K + NaCl treatment had the greatest impact on needle S concentrations in jack pine, with a 31% reduction in S concentrations compared with the control (Fig. 3.11).

3.3.4 Sodium and chloride tissue concentrations

Sodium accumulation patterns were similar in jack pine and white spruce. For both species, the treatments of 10% P + NaCl and 10% K + NaCl resulted in the greatest accumulation of Na in needles compared to the other treatments (Fig. 3.12). The shoot Na concentrations ranged from 0.04 mg g^{-1} to 12.41 mg g^{-1} in white spruce and from 0.08 mg g^{-1} to 30.10 mg g^{-1} in jack pine.

In the NaCl treatment, with full nutrient application, jack pine seedlings accumulated about 24% to 30% less Na and white spruce had about 42% to 47% less Na, compared with the NaCl treatment combined with P or K deficiency (Fig 3.12).

High accumulation of Cl occurred in the needles of all NaCl-treatments. White spruce had concentrations ranging from 0.48 mg g^{-1} to 21.0 mg g^{-1} and jack pine from 0.36 mg g^{-1} to 46.50 mg g^{-1}, showing increased Cl associated on the 10% N treatments (Fig. 3.13).

3.4 Discussion

Physiological and biochemical responses in plants, including ion toxicity, nutrient deficiency, and interactions between these factors may provide important information to understand the effects of salinity (Munns 1993, 2002, Neumann 1997, Yeo 1998, Hasegewa et al. 2000).

My results support previous studies that growth reduction is related to Na and Cl accumulation in plants (Flowers and Yeo 1981, Shannon et al. 1998). Nevertheless, NaCl stress is aggravated if a nutrient is limiting and higher NaCl treatments could be lethal or severely growth-limiting (Grattan and Grieve 1992).

In my study, NaCl caused reductions in stem diameter, height growth, as well as dry weight of plants, as was frequently reported in other studies (Renault et al. 1998, 2000, Singla and Garg 2005, Khan et al. 2013). This could partly be the effect of inadequate amount of essential nutrients in the expanding region that may limit cell division and elongation when plants are exposed to NaCl (Berstein et al. 1995). Also, a reduction in photosynthetic pigment concentrations by NaCl treatments can have a major impact on photosynthesis and, subsequently, growth.

Growth conditions in the controlled-environment growth room in the present study could have also affected plant responses to NaCl. It has been reported that the salinity effects on photosynthetic pigments concentrations may also depend on light intensity. Morales et al. (1992) demonstrated that chlorophyll composition in barley did not change in response to salt stress when grown under low light conditions. However, in the presence of high light (up to 1500 μmol m^{-2} s^{-1} PPFD) conditions NaCl treatments induced significant changes in photosynthetic pigment levels in sorghum (Masojidek et al. 1991). In my study, seedlings were grown and treated in approximately 300 μmol m^{-2} s^{-1} PPFD and white spruce and jack pine showed a significant decrease in total chlorophyll concentration in 10% N + NaCl and 10% P + NaCl treatments, like shown in many studies (Sultana et al. 1999, Tort and Turkyilmaz, 2004, Misra et al. 2006, Murillo-Amador et al. 2007, Taffouo et al. 2010, Qados 2011). The decrease in chlorophyll concentration leads to a reduction of electrons flow through the photosystems, helping prevent photodamage to PS2 when

salt-stressed plants are exposed to high light (Mishra et al. 1991, Morales et al. 1992, Belkhodja et al. 1994).

Saline soils usually have higher concentrations of Na than K and Ca resulting in passive accumulation of Na in shoots and roots (Bohra and Doerffling 1993). Therefore, there is an inverse correlation between shoot Na concentration and salt tolerance of plants (Yeo and Flowers 1986). Higher amounts of Na interfere with nutrient absorption (Marschner 1995, Grattan and Grieve 1999, Ashraf and Harris 2004, Flowers and Flowers 2005). In my study, the levels of Na were significantly higher in jack pine in the 10% P + NaCl treatment compared to the control + NaCl treatment.

Chloride is an important factor contributing to salt injury in conifers (Franklin et al. 2002, Franklin and Zwiazek 2004) mostly because plants can absorb a substantial amount of Cl, when its concentration in the soil solution is high (Christensen et al. 1981) and they cannot successfully regulate Cl entry into the shoot (Jacoby 1994, Franklin and Zwiazek 2004). However, there is no general agreement about the control of Cl uptake and transport in plants (Mansour 2014). The highest needle concentrations of Cl were found in jack pine compared with white spruce seedlings.

The plants exposed to 10% P + NaCl accumulated more Na, while seedlings treated with 10% N + NaCl accumulated more Cl (Fostad and Pedersen 2000). Although some more Cl was present in the nitrogen treatment due to slightly different nutrient composition, this is a relatively small amount that should not affect the interpretation of the results. Smith et al. (1987) found that an increase in Cl concentration in leaves of kiwifruit (*Actinidia deliciosa*) resulted in an equivalent decrease of NO_3^- and did not change the concentrations of P or S. In the present study, increased Cl concentrations were found when N was deficient, especially in the presence of NaCl. However,

total N concentrations in the other treatments did not decrease in response to NaCl suggesting that NO_3^- absorption did not compete directly with Cl (Ourry et al. 1992, Liu and Shelp 1996).

The inhibition of NO_3^- absorption by Cl depends on the plant species and the concentrations of both ions in the soil solution (Cerezo et al. 1997). The antagonistic effects between NO_3^- and Cl were reported in many plant species (Fuqua et al. 1976, Liu and Shelp 1996, Bar et al. 1997). The competition between Cl and NO_3^- is thought to be more severe in salt-sensitive plants than in salt-tolerant plants, such as cotton (*Gossypium* spp.) (Leidi et al. 1992).

Because mineral nutrients also contribute to salinity, high salinity can disturb the nutrient balance in the plant or interfere with their absorption (Blaylock et al. 1994). It has been also reported that high NaCl concentration induces P and K deficiency in plants, including tomato (*Solanum lycopersicum*) (Adams 1988, 1991), cucumber (*Cucumis sativus*) (Sonneveld and Kreij 1999), and spinach (*Spinacia oleracea*) (Kaya et al. 2001).

In the present study, NaCl exposure caused a significant decrease in K and Ca concentrations in jack pine needles, especially in the 10% K + NaCl treatment, and 10% P + NaCl in white spruce. It has previously been reported that leaf K levels declined in maize (*Zea mays*), barley (*Hordeum vulgare*) (Benes et al. 1996), and sorghum (*Sorghum bicolor*) (Khan et al. 1995). Indeed, many studies support the notion that salinity inhibits P uptake (Treeby and van Stevenick 1988, Martinez and Läuchli 1994, Martinez et al. 1996, Navarro et al. 2001). Moreover, the uptake and transport of P depends on the external concentration of P (Jungk et al. 1990), and regulated by multiple transporters in the plasma membrane (Schachtman et al. 1998). Treeby and van Steveninck (1988) also found that P uptake depended on the P in the solution, and at low concentration, salinity decreased the uptake in lupin (*Lupinus albus*) roots. Therefore, a probable

explanation for the decrease in P uptake in the presence of NaCl reported in the present study could be the competition between P and Cl for the transport sites (Navarro et al. 2001).

When the supply of P is limited, plants may develop more roots than shoots (Schachtman et al. 1998, IPNI 1999). Salt uptake by roots and its translocation to shoots is a result of the transpirational flux, essential to maintain the water status in the plant and high transpiration rates may cause accumulation of toxic levels of ions in the shoots (Renault et al. 1998, Yeo 1998). It is likely that low transpiration rates reduce the translocation of ions to shoots (Orsini et al. 2012). However, Franklin and Zwiazek (2004) did not find a correlation between transpiration rates and Na uptake in jack pine.

Although the question of the effect of salinity on P is not fully resolved (Zribi et al. 2014), it is well established that P has an important role in alleviating the adverse effects of salinity on plant growth. P uptake can be stimulated by small amounts and suppressed by higher amounts of Cl (Wang et al. 1989). In fact, many studies described a decline in P absorption when plants were exposed to Cl (Kafkafi 1984). In the present study, when P was not deficient, the impact of NaCl on root dry weights in white spruce were less prominent. In the NaCl treatment where P was deficient, the concentrations of Na in needles were higher, dry weights, stem diameters, and heights were smaller, and net photosynthesis and transpiration rates were reduced. These findings agree with those of Knight et al. (1992) and Khan et al. (2013).

Plant tissue comprises about 1-10% K of the dry matter and K is one of the most abundant nutrients in plants (Epstein and Bloom 2005). In the present study, K was the second most abundant mineral nutrient in the tissue, with higher levels in white spruce compared with jack pine. In plants exposed to salinity, inhibition of K uptake by Na can be the major cause of the reduced K concentration in cells (Kronzucker et al. 2008). However, another possible cause may be a

stimulation of K efflux by Na (Lynch and Läuchli 1984, Cramer et al. 1985, Kaya et al. 2002). K deficiency can reduce net photosynthesis rate, probably because of the dependence of protein synthesis and development processes on K and interference with the photosynthetic system (Huber 1985).

In addition to reducing K absorption, salinity also interferes with K translocation from roots to shoots, which results in a lower K shoot content (Kant and Kafkafi 2002). The results of this study showed that K deficiency also significantly reduced net photosynthesis and transpiration rates in white spruce, but in jack pine this effect was present only in the NaCl treatment. Inhibitory effects of salinity on biochemical photosynthesis have been commonly reported. Nazir et al. (2001) found a significant reduction in photosynthesis in *Brassica* sp. and Raza et al. (2006) in wheat (*Triticum aestivum*) species under salt stress. NaCl was more effective in reducing photosynthesis and transpiration rates in plants than nutrient deficiency. In this study, photosynthesis declined about 77% in white spruce and 41% in jack pine, in the presence of 60 mM NaCl, and was reduced more than 90% when P deficiency was also present. Sultana et al. (1999) found an extreme reduction on photosynthetic rate in *Oryza sativa* treated with just 61 mM NaCl, partly attributed to the reduction in photosynthetic pigments (Kolchevskii et al. 1995) and partly to the increased ion concentrations (Khan et al. 1997).

Certainly, there is much evidence that salinity affects photosynthetic parameters, like osmotic and leaf water potential, transpiration rate, and leaf water content (Sultana et al. 1999), mostly because of the reduction in leaf area, chlorophyll content, and stomatal conductance (Downton et al. 1985, Yeo et al. 1985, Netondo et al. 2004). An immediate response to salinity is stomatal closure (Kaya et al. 2001). Other investigators also attributed the decrease in net photosynthesis to a direct effect of salt on stomatal resistance via reduction in guard cell turgor,

causing a reduction in intercellular CO_2 (Dionisio-Sese and Tobita 2000). Nevertheless, Koyro (2006) suggested that a reduction in photosynthesis and transpiration rates may represent an adaptive mechanism to deal with salinity, not just a negative consequence of excessive salt (Flanagan and Jefferies 1989, Clark at al.1999). This strategy could decrease salt accumulation and maintain ions longer at subtoxic levels (Everard et al. 1994).

It has been also reported that the sensitivity of some plants to salinity is due to their inability to maintain Na and Cl out of the transpiration stream (Gorham et al. 1990). In fact, the present study found statistically significant decreases in transpiration rates in the presence of NaCl, with a more pronounced influence when N, K, and K were deficient.

Salinity also can cause changes in membrane properties through a displacement of Ca by Na at a high NaCl concentration (Cramer et al. 1985), consequently affecting the selectivity for K uptake (Cramer et al. 1985, 1987). Certainly, when the concentration of Na increases in leaves, Ca levels decreases (Shibli et al. 1998), similarly to what I observed in jack pine in this study.

In conclusion, the results of the study demonstrated that P deficiency aggravated the effects of NaCl in white spruce and jack pine seedlings. The effects of 10% P and NaCl treatments included growth and dry weight decrease, profound decreases in chlorophyll concentrations, net photosynthesis and transpiration rates, and higher concentration of Na in needles. Since low soil P levels may aggravate the effects of salt stress, P fertilization should be considered as an important factor in revegetation areas.

3.5 Tables

Table 3.1 Concentration of selected essential elements added to the hydroponic system nutrient solution in the in the present study

Treatment	Element (mM)					
	N	P	K	Ca	Mg	S
Control	8.0	1.0	3.0	2.0	0.5	0.5
Control + NaCl	8.0	1.0	3.0	2.0	0.5	0.5
10% N	0.8	1.0	3.0	2.0	0.5	0.5
10% N + NaCl	0.8	1.0	3.0	2.0	0.5	0.5
10% P	8.0	0.1	3.0	2.0	0.5	0.5
10% P + NaCl	8.0	0.1	3.0	2.0	0.5	0.5
10% K	8.0	1.0	0.3	2.0	0.5	0.5
10% K + NaCl	8.0	1.0	0.3	2.0	0.5	0.5

Table 3.2 Effects of mineral nutrition and NaCl treatments on relative stem diameter growth (RSDG) and relative shoot height growth (RSHG) in white spruce and jack pine seedlings. Different letters beside the values indicate significant differences ($\alpha = 0.05$) between treatments. Means (n = 30) are shown

Treatments	RSDG (%)		RSHG (%)	
	White spruce	Jack pine	White spruce	Jack pine
Control	5.28a	4.08a	34.89a	33.80a
Control + NaCl	3.94ab	3.47ab	30.29bc	32.89a
10% N	5.04a	3.47ab	34.70ab	33.32a
10% N + NaCl	3.65ab	2.78bcd	28.97c	28.00b
10% P	3.92ab	3.31abc	29.92bc	29.75ab
10% P + NaCl	2.65b	1.96d	26.53c	22.38c
10% K	5.20a	3.40abc	30.13bc	30.16ab
10% K + NaCl	3.79ab	2.45cd	30.09bc	28.53b

3.6 Figures

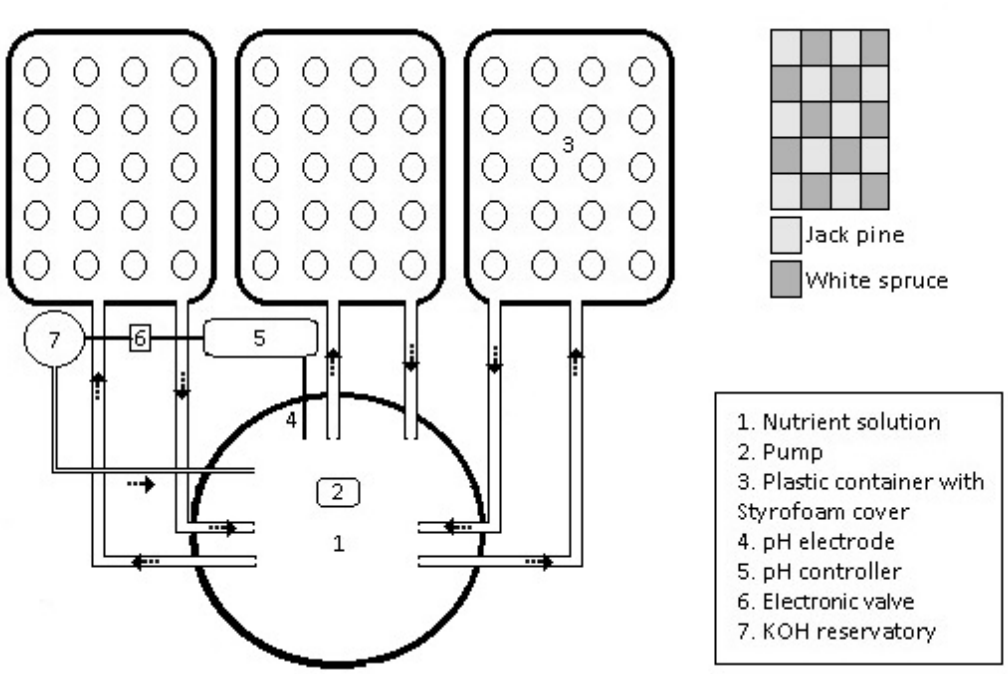

Figure 3.1 Hydroponics experiment set up

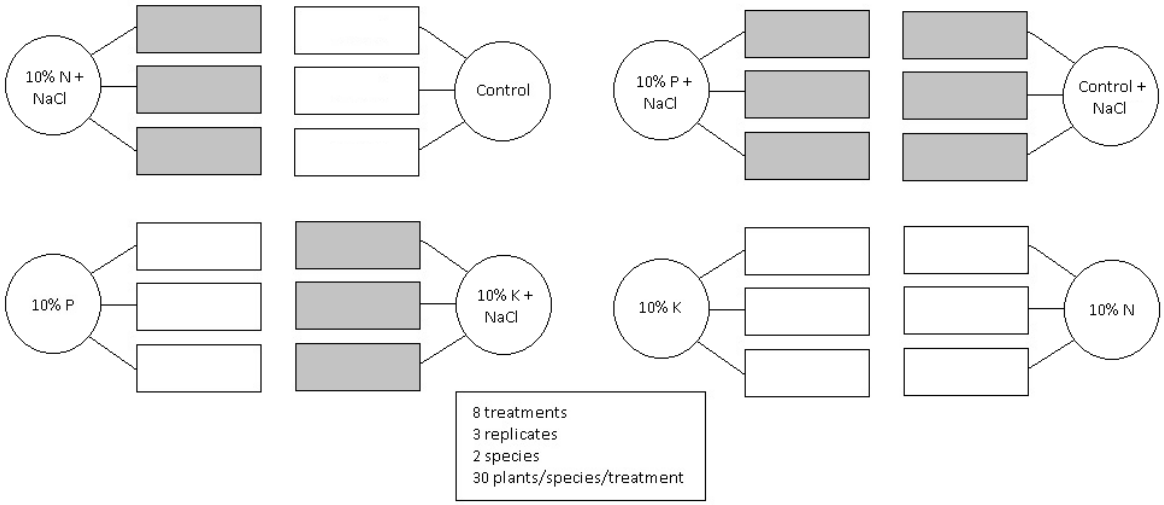

Figure 3.2 Randomized complete block design arrangement in the growth room

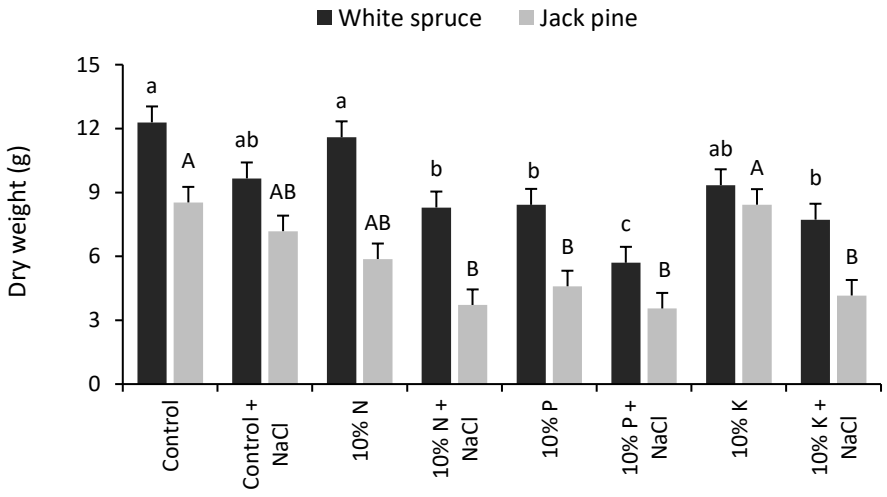

Figure 3.3 Effects of mineral nutrition and NaCl treatments on total dry weights in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments. Means ($n = 6$) ± SE are shown

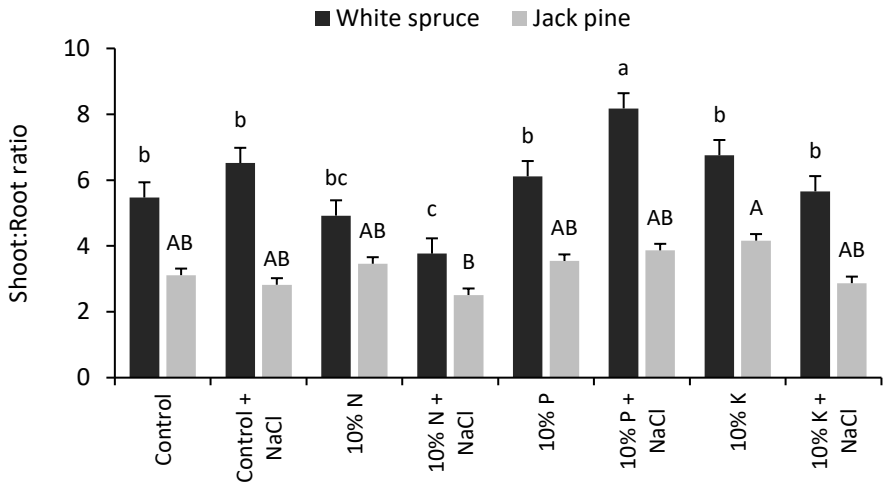

Figure 3.4 Effects of mineral nutrition and NaCl treatments on shoot:root dry weight ratios in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences (α = 0.05), between treatments. Means (n = 6) ± SE are shown

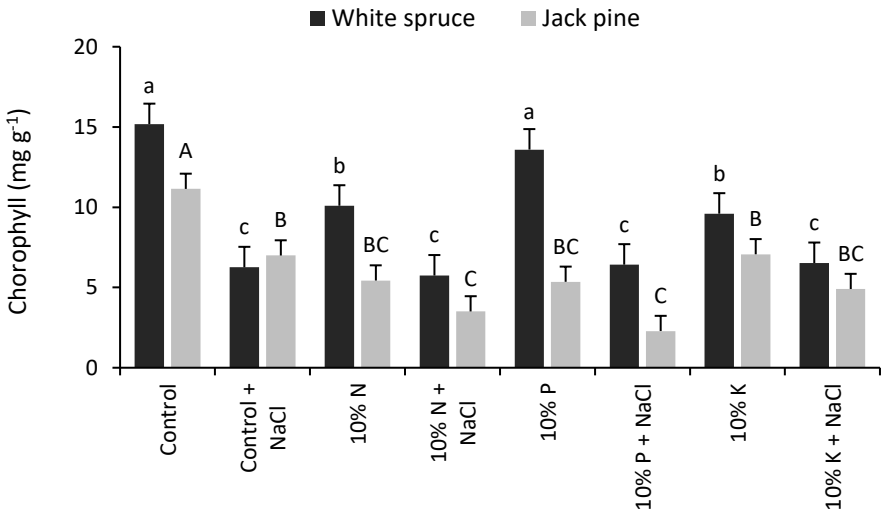

Figure 3.5 Effects of mineral nutrition and NaCl treatments on total chlorophyll in needles of white spruce and jack pine seedlings. Different letters above the bars indicate significant differences (α = 0.05) between treatments within each plant species. Means (n = 6) ± SE are shown

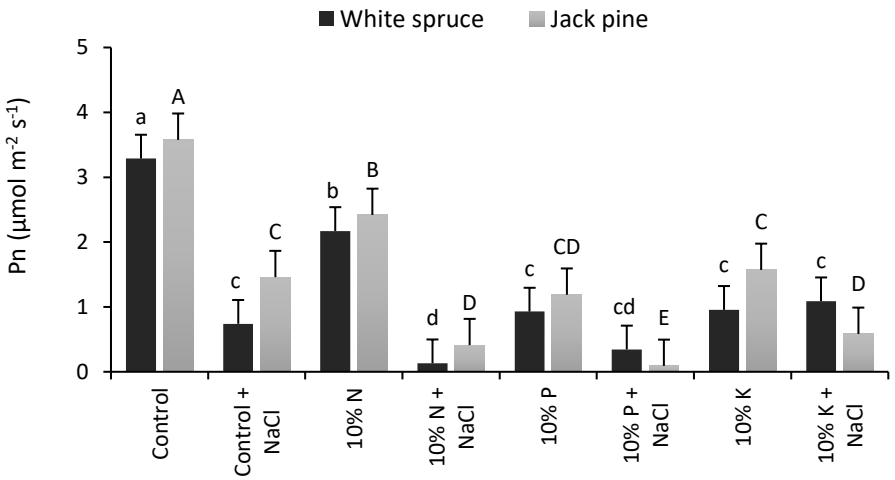

Figure 3.6 Effects of mineral nutrition and NaCl treatments on net photosynthesis rates in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments within each plant species. Means ($n = 6$) ± SE are shown

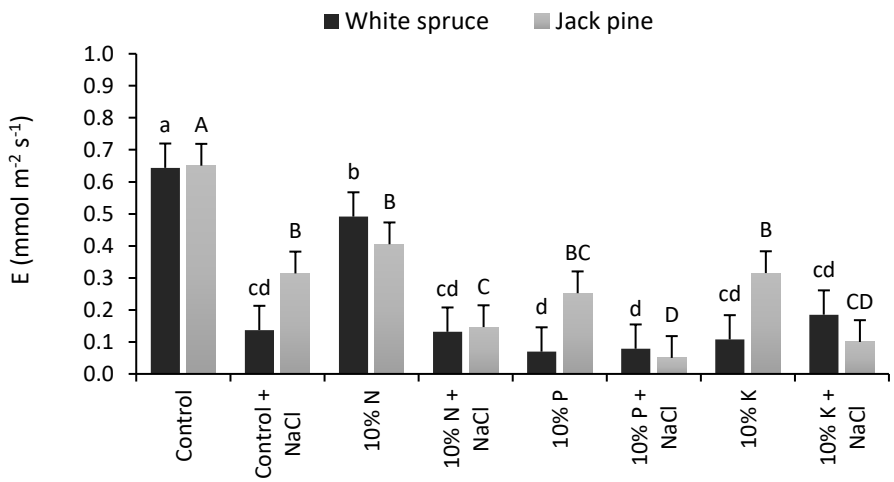

Figure 3.7 Effects of mineral nutrition and NaCl treatments on transpiration rates in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments within each plant species. Means ($n = 6$) ± SE are shown

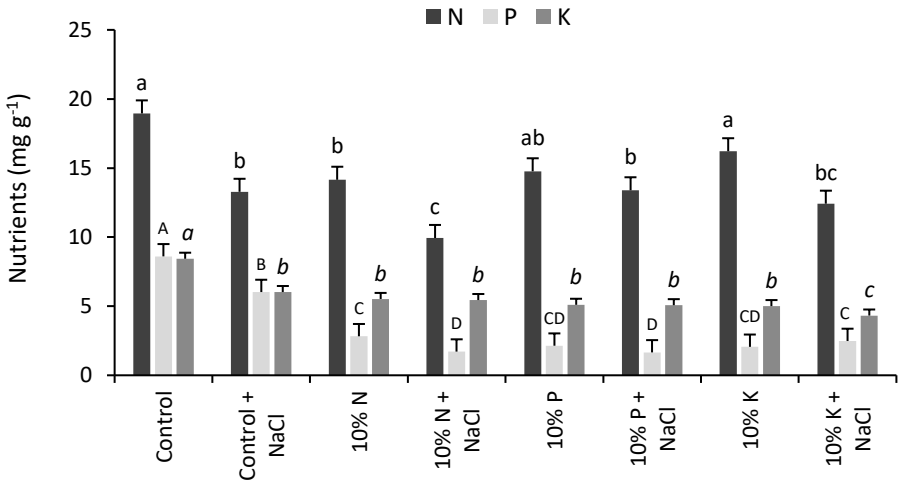

Figure 3.8 Effects of mineral nutrition and NaCl treatments on N, P, and K needle concentrations in white spruce seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments. Means ($n = 4$) ± SE are shown

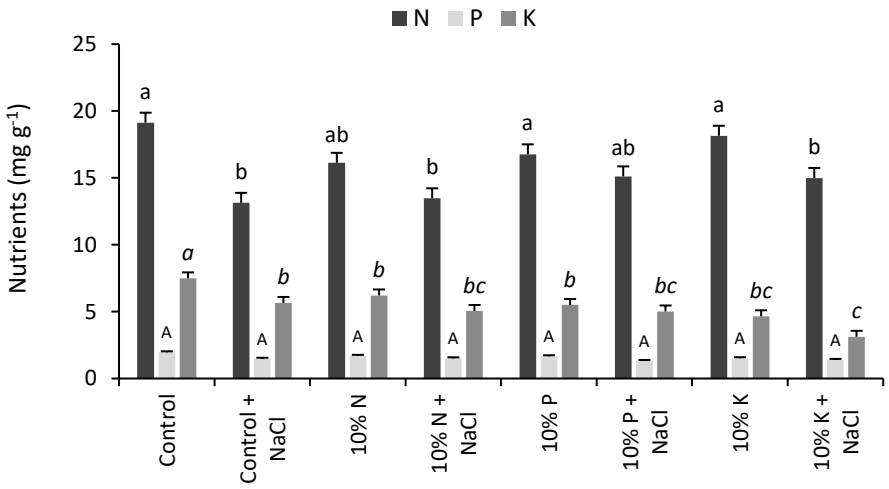

Figure 3.9 Effects of mineral nutrition and NaCl treatments on N, P, and K needle concentrations in jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments. Means ($n = 4$) ± SE are shown

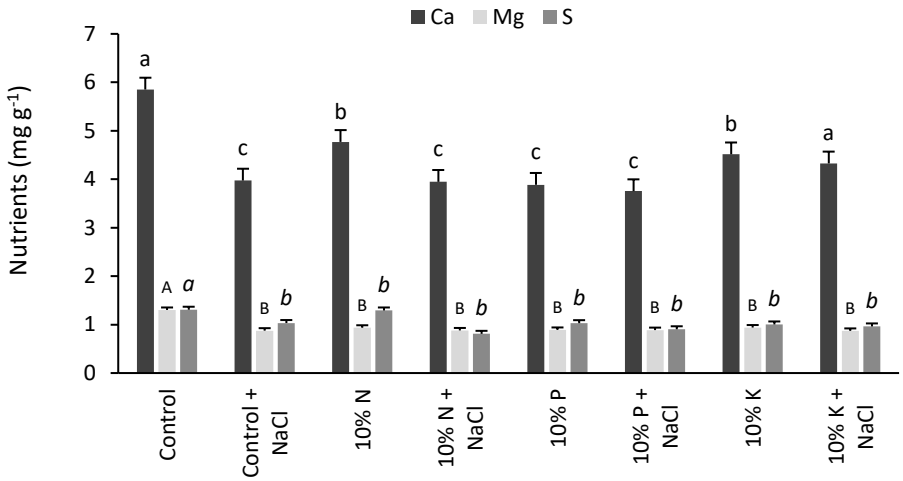

Figure 3.10 Effects of mineral nutrition and NaCl treatments on Ca, Mg, and S needle concentrations in white spruce seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments. Means ($n = 4$) ± SE are shown

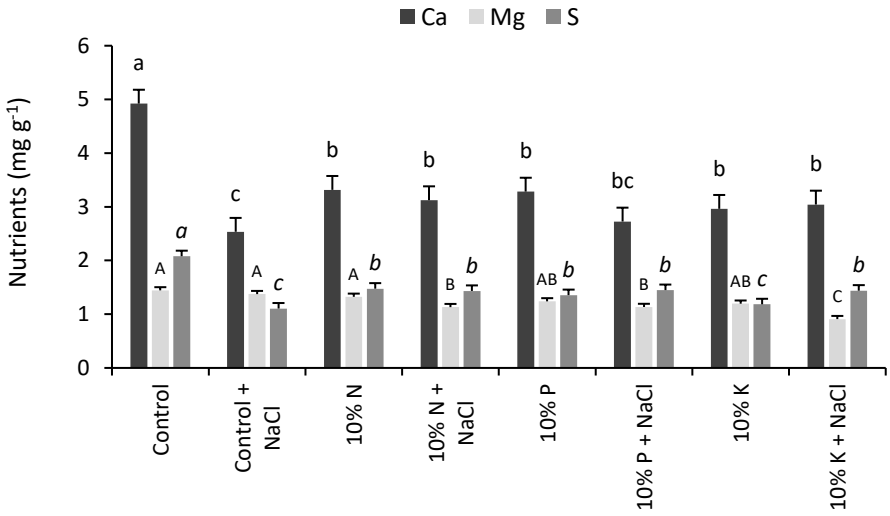

Figure 3.11 Effects of mineral nutrition and NaCl treatments on Ca, Mg, and S needle concentrations in jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments. Means ($n = 4$) ± SE are shown

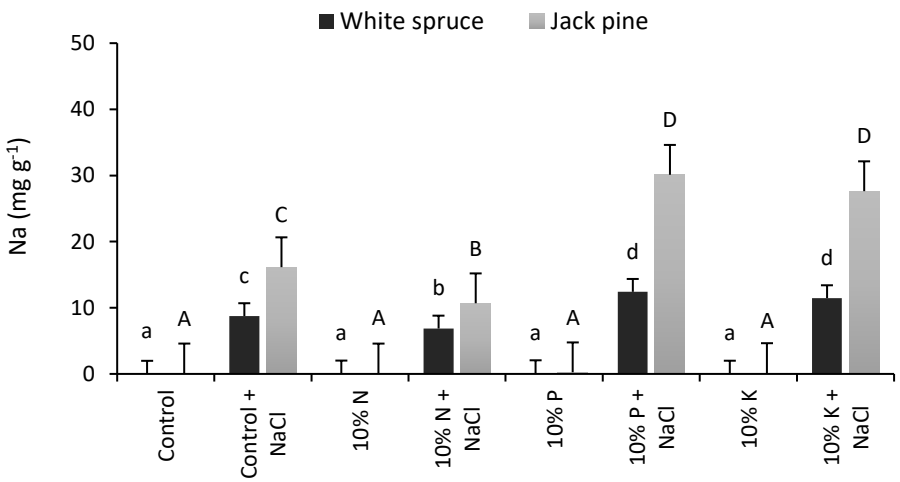

Figure 3.12 Effects of mineral nutrition and NaCl treatments on Na needle concentrations in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments within each plant species. Means ($n = 4$) ± SE are shown

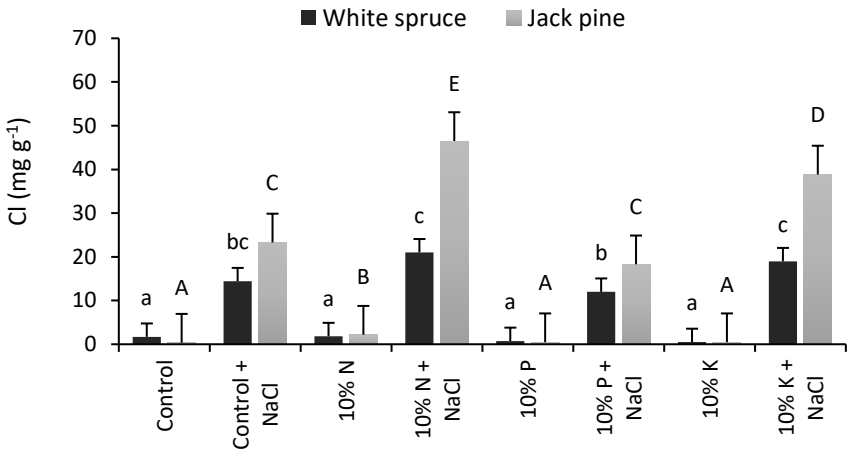

Figure 3.13 Effects of mineral nutrition and NaCl treatments on Cl needle concentrations in white spruce and jack pine seedlings. Different letters above the bars indicate significant differences (α = 0.05) between treatments within each plant species. Means (n = 4) ± SE are shown

Chapter 4

General discussion and conclusion

4.1 Outcomes of the studies

Salinity affects important plant processes like water and ionic relations and nutrient uptake. When exposed to salinity, plants experience water stress, which immediately affects cell expansion. Under longer-term exposure, plants undergo ionic stress, which can lead to early senescence and, consequently, a reduction in the photosynthetic area available to support growth (Cramer and Nowak 1992). Salt affects photosynthetic components such as enzymes, chlorophyll, and carotenoids. These changes depend on the severity and extent of the stress (Lakshmi et al. 1996, Misra et al. 1997) and the plant species (Dubey 1994). Concurrently, soil salinity imposes osmotic stress, nutrient deficiency (N, P, K, Ca), and oxidative stress (Bano and Fatima 2009).

Stem diameters and heights of black spruce, white spruce, and jack pine were significantly reduced at 30 mM NaCl and higher, and white spruce and jack pine also exhibited reduced biomass. Chlorophyll concentrations decreased in response to NaCl treatments in all species. Symptoms of salt injury were observed in young and old needles of the plants treated with NaCl and significant needle necrosis in most plants under 90 mM NaCl treatment. The greatest effect was observed in white spruce, with an average of 58% of the needles injured, however, jack pine had higher accumulation of Na and Cl in needles, confirming the hypothesis that the level of salt injury can be correlated, and therefore, predicted from shoot Na and Cl concentrations.

From the results of this experiment, it can be concluded that the presence of NaCl in the nutrient solution strongly affected growth (stem diameters, heights, and dry weights), and chlorophyll concentrations in jack pine, black spruce, and white spruce seedlings. NaCl can lead

to Na and Cl accumulation in needles and cause injury and necrosis. In my study, there was a strong positive correlation between necrosis and Na and Cl needle concentrations, suggesting that plant necrosis is closely related to high concentrations of Na or Cl.

The critical Na concentration, beyond which plant injury was established, differed between species. In black spruce, a concentration of 2.67 mg g^{-1} of Na in needles, treated with 30 mM NaCl, was responsible for up to 10% of necrosis while 17.72 mg g^{-1} of Na in needles, treated with 90 mM NaCl, caused 34% overall plant necrosis. In white spruce and jack pine, on the other hand, a minimum of 11.62 mg g^{-1} and 10.91 mg g^{-1}, respectively, of Na in needles of seedling treated with 30 mM NaCl was necessary to cause 10% and 18% necrosis. In both species studied, when treated with 90 mM NaCl, 21.8 mg g^{-1} and 22.6 mg g^{-1} of Na in needles was responsible for 45% and 47% necrosis. The concentrations of over 30 mM NaCl became critical, with more than 25% of the needles showing injury and necrosis. Thus, the needle Na concentrations analysis can help to predict imminent plant mortality and it is a non-destructive method, since only some needles from the tree can be removed to be analyzed.

Adequate concentrations of nutrients are required for plant growth, and below this threshold, plant growth and development are reduced. Nutrient availability decreases under salt stress conditions, and the essential nutrient requirements increase (Marschner 1995, Khan et al. 2013). Therefore, the concentrations of essential elements in conifer needles can be used as indicator of biotic or abiotic stresses (Lombardo et al. 2001, Poykio and Torvela 2001, Rautio and Huttunen 2003, Ábrahám et al. 2010).

The results of my study on the effects of mineral nutrition on salt tolerance in forest plants showed that salinity limits growth more than nutrient deficiency. Nevertheless, plant development would always exhibit negative effects if a nutrient was limiting and higher NaCl treatments could

be lethal (Grattan and Grieve 1992). The effects of nutrients and NaCl treatments varied between white spruce and jack pine but, in general, stem diameters, seedlings heights, total dry weights, chlorophyll concentrations, net photosynthesis and transpiration rates were reduced in the presence of NaCl. Moreover, P deficiency aggravated the effects of NaCl in white spruce and jack pine seedlings. The effects of 10% P + NaCl treatment included a decrease in growth, chlorophyll concentrations, net photosynthesis and transpiration rates, and higher concentration of Na in needles, with greater impact in jack pine.

In general, plants exposed to 10% P + NaCl accumulated more Na while seedlings treated with 10% N + NaCl accumulated more Cl. The results of studies concerning the effects of P nutrition and salinity in plant growth are contradictory. However, many of these studies emphasized the importance of P for the alleviation of the adverse effects of salinity stress in plants (Qados 2011, Shahriaripour et al. 2011). P uptake can be stimulated by small amounts of Cl and suppressed by higher amounts of Cl (Wang et al. 1989).

Under stressful salt conditions, the availability of P can be reduced, because of ionic-strength effects that interfere on P activity or because P concentration are strongly influenced by absorption processes and low solubility of Ca-P minerals, which can decrease P availability under saline conditions (Grattan and Grieve 1999). NaCl also reduced K uptake, interfering with K translocation from root to shoot, which resulted in lower K needle concentrations (Cakmak 2005). An optimum mineral-nutrient status of plants plays critical role in determining plant tolerance to various stresses (Umar et al. 2011). Therefore, white spruce and jack pine seedlings under salt stress will respond positively to P and K increase (Hu and Schmidhalter 2005) as these nutrients may help to alleviate the detrimental effects of salt stress.

4.2 Perspectives for future studies

The extent and impacts of salinity in plants depend on the other environmental factors such as temperature, light, and nutrients (Shannon et al. 1994). Na competes with K uptake (Britto et al. 2010) and plants need to maintain high concentrations of K and Ca to grow successfully in saline environments (Greenway and Munns 1980). Additionally, supplementary K, Ca, and P may decrease the impacts of salinity on plant growth, water use, and membrane permeability, accentuating the critical role of these nutrients in protecting plants from salt stress (Cramer et al. 1985, Rengel and Elliott 1992, Marschner 1995).

Posphorus and salinity act antagonistically, and it has been observed that P fertilization reduces the concentration of Na in shoots (Aslam et al. 1996, Asch et al. 1999, Malik et al. 1999, Kaya et al. 2001, Abid et al. 2002), resulting in greater survival, growth (Qadar 1998), and inducing salt tolerance in plants (Salim et al. 1999).

Contradictory results have been reported, indicating that K and Ca treatment had no effect on Na influx (Cramer et al. 1987). Therefore, future research can reveal whether or not higher K, Ca, and P concentrations may compete with Na and reduce its uptake by conifers under saline-sodic conditions (Wakeel 2013). Increasing K concentration in the root zone may not only decrease Na uptake, but can increase K uptake and improve plant survival under saline conditions (Maathuis and Amtmann 1999, Demidchik and Tester 2002, Cuin et al. 2003, Cuin and Shabala 2005, Chen et al. 2007). Also, a comprehensive analysis of K and Na interaction in soil and plants is necessary to reveal the mechanisms of their uptake from sodic and saline-sodic soils and their transport in plants.

Therefore, fertilization with K, Ca, and P could be an alternative strategy to reduce the adverse effects of salinity (Nieman and Clark 1976, Cerda et al. 1977, Kaya et al. 2001, Kaya et al. 2003, Garg al. 2005, Khan et al. 2013) in plants. However, there is no evidences in the literature that conifers suffering from environmental stresses like salinity have a larger internal requirement for K, Ca, and P, nor of how the different species change their cellular metabolism in terms of accumulation of osmoprotective compounds and their potential to develop or improve stress tolerance.

Additional studies that could help solve the questions raised through this research are:

1) Analyze individually the uptake, distribution, and contribution of Na and Cl in plants under high-salt stress conditions;

2) Examine the effects of macronutrients addition to alleviate the impacts of salt stress;

3) Relationship and interaction between Na, K, and Ca in plants under salinity stress;

4) Distribution and roles of osmoprotective compounds accumulated in conifers under abiotic stress.

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Appendix 1

Table a1.1 ANOVA table showing effects of NaCl treatments on the measured parameters

Parameters	Black spruce	White spruce	Jack pine
Relative stem diameter growth	<0.0001	<0.0001	0.089
Relative shoot height growth	0.301	0.243	0.003
Dry weight	0.094	<0.0001	<0.0001
Shoot:Root ratio	0.103	0.968	0.011
Chlorophyll	0.017	0.004	0.004
Sodium - needles	0.006	<0.0001	<0.0001
Sodium - roots	<0.0001	<0.0001	<0.0001
Chloride - needles	<0.0001	<0.0001	<0.0001
Chloride - roots	<0.0001	<0.0001	<0.0001

Appendix 2

Table a2.1 Substitute chemical combinations to achieve the final concentration of macronutrients required for the treatments

Treatment	Compound	Concentration stock solution (M)	Volume of stock solution taken (mL L^{-1})	Final concentration of element (mM)	
Control	KNO_3	1	3.0	N	8.0
	$Ca(NO_3)_2.4H_2O$		2.0	P	1.0
	$NH_4H_2PO_4$		1.0	K	3.0
	$MgSO_4.7H_2O$		0.5	Ca	2.0
				Mg	0.5
				S	0.5
10 % N	KNO_3	1	0.8	N	0.8
	KH_2PO_4		1.0	P	1.0
	KCl		1.2	K	3.0
	$CaCl_2.2H_2O$		2.0	Ca	2.0
	$MgSO_4.7H_2O$		0.5	Mg	0.5
				S	0.5
				Cl	5.2
10% P	KNO_3	1	3.0	N	8.0
	$Ca(NO_3)_2.4H_2O$		2.0	P	0.1
	$NH_4H_2PO_4$		0.1	K	3.0
	NH_4NO_3		0.45	Ca	2.0
	$MgSO_4.7H_2O$		0.5	Mg	0.5
				S	0.5
10% K	KNO_3	1	0.3	N	8.0
	$Ca(NO_3)_2.4H_2O$		2.0	P	1.0
	$NH_4H_2PO_4$		1.0	K	0.3
	NH_4NO_3		1.35	Ca	2.0
	$MgSO_4.7H_2O$		0.5	Mg	0.5
				S	0.5

Table a2.2 ANOVA table showing effects of mineral nutrition and NaCl treatments on the measured parameters

Parameters	**White spruce**	**Jack pine**
Relative stem diameter growth	<0.0001	<0.0001
Relative shoot height growth	<0.0001	<0.0001
Dry weight	0.0003	0.0088
Shoot:Root ratio	0.0160	0.0070
Chlorophyll	<0.0001	<0.0001
Photosynthesis	<0.0001	<0.0001
Transpiration	<0.0001	<0.0001
Nitrogen	<0.0001	<0.0001
Phosphorus	<0.0001	<0.0001
Potassium	<0.0001	<0.0001
Calcium	0.0720	<0.0001
Magnesium	0.0080	0.0050
Sulfur	0.0170	<0.0001
Sodium	<0.0001	<0.0001
Chloride	<0.0001	<0.0001