Evaluating Jack Pine Seedling Characteristics in Response to Drought and Outplanting

by

Alexander Eric Goeppel

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Department of Renewable Resources
University of Alberta

Abstract

This thesis explores the influence of non-structural carbohydrates (NSC), seedling size, and root:shoot ratio (RSR) on jack pine (*Pinus banksiana* Lamb.) seedling performance under different drought intensities in a growth chamber and after outplanting on a reclamation site. NSC content, size, and RSR are seedling characteristics which could improve drought tolerance of jack pine and thus outplanting success. During seedling production, characteristics were altered by growing seedlings in a greenhouse, incorporating a period of outdoor growth, and staggering germination. Generally, smaller outside grown seedlings with initially high RSR, allocated more growth to aboveground organs whereas large greenhouse grown seedlings demonstrated greater growth allocated to roots. In the growth chamber, large seedlings exhibited less water stress under severe drought. On the reclamation site, seedlings were outplanted on different aspects and seedlings on the warmer and drier south-facing aspects had increased needle production and stomatal conductance.

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Chapter 1: Introduction

1.1 Boreal Forest and Jack Pine

The boreal forest is a widespread biome in the northern latitudes, and trees growing there often contend with harsh growing conditions. The boreal forest accounts for roughly a third of global forested areas (Natural Resources of Canada, 2013). This biome is composed of conifer and broadleaf trees, and the climate is characterized by short summers and long, cool winters. Low temperatures and nitrogen are generally the main limiting factors for tree growth in these forests (Näsholm et al., 1998; Baldocchi et al., 2000).

Among the tree species that grow under these challenging conditions is jack pine (*Pinus banksiana* Lamb.) which is an evergreen conifer, and it has the most widespread range of any pine species in Canada (Burns and Honkala, 1990). It is a fast-growing and shade intolerant pioneer species that can be found on dry and oligotrophic boreal sites, including well-drained upland sites composed of coarser soils where most other trees have difficulty growing (Burns and Honkala, 1990). In the boreal forest, when jack pine are not growing in pure, even-aged stands, they occur in mixed stands with trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh), black spruce (*Picea mariana* Mill.), and balsam fir (*Abies balsamea* (L.) Mill.) (Burns and Honkala, 1990). Similarly to other pine species, jack pine has adapted to persist after large disturbances such as forest fires by means of serotinous cones. Adaptations well-suited for colonization after disturbances such as rapid seedling growth rates and ability to withstand stressful growing conditions make jack pine an ideal candidate for reclamation sites.

1.2 Reclamation and Site Conditions

In addition to an abundance of renewable resources, the boreal forest is rich in other resources that have led to many forms of anthropogenic disturbances, including open pit mining for bitumen extraction in the oil sands region of northeastern Alberta (Canada). Disturbances from surface mining can be extensive owing to deep excavations that disrupt the integrity of the soil and results in the complete removal of surrounding vegetation. In Alberta, the Conservation and Reclamation Regulations (AR 115/1993) of the Environmental Protection and Enhancement Act states that the goal of conservation and reclamation is "to return the specified land to an equivalent land capability," (Government of Alberta, 1993). "Equivalent land capability" is defined in greater detail as "the ability of the land to support various land uses after conservation and reclamation is similar to the ability that existed prior to an activity being conducted on the land, but that the individual land uses will not necessarily be identical," (Government of Alberta, 1993). The goal of reclamation is to achieve a self-supporting ecosystem similar to what had persisted previous to the disturbance.

The process of reclaiming these sites can be lengthy, beginning with soil reconstruction. Reclamation begins with laying down the overburden. Under circumstances where the overburden is saline and slightly alkaline, it is covered with approximately 80 cm subsoil that has no salinity and a more neutral pH for root growth (Rowland et al., 2009). A thinner layer of subsoil is applied when overburden bears more desirable characteristics for plant growth. An organic capping material such as peat-mineral-mix (PMM) or forest floor material (FFM) is placed overtop as a planting medium. Peat mineral mix is composed of salvaged peat from low-lying forest, fens, or bogs and underlying mineral material while FFM is generally composed of salvaged topsoil including the A and B soil horizons from an upland site. Seed sources for tree species can be too distant for natural seed dispersal due to the considerable scale of oil sands mine sites and boreal tree seeds are not found in the soil seed bank. Also, reclamation sites may not offer suitable conditions for seeds to germinate and grow due to unfavorable microsite conditions or inadequate nutrients (Landhäusser et al., 2010; Wolken et al., 2010; Pinno and Landhäusser, 2012). Consequently, nursery-grown tree seedlings are typically outplanted on these disturbed sites to begin reestablishment of forests (Macdonald et al., 2012).

As seedlings, trees are at a vulnerable stage and must overcome challenges including drought stress caused by planting stress, and/or site conditions after outplanting. In reclamation sites, planted seedlings may experience stress due to low root permeability (more suberized roots), root confinement, and/or poor contact between the soil and roots (Kozlowski and Davies, 1975; Rietveld, 1989; Burdett, 1990). These factors restrict the supply of water to transpiring aboveground organs causing stress. In boreal reforestation sites, seedlings can be subjected to high vapor pressure deficit (VPD) after the canopy has been removed (Groot et al., 1997). Transpiration is driven by VPD and stomatal conductance, and it is likely that seedlings planted in reclamation sites also contend with high VPD when there is no vegetation cover. In addition to low soil water availability, high VPD can exacerbate moisture constraints for planted seedlings.

Seedlings can be inherently susceptible to drought stress due to morphological characteristics. For example, shade intolerant boreal tree species have relatively high leaf area as seedlings (Ewers et al., 2005). High leaf area in addition to limited root access to water (Christina et al., 2011) makes seedlings particularly susceptible to hydraulic failure(McDowell et al., 2008).Hydraulic failure occurs when high evaporative demand in addition to low water availability cause cavitation to occur within the xylem, preventing the flow of water and hydration of cells (Sperry et al., 1998).

1.3 Mechanisms of Drought Avoidance

Site conditions place considerable challenges on reclamation efforts, and this is why it is important to have a comprehensive understanding of the mechanisms trees have to avoid drought. Seedlings contend with drought by utilizing stomatal regulation, leaf area and shedding, root growth, and/or osmotic adjustment.

1.3.1 Stomatal Regulation

The benefits and drawbacks of stomatal regulation can help explain which strategies trees adopt when faced with drought. Seedlings may reduce stomatal conductance by closing stomata to conserve water and prevent the xylem from reaching water potentials that cause cavitation (Sperry and Pockman, 1993), but this may also decrease photosynthesis (Hsiao et al., 1976; Sperry and Pockman, 1993; Meinzer et al., 2001). There are two main ways in which trees regulate their stomata: anisohydric and isohydric regulation (Tardieu and Simonneau, 1998). Anisohydric regulation is characterized by stem water potential declining with soil water potential as the soil dries because of higher stomatal conductance. In other words, these trees will not close their stomata unless there is an immediate risk of cavitation, and this allows for more photosynthesis during drought. Trees that occur in xeric conditions and exhibit anisohydric regulation generally have xylems that are more resistant to cavitation (McDowell et al., 2008).

On the other hand, trees that experience isohydric regulation reduce their stomatal conductance when soil water potential decreases. As a result, these trees exhibit stem water potentials that fluctuate less in relation to changes in soil water potential. Studies have documented pine species to experience isohydric stomatal regulation including Colorado piñon pine (*P. edulis* Englem.) (West et al., 2008), smooth-bark Mexican pine (*P. pseudostrobus* Lindl.) (Himmelsbach et al., 2011), Aleppo pine (*P. halepensis* Miller) (Klein et al., 2011), and maritime pine (*P. pinaster* Ait.) (Ripullone et al., 2007). Pine have higher vulnerability to cavitation compared to other conifers and thus they have a higher level of stomatal control (Piñol and Sala, 2000; Martínez-Vilalta et al., 2004).

1.3.2 Leaf Area and Shedding

Aside from stomatal regulation, producing smaller leaves or shedding leaves can help trees mitigate drought stress. Leaf area typically decreases in drier habitats among species and communities (Fonseca et al., 2000; Ackerly and Cornwell, 2007). Smaller leaf area improves convective cooling and minimizes water loss through transpiration (Vogel, 1970; Parkhurst and Louks, 1972; Gibson, 1998; Vogel, 2009). Leaf shedding is an alternative strategy that lowers total plant transpiration, and it prevents stems from experiencing exceedingly low water potentials (Tyree et al., 1993).

1.3.3 Root Growth

Pine exhibits other traits that help maintain high water potential such as increasing water uptake through root growth, thus increasing root to shoot ratio (RSR)

(Kozlowski and Pallardy, 2002). RSR is representative of the balance between above- and belowground dry mass, and although high RSR has been observed to be beneficial for seedlings, there are conflicting results on this topic. Outplanted temperate tree species such as ponderosa pine (*Pinus ponderosa* Dougl.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) exposed to dry summers had greater survivorship and increased stem mass when they bore high RSR and large roots (LopushInsky and Beebe, 1976). In drier climates such as the Sudanian savanna or the Mediterranean, high RSR increased survival of outplanted seedlings in xeric sites (Zida et al., 2008; Del Campo et al., 2010). On the contrary, one study found holm oak with lower RSR translated to higher survival during dry summer conditions (Villar-Salvador et al., 2004b). Another study found that seedlings with greater rooting depth had higher survival under drought whereas high RSR was not beneficial (Padilla and Pugnaire, 2007). In the Mediterranean, seedlings with initially high RSR did not perform well which corresponds to evidence that more drought tolerant species have low RSR (Valladares and Sánchez-Gómez, 2006).

This may not hold as a general rule, however. A study by Lloret et al. (1999) found that shrub species with roots rarely penetrating below the first 10 cm of soil relied on high RSR. It is plausible that freshly planted seedlings rely on intercepting water at shallower soil depths since they cannot penetrate deeper sources of water without root growth. Adapted to conditions similar to the Mediterranean, three Canary Island pine (*Pinus canariensis* Smith) provenances were found to increase RSR in a slowly imposed moderate drought treatment while one provenance increased RSR in both slowly imposed moderate and fast imposed severe drought treatment (López et al., 2009). A fifth provenance did not change RSR and had low field survival rates (López et al., 2009). The discrepancy may suggest that increasing RSR is beneficial within certain drought intensities specific to species or even populations, but this remains to be confirmed.

1.3.4 Osmotic Adjustment

Soluble sugars and other solutes aid in tree survivorship under drought conditions through osmotic adjustment. Solutes are accumulated in leaves and the meristems of roots (Kozlowski and Pallardy, 2002) in order to maintain turgor at low water potentials (Hsiao et al., 1976; Morgan, 1984; Munns, 1988). Osmotic adjustment does not occur in all species, but it has been observed in pine trees, including jack pine (Koppenaal et al., 1991).

1.3.5 Other Beneficial Characteristics

Even though non-structural carbohydrates (NSC) which include water-soluble sugars and starch—can directly contribute to increased seedling survivorship under drought via osmotic adjustment, NSC can also benefit seedlings indirectly. Starch is often the largest constituent of reserve carbon, and both soluble sugar and starch serve as a source of carbon and energy for structural growth and respiratory needs when photosynthesis has diminished or stopped (Kozlowski, 1992). They are therefore viewed as a buffer for periods of stress (Chapin et al., 1990; Kozlowski and Pallardy, 2002), which may explain why trees inhabiting stressful environments are associated with high NSC concentrations (Körner, 2003). For these reasons, seedlings with either high NSC concentrations or large NSC reserves may perform better under drought.

If there are circumstances where seedlings do not differ in NSC tissue concentration, large seedling size may reflect high NSC content. High content allows for mobilization of adequate NSC to facilitate healing and restoring of damaged tissue, thus ensuring seedling survival (Canham et al., 1999). Large seedlings may have a greater capacity to grow and mobilize resources including NSC and nutrients (Villar-Salvador et al., 2012). Greenhouse studies that had restricted irrigation produced drought resistant seedlings which happened to be smaller in size (Van Den Driessche, 1991; Royo et al., 2001). However, there is evidence that larger seedlings have lower mortality and greater growth after outplanting. Growth was greater in larger seedlings among two Mediterranean species that are considered drought tolerant: Aleppo pine (*Pinus halepensis* M.) and holm oaks (*Quercus ilex* L.) (Puértolas et al., 2003; Villar-Salvador et al., 2004a; Cuesta et al., 2010). Larger Aleppo pine seedlings had lower mortality two to seven years after outplanting (Del Compo et al., 2007; Oliet et al., 2009). Interestingly, these results are generally found among Mediterranean species, and there is little evidence to suggest that larger seedling size is beneficial under drought among species from other biomes, such as jack pine in the boreal forest.

1.4 Manipulating Seedling Characteristics

It may be possible to manage susceptibility to drought stress by creating seedlings with characteristics that increase survivorship and growth under xeric conditions. These characteristics include high stomatal regulation, root:shoot ratio (RSR), and non-structural carbohydrates (NSC), as well as large size. In a nursery setting the target seedling approach can be used to promote these potentiality beneficial characteristics. The target seedling concept originated from the late 1970s and early

1980s, and it entails creating seedlings with specific morphological and physiological characteristics that can be correlated to effective seedling establishment in the field (Rose et al., 1990; Rose and Haase, 1995; Landis, 2003; Landis and Dumroese, 2006). Many characteristics should be taken in account since the target seedling concept assumes multiple characteristics contribute to better performance (Rose et al., 1990). This concept requires going beyond nursery measurements and assessing seedling quality after outplanting to ensure they are able to tolerate stress and demonstrate high growth (Johnson and Cline, 1991). In addition, there is no single way to validate the effectiveness of seedling characteristics to high seedling survival, as seedling quality is both species- and site-specific (Puttonen, 1996). Identifying limiting factors that may inhibit successful seedling establishment of a particular site is thus necessary (Landis, 2003; Landis and Dumroese, 2006). Drought stress is potentially a limiting factor for afforestation efforts in the boreal, and it is therefore necessary to test seedling quality in the context of drought stress.

Since nursery practices have a strong influence on seedling characteristics, testing seedling quality under drought requires an understanding of the mechanisms that promote the development of potentially beneficial characteristics. Drought hardening, or the deliberate exposure of seedlings to drought, is an effective way to increase drought tolerance, but its influence on seedling characteristics appears variable in pine species and depends on drought intensity. Drought hardening has been observed to reduce needle production and increase RSR in black pine (*Pinus nigra* Arnold) (Biel et al., 2004), whereas RSR remained unaffected with lodgepole pine (*Pinus contorta* Dougl.) or decreased with stone pine (*Pinus pinea* L.) (Stewart and Lieffers, 1993; Villar-

Salvador et al., 2013). Drought hardening also influences starch and sugar concentrations; drought-hardened black pine only experienced reductions in starch concentrations (Guehl et al., 1993). A study on Aleppo pine with four different hardening intensities observed low root and shoot soluble sugar concentrations in two moderate intensities, and the most and least intense hardening treatments had the lowest root starch concentrations (Royo et al., 2001). Conversely, stone pine starch and soluble sugar concentrations increased under drought hardening (Villar-Salvador et al., 2013).

Alternative methods that change seedling characteristics may provide less variable results. Trembling aspen seedlings gown outside had significantly higher NSC concentrations and slightly higher RSR relative to greenhouse grown seedlings, which may be attributed to larger fluctuations in wind, VPD, temperature, and light intensity (Landhäusser et al., 2012). Likewise, growing Loblolly pine (*Pinus taeda* L.) seedlings under greenhouse conditions produced large seedlings whereas seedlings grown outside inhibited shoot elongation and increased the growth of roots, thus increasing RSR (Retzlaff et al., 1990). Whether placing pine seedlings outside can change NSC concentrations has not been tested.

1.5 Objectives

The first objective of this research project was to determine whether altering growing conditions would promote different seedling RSR, size, and NSC reserves in jack pine. If successful, the next objective was to assess how differences in seedling RSR, size, and/or NSC reserves influence pine seedling performance under different drought intensities in a growth chamber setting. The final objective was to test how these results translate after outplanting seedlings with the same differences in a reclamation site. In addition, the influence of south and north-facing aspect and hydrogel-amended soil on outplanted seedlings was also assessed.

Chapter 2: Evaluating Jack Pine Seedling Characteristics in Response to Drought

2.1 Introduction

Worldwide, drought causes substantial reductions in tree growth, and when severe, it can even cause massive mortality (Allen et al., 2010). Small trees and seedlings appear to be particularly vulnerable to drought (Condit et al., 1995; Hanson et al., 2001; Wyckoff and Clark, 2002). Under non-lethal circumstances, drought reduces growth, impacting seedling survival and long-term recruitment (Anderson-Teixeira et al., 2013) and consequently influencing forest regeneration and reclamation efforts. Therefore, understanding how drought impacts seedlings is crucial for making seedlings that can withstand drought better.

Drought stress can negatively influence seedling water and carbon balance. Drought reduces soil water content and soil water potential and may be accompanied by high vapor pressure deficit which exposes the xylem to exceedingly low water potentials. Seedlings may be particularly susceptible to hydraulic failure due to their small rooting volume and thus inclined to experience more negative water potentials than larger trees (McDowell et al., 2008). Declining hydraulic conductivity can result in cavitation which may cause further reductions in water potential (Tyree and Sperry, 1989; Tyree and Ewers, 1991; Meinzer et al., 2001) and ultimately catastrophic hydraulic failure for seedlings (Williams et al., 1997; Tyree and Zimmermann, 2002). Seedlings can reduce stomatal conductance to prevent excessive water loss and xylem water potentials that cause cavitation (Sperry and Pockman, 1993), but this may cause reductions photosynthesis (Hsiao et al., 1976; Sperry and Pockman, 1993; Meinzer et al., 2001). However, cell division and cell wall synthesis are more sensitive to water stress than assimilation of carbon (Hsiao, 1973; Hsiao et al., 1976). Ultimately, drought leads to reduced growth which may either result from reduced turgor or carbon availability.

Knowledge of the characteristics that benefit planted seedlings when exposed to drought, may aid reforestation and forest restoration efforts and help us understand how tree growth and survival are limited under drought. This agrees with the target seedling concept where the goal is to have seedlings with specific morphological and physiological characteristics that can be correlated to effective seedling establishment (Rose and Haase, 1995). Non-structural carbohydrates (NSC) may be used by seedlings as a safeguard against periods of stress (Chapin et al., 1990; Kozlowski and Pallardy, 2002) and is used for osmotic adjustment to maintain turgor under drought conditions (Hsiao et al., 1976; Morgan, 1984; Munns, 1988). Therefore, high NSC may be beneficial for seedlings under drought. Seedlings with higher root:shoot ratio (RSR) may achieve a better balance between water uptake and water loss through transpiration, and higher RSR may also help seedlings in tolerating drought. Although, one study found holm oak (*Quercus ilex* L.) with lower RSR translated to higher survival during dry summer conditions (Villar-Salvador et al., 2004a). Large seedling size may also be beneficial since it is hypothesised that large seedlings have higher photosynthetic capacity per plant and can store more total NSC and nutrients (Villar-Salvador et al., 2012).

The first objective of this study was to determine if NSC reserves, RSR, and size can be modified in jack pine (*Pinus banksiana* Lamb.) seedlings by changing seedling growing conditions during seedling production. It was hypothesized that placing seedlings outside during the growing season would expose them to moisture constraints driven by higher vapor pressure deficit which may reduce shoot growth and allow more photosynthates to be allocated to root growth (and increase RSR) and/or NSC reserves. It was also hypothesized that staggering seedling germination would produce seedlings of varying sizes. In other words, germinating seedlings at a later date would likely produce smaller seedlings. If the change in seedling characteristics was successful, the next objective was to determine how these different characteristics influence pine seedling performance under different drought intensities.

2.2 Material and Methods

2.2.1 Seedling Stock Production

Jack pine seed for this experiment was collected from open pollinated populations located near Fort McMurray, AB, Canada (56.727680° N, 111.389749° W). All seedlings were cultivated at the Crop Diversification Centre North (CDC North) in Edmonton, AB, Canada (53.643162° N, 113.359455° W) in styroblock containers (4-12A; Beaver Plastics Ltd, Edmonton, AB, Canada). Each stryroblock has a total of 77 cavities (4 cm wide and 12 cm deep; 125 ml) which were filled with growing substrate (Professional Growing Mix, Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) composed of 60-75 % sphagnum peat moss, perlite, and dolomite limestone. Two weeks after germination, all seedlings were fertigated twice a week with a commercial nursery blend used for pine seedlings of 96 ppm of N, 76 ppm of P, and 164 ppm of K, supplemented with a blend of chelated micronutrients. At other times seedlings were watered daily or more often when needed. The greenhouse conditions were maintained at 50 % relative humidity with natural temperatures, and when needed, natural light was supplemented with fluorescent lights to extend the day period to 16 hours.

To create seedling stock types with different characteristics we grew pine seedlings under different growing conditions. For the greenhouse (G) stock type, seeds were germinated on April 11, 2011, and seedlings were grown under greenhouse conditions through the entire growing season (Figure 2.1). For the outside (O) stock type, seeds were also germinated on April 11 under greenhouse conditions, but seedlings were moved outside eight weeks later (June 7), where they continued their growth and development for the rest of the growing season (Figure 2.1). For the outside late-germination stock type (OL), seeds were germinated four weeks later (May 8), and seedlings were grown under greenhouse conditions for four weeks and moved outside on the same day as the O seedlings (Figure 2.1). During the growth phase all seedlings were fertigated twice a week until August 13, using the nutrient solution described above. During the last two weeks of August the seedlings were fertigated with only half the nitrogen concentration, and after August 31, all fertilization was suspended. At that time, the seedlings of the G stock type were moved outside to naturally harden and induce dormancy in preparation for frozen storage. Prior to frozen storage, 24 seedlings from each stock type were destructively harvested and initial seedlings characteristics such as stem height, root collar diameter, needle dry mass, stem dry mass, and root dry mass were measured. On November 11, the remaining seedlings were lifted and placed into plastic bags and waxed cardboard boxes and put into frozen storage at -3 °C for 13 weeks.

2.2.2 Growth Chamber Experiment

On February 4, 2012, 75 seedlings of each stock type were taken from frozen storage and slowly thawed over three days. Fifteen seedlings of each stock type were randomly selected to determine initial non-structural carbohydrate (NSC) reserves in roots, stems, and needles. The remaining 60 seedlings of each stock type were then potted in square planting pots 13.7 cm wide and 15.6 cm deep. Pots had a maximum volume of 2 L with eight holes at the bottom to allow free drainage. The seedlings were potted in a soilless mixture of peat moss (Premier Horticulture Ltd, Rivière-du-Loup, QC, Canada), expanded vermiculite (W.R. Grace & Co.-Conn., Cambridge, MA, USA), and turface clay (PROFILE Products LLC, Buffalo Grove, IL, USA) (2:1:1 based on volume). During the potting process, the soil was mixed with water containing 2 g L^{-1} of 10-52-10 (N-P-K) water soluble fertilizer with chelated micronutrients (Plants Products Co. Ltd., Brampton, ON, Canada) and reached 67 % gravimetric soil moisture content. After planting, the initial stem height from the root collar to the terminal bud, root collar diameter (RCD), and total pot mass (weight of pot containing the substrate and plant) of all potted seedlings were measured. All seedlings were grown in a growth chamber at the University of Alberta in Edmonton, AB, Canada. In the growth chamber, seedlings were illuminated with fluorescent lights for 18 hours each day and kept at 19 ± 1.2 °C with a relative humidity of 53 ± 8 %. Seedlings received approximately 350 µmol $m^{-2}s^{-1}$ of photosynthetic active radiation (PAR) at the pot level and were rotated every month to compensate for heterogeneous conditions that may exist in the growth chamber. Terminal buds were flushing across all stock types and treatments by February 16, 2012.

2.2.3 Drought Treatments

Three levels of drought were imposed on the three stock types: mild drought (MLD), severe drought (SEV), and a well-irrigated control (CON). Seedlings from each stock type were randomly distributed among treatments, and for each stock type, seedlings did not differ in initial RCD and shoot height (both *P*≥0.174) among drought treatments. The target drought conditions were based on a xylem vulnerability curve developed for jack pine grown under open conditions (Schoonmaker et al. 2010) and a moisture retention curve derived for the potting mixture (Figure 2.2 and also Appendix 1). The target water potential for the SEV drought was -1.4 MPa, at which point cavitation in the stem xylem begins to occur (Appendix 1); the target for the MLD drought was –0.5 MPa.

After selecting the SEV and MLD water potential targets, the soil moisture retention curve of the potting mixture was used to determine the corresponding gravimetric water content for both treatments. The soil moisture retention curve was developed by first oven drying the potting mixture used in this study for at least 48 hours at 60 ^{0}C. Afterwards, the mixture was separated into individual Ziploc bags where a measured water quantity was added to obtain predetermined gravimetric water content. The bags were stored at 4 °C for 24 hours to allow the samples to equilibrate. Two samples were taken from each bag and placed in stainless steel sample cups, and soil water potential was measured using a dewpoint potentiameter (WP4, Decagon devices, Pullman, WA, USA). Before measuring soil water potential, the cups were placed on a temperature equilibration plate (Decagon devices, Pullman, WA, USA) to ensure that samples were maintained at 19 °C before being put into the WP4 which had a temperature setting of 21 °C. The WP4 temperature setting was comparable to growth chamber conditions, and maintaining samples initially at a slightly cooler temperature prevented condensation from occurring within the WP4 chamber. The soil moisture retention curve was derived by first plotting the soil water potential with the corresponding gravimetric water content, and the soil measurements were then used to fit the van Genutchen model (Figure 2.2; refer to Appendix 1 to view the model) to the data. The fitted model was used to generate corresponding gravimetric water contents for the soil water potential targets and thus, gravimetric water content was used as a proxy for the different levels of drought. The SEV target of -1.4 MPa was equivalent to a 40 % gravimetric soil water content, while the MLD target of –0.5 MPa was equivalent to a 60 % gravimetric soil water content.

To ensure that soil moisture targets for each of the drought treatments were maintained, pots were weighed using a digital balance (PGW 4502e, Adam Equipment, Danbury, CT, USA) to determine daily water loss. Forty two MLD and SEV pots from different positions within the growth chamber were randomly selected and weighed, and the average water loss for the MLD and SEV treatments was calculated relative to the location of the pots within the growth chamber (i.e. front, middle, and back). This was done to compensate for subtle differences in temperature and humidity and their effect on water loss within the growth chamber. To achieve the drought treatments gradually, trees were watered with a quantity approximately half the daily weight loss to ensure a steady and slow decrease in gravimetric water content. The MLD treatment reached the target water content after 11 days while the SEV treatment reached its target water content after 20 days. After reaching treatment targets, the soil water content was kept constant by watering all seedlings daily with the full amount of water that was lost over the last 24 hours. To ensure that soil moisture targets were met, soil water content was also measured in soil cores taken from a subsample of pots on two separate occasions after 65 and 92 days into the study at which time seedlings were destructively sampled. During the entire experiment, controls were well-watered when needed. There were no screens in the pots to prevent the loss of the potting material, so seedlings were watered slowly to minimise the loss of material especially for well-watered seedlings that received a greater volume of water.

2.2.4 Seedling Measurements

2.2.4.1 Growth Variables

To determine initial seedling characteristics prior to cold storage and the drought experiment, 24 seedlings from each stock type were destructively sampled. Stem height from the root collar to the terminal bud was determined for each seedling, and dry mass was determined for needles, stems, and roots by oven drying samples at 70 °C for at least 72 hours. Prior to drying, roots were carefully washed to remove potting material. At the end of the drought experiment (92 days into the study), the same measurements were made on 10 seedlings of each stock type and treatment combination. In addition, needle mass was separated into needles of the past growing season and needles formed during the experiment.

Seedling growth response was investigated in several ways. Height growth of each potted seedling was calculated as the difference between its initial stem height measured at the time of potting and its final stem height. Relative height growth was also calculated as height growth divided by initial height of each seedling to measure how much a seedling grew relative to initial height over one growing season. Needle growth was estimated as the dry mass of new needles. Root growth and root to shoot ratio (RSR) change were calculated by subtracting the average initial, pre-cold storage dry mass or ratio of each stock type from the final dry mass or ratio of each potted seedling. Unlike root growth, the difference in RSR values was divided by the initial value to give a relative change.

2.2.4.2 Physiological Variables and Tissue Analysis

Just prior to the end of the study, stomatal conductance and shoot water potential were measured on the 10 potted seedlings of each stock type and treatment combination that were subsequently harvested. Stomatal conductance was measured using LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). The reference level for CO_2 was set at 385 ppm, and relative humidity was set at 45 %. The LI-6400 sensor head was fitted with 6400-2B red/blue LED light source, and light conditions were set at 1000 $\mu mol^{-2}s^{-1}$. Physiological measurements were taken between 08:00 and 16:00 hours. New needles near the terminal bud were selected for measurements. Needles were in the leaf chamber for approximately four minutes to allow stomatal conductance readings to stabilize. Needles were marked on the outside of the leaf chamber gasket with permanent marker. After the shoot water potential was taken, the needles were cut and projected leaf area was estimated from a scanned image using winSEEDLE software (winSEEDLE 2006 Régent Instruments Inc., Sainte-Foy, QC, Canada). Stomatal conductance was scaled by needle surface area to account for differences in leaf area placed inside the chamber. Shoot water potential was then measured on the same seedlings by cutting the seedlings just above the root collar and placing the shoot in a Scholander pressure chamber (Compact Water Status Console, Soilmoisture Equipment Corp., Goleta, CA, USA). In addition, shoot water potential was measured on another 10 potted seedlings per stock type and treatment combination at the halfway mark of the study to ensure the drought treatment targets were achieved.

Non-structural carbohydrate (NSC) concentrations were determined using the method of Chow and Landhäusser (2004) for root, stem, and needle tissues of 15 seedlings of each stock type after cold storage and from the 10 harvested seedlings of each stock type and treatment combination at the end of the study. All dried tissues were ground to pass 40 mesh (0.4 mm) using a Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA). To determine water soluble sugar concentrations, samples were extracted three times with 80 % ethanol at 95 ^{0}C. Extracts were reacted with phenol-sulfuric acid, and soluble sugar was measured colorimetrically at 490 nm using a spectrophotometer (Chow and Landhäusser, 2004). Afterwards, the remaining residue was digested using α-amylase (from *Bacillus licheniformis*, ICN-190151, ICN Biomedicals, Aurora, OH, USA) and amyloglucosidase (from *Aspergillus niger*, Sigma A-1602, Sigma-Aldrich Inc., St Louis, MO, USA) for starch determination. After the addition of a peroxidase–glucose oxidase*/o*-dianisidine reagent (Sigma P-7119 and Sigma D-3252, Sigma-Aldrich Inc. , St Louis, MO, USA), starch concentration was measured colorimetrically at 525 nm (Chow and Landhäusser, 2004). The NSC (soluble sugar and starch) content was calculated by multiplying the tissue concentration by the dry mass of the respective tissue. To estimate whole tree NSC, soluble sugar, and starch concentration for each seedling, the whole tree NSC, soluble sugar, and starch content was divided by seedling total dry mass. Percent change in NSC content was estimated as the difference between NSC content of each seedling at the end of the drought experiment and the average content of each stock type after frozen storage. The difference was divided by the average content of each stock type after frozen storage to give a relative change.

2.2.5 Data Analysis

One-way ANOVA was used to compare initial seedling measurements of the three stock types (G, O, and OL). Stock type and drought treatment effects on growth and physiological variables were analyzed using a two-way ANOVA with the exception of stem water potential which was analyzed with a three-way ANOVA to include the effect of measuring period. PROC MIXED (SAS 9.2, SAS Institute, Cary, North Carolina, USA) was used for all analyses. Prior to all statistical analyses, tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and equal variance (Levene's) of the residuals were performed. Initial root collar diameter, initial needle dry mass, initial total dry mass, initial NSC concentration, RSR change, whole tree soluble sugar concentration and root soluble sugar concentration had equal variance. For variables with unequal variance, ANOVA models that assumes unequal variance were used, and the unequal variance model with the best fit was determined using Bayesian information criterion (BIC) (Littell et al., 2006). Treatment and stock type averages were compared using Tukey-Kramer adjustment with an $\alpha=0.05$ as the significance level.

2.3 Results

2.3.1 Initial Seedling Characteristics

Stock types varied considerably in initial size and dry mass allocation. The full greenhouse conditions (G) stock type produced a large seedling with high needle, stem, and root mass. However, the G stock type had the lowest RSR of all stock types (Table 2.1). The Outside (O) stock type had the next largest total mass despite being shorter in height than the OL stock type. Seedlings of this stock type also had the highest RSR, as their root mass was similar to the G stock type. Late-germination (OL) created a small seedling with a total mass less than half of the G seedlings; however the OL stock type had a RSR between that of the O and G stock types (Table 2.1).

Due to their large size, seedlings of the G stock type had the highest NSC content compared to the other stock types. However, the G stock type generally had lower organ level NSC concentrations compared to the other stock types (Table 2.1) even though whole seedling NSC concentrations were not significantly different among stock types (P=0.122; Appendix 2). The O stock type had the next highest NSC content, with high stem and root NSC concentrations but lower needle NSC concentration (Table 2.1). Finally, the small seedlings of the OL stock type had the smallest NSC content, but high NSC concentrations in the needles, stem, and roots (Table 2.1). However, the differences in organ-level NSC concentrations were balanced out by the differences in RSR, leading to relatively similar whole seedling NSC concentrations among stock types.

2.3.2 Impact of Drought on Seedling Growth Performance

Overall, height growth declined with increasing drought severity in all three stock types (Figure 2.3a). However, the extent of this decline varied with stock type (stock type*drought treatment: P<0.001; Appendix 3). Under well-watered conditions, G and O stock types had greater height growth than the OL stock type; however stock types did not differ significantly under drought (both MLD and SEV). In fact, the G stock type, though not statistically different, tended to have the lowest height growth under drought, particularly under severe drought where its growth was less than half of that of the other stock types. Compared to control conditions, severe drought reduced the height growth of G stock type by 89 %, O stock type by 71 %, and OL stock type by 55 %.

Relative height growth also decreased with drought (Figure 2.3b; Appendix 3). Well-watered seedlings were able to double their height relative to their initial size, while seedlings under drought (regardless of severity) grew only 30% of their initial height. Among stock types, the O stock type with the high RSR had the highest relative growth of 74 % followed by the OL and then G stock types with 54 % and 34 %, respectively (Figure 2.3c).

Similar to height growth, new needle mass production decreased with drought (Figure 2.4a). However, unlike height growth, there was a clear difference between the MLD and SEV treatments, with the exception of the G stock type. Under well-watered conditions, the G stock type produced more new needles than any other seedling. But the G stock type also suffered a greater reduction in new needle mass in response to drought compared to other stock types (stock type*drought treatment: *P*=0.001; Appendix 3): the G stock type in the SEV treatment had a 90 % reduction in new needle mass compared to well-watered seedlings, while the O and OL in the SEV treatments only had a 85 % and 82 % reduction, respectively. The G stock type also tended to have lower—though not significantly different—new needle mass in the MLD treatment than the other stock types.

The G stock type generally had greater root growth than the other stock types despite having the largest reduction in growth under drought stress (as indicated by stock type*drought treatment: *P*<0.001; Appendix 3). Under well-irrigated conditions, the G stock type grew twice as many roots as the other two stock types (Figure 2.4b). Mild drought reduced root growth of all seedlings, but the G stock type grew at least 41 % more than the other two stock types and similar to the root growth of O and OL CON seedlings. Severe drought tended to further decrease root growth, but G seedlings still grew 85% more than the OL stock type though not significantly more than the O stock type (*P*=0.168).

After the experiment RSR change was higher in the G stock types compared to other two stock types, but the effect of drought on RSR change varied with stock type (Figure 2.4c). Both O and OL stock types had decreased RSR while the G stock type increased in RSR. The reduction in RSR tended to decline with drought severity for O seedlings, but there was no effect of drought treatment in G or OL stock types.

2.3.3 Impact of Drought on Seedling Physiology and NSC Reserves

Shoot water potential decreased with increasing drought severity. However, the degree to which water potential declined varied among stock types (stock type*drought treatment: *P*=0.001, Figure 2.5; Appendix 3). In fact, the only differences among stock types in shoot water potential were detected in the SEV treatment where the G stock type had the highest water potential (less negative) which was significantly different from the OL stock type with the O stock type being marginally significant (*P*=0.063) (Figure 2.5).

Seedlings under well-watered conditions had the highest stomatal conductance regardless of stock type, which was more than three times greater than that of seedlings exposed to the SEV treatment (Figure 2.6a). There was a linear decline in stomatal conductance as drought intensity increased. Across treatments, the O and OL stock types had similar but higher (31%) stomatal conductance than the G stock type (Figure 2.6b).

After the 13-week drought, there were only subtle differences in concentrations among the stock types. The G stock type had higher whole seedling sugar concentrations than the other stock types, but starch concentrations were similar among all stock types (Figure 2.7). There was no difference in total NSC concentration, and at the tissue level, there were no differences in needle and root sugar and starch concentrations among stock types.

Drought reduced NSC concentrations, but it also affected the balance between starch and soluble sugar. Whole seedling starch concentration was highest among well-watered seedlings while the lowest concentrations were found among SEV seedlings and MLD seedlings had moderate levels (Figure 2.8a). The MLD treatment reduced whole seedling sugar concentrations whereas the SEV treatment did not. Well-watered seedlings had higher needle sugar and starch concentrations than seedlings under drought (Figure 2.8b). Roots had high starch concentrations which declined as drought intensity increased (Figure 2.8c). In contrast, root sugar concentrations increased with drought intensity.

The percent change in NSC content of seedlings from after cold storage to the end of drought experiment also declined with increasing drought intensity (Figure 2.9a). Well-watered seedlings nearly doubled their NSC content relative to initial levels whereas seedlings under mild drought had levels similar to those prior to the experiment. Seedlings from the SEV treatment experienced 38 % reduction compared to pre-drought treatment levels. Across treatments, the G stock type had a greater relative increase in NSC content than the O stock type, and the OL stock type had a moderate increase in content that was not significantly different from either the G or O stock type (Figure 2.9b).

2.4 Discussion

2.4.1 Seedling Response to Drought

Stock type growth allocation differences suggest different limitations; plants typically allocate more growth to organs responsible for the acquisition of resource that may be limiting (Chapin et al., 1987). The G stock type with initially low RSR allocated more carbon to root growth and had smaller reductions in root growth under severe drought compared to well-watered conditions relative to other stock types suggesting that these large seedlings had greater limitation in water uptake. In contrast, the negative change in RSR indicates that outside seedlings allocated relatively more to photosynthesizing tissue.

Not only did initial stock type characteristics influence allocation between needles and roots, they also influenced physiological responses to drought. Less needle area of seedlings grown outside allowed for greater supply of water per needle resulting in higher stomatal conductance. Alternatively, greenhouse seedlings had more needle area thus reducing water supply per needle and suppressing stomatal conductance. Differences in shoot water potential were not observed among stock types except under severe drought. The greenhouse seedlings had less negative shoot water indicating less water stress while seedlings grown outside with initially high RSR had lower shoot water potential. It is plausible that the greater root growth observed among greenhouse seedlings may have been effective in increasing water uptake. Another explanation for the more negative shoot water potential of outdoor-grown seedlings is that they had reduced cavitation vulnerability as a result of acclimation to the greater water stress they initially experienced. This is unlikely though as, for many pine species, there appears to be no difference in cavitation vulnerability between populations occupying xeric and mesic sites (Mencuccini and Comstock, 1997; Maherali and DeLucia, 2000; Martínez-Vilalta and Piñol, 2002; Martínez-Vilalta et al., 2009) with the exception of Canary Island pine (*Pinus canariensis* Sm.; López et al., 2013).

Drought had negative effects on both water and carbon balance in pine seedlings. The reductions in stomatal conductance and shoot water potential led to reduced growth, NSC concentration and content. Natural and experimental drought has often been found to decrease NSC concentrations in seedlings and trees (Parker and Patton, 1975; Guehl et al., 1993; Sayer and Haywood, 2006; Galvez et al., 2013). NSC concentrations declined with drought due to the water conservation strategy of pine. The genus *Pinus* has relatively high vulnerability to cavitation relative to other conifers (Martínez-Vilalta et al., 2004). To mitigate this vulnerability, pines utilize an isohydric regulation whereby stomata close early on as drought progresses to limit water loss when evapotranspiration rates are high, thus preventing severe cavitation (Tardieu and Simonneau, 1998). However, reduced stomatal conductance reduces photosynthesis (Cowan and Farquhar, 1977). Mitchell et al. (2013) demonstrated the importance of stomatal regulation on changes in NSC over time. They found that radiata pine (Pinus radiata D. Don) conserved water by reducing stomatal conductance with the onset of drought, eventually leading to a decline in NSC concentration—especially starch—at the time of death. By integrating earlier literature, McDowell (2011) hypothesized that growth declines during drought at a faster rate than photosynthesis, resulting in higher NSC concentrations; however, if drought conditions persist, photosynthesis will decline further and eventually result in decreased NSC. This decline in NSC has been demonstrated for aspen seedlings (Galvez et al., 2013). Although seedling mortality was not the intention of our drought experiment, it demonstrated that prolonged drought conditions can cause a decline in NSC without intensive drought that can induce mortality. Interestingly, different growth and physiological responses did not encourage differences in NSC concentrations at the end of the drought experiment. This suggests that drought or response to drought may have stronger influence over NSC concentrations.

In contrast, a study by Galvez et al. (2011) observed drought stress to increase NSC concentrations among trembling aspen (*Populus tremuloides* Michx.) seedlings under glass house conditions. However, this was attributed to the well-watered controls having no environmental cue to set bud, so they continued to grow while seedlings under drought stopped growing and increased their NSC reserves (Galvez et al. 2013). In our experiment, only root sugar concentrations increased with drought intensity which could be attributed to osmotic adjustment which has been documented in jack pine (Koppenaal et al., 1991).

2.4.2 Management Recommendations

Large greenhouse seedlings are perhaps the preferred “all-purpose” choice for sites that exhibit both ideal and drought conditions. They had a greater capacity to grow under well-watered conditions that may have been due to higher photosynthesis per plant. This stock type had higher needle mass (data not shown), and this may have translated to higher photosynthesis per plant despite lower stomatal conductance per needle. Although we could not determine how much of the growth was supported by photosynthesis or NSC reserves, ^{14}C labelling experiments indicate that a related species Scots pine (*Pinus sylvestris* L.) relies largely on current photosynthesis for shoot elongation during bud break (Hansen and Beck, 1994; Lippu, 1994). Other studies on Scots pine found NSC reserves are used for growing root tips before root elongation, but root elongation relies on current photosynthesis (Vapaavuori et al., 1992; Iivonen et al., 2001). Our study supports this notion as jack pine seedlings appear to have reduced growth under drought regardless on the amount of carbon stored in its tissues assuming that there is no sink limitation, so having greater photosynthetic capacity via greater leaf area can therefore be advantageous by compensating for lower photosynthetic rates and facilitating more growth under ideal conditions.

Seedlings grown outside with high RSR may be suited for sites prone to drought. Although, the outside seedlings did not outperform the greenhouse seedlings, the relatively large root systems of outside seedlings may be advantageous under greater drought severity. This is consistent with studies that have found high root area in relation to leaf area makes seedlings less susceptible to drought stress (Sperry et al., 1998; Ewers et al., 2000; Hacke et al., 2000; Addington et al., 2006).

This experiment was successful in manipulating jack pine seedling size and RSR through altering growing condition, but it did not change whole tree NSC concentration. Later germination produced the smallest seedlings in terms of dry mass while ideal greenhouse conditions produced large seedlings with the lowest RSR. The outside (O) and outside late-germination (OL) stock types were exposed to greater moisture constraint due to higher evaporative demand which was mitigated by allocating more growth to roots resulting in high RSR during initial growing conditions.

Manipulation of NSC concentration was unsuccessful and this is likely linked to jack pine being an evergreen. Deciduous trees such as trembling aspen appear to be more difficult in suppressing aboveground growth, but in doing so; these trees allocate more to root growth and increase NSC concentrations (Landhäusser et al., 2012). This experiment demonstrates that growing pine seedlings outside is an effective method in reducing shoot elongation as well as increasing root mass and consequently RSR, but it did not translate to increased NSC concentrations. No change in NSC concentration may be due to pine having a longer supply of photosynthates throughout the year which prevents large fluctuations in NSC concentrations and greater distribution of NSC reserves in the foliage whereas aspen roots facilitate greater storage (Landhäusser and Lieffers, 2012).

Though there were no initial differences in whole tree NSC concentrations between the stock types, the large G stock type had lower NSC concentrations at the tissue level likely due to favorable growing conditions. A study by Landhäusser et al. (2012) found greenhouse grown trembling aspen seedlings had lower stem and root NSC concentrations compared to seedlings grown outside. It was concluded that the early termination of height growth drove outside grown seedlings to allocate photosynthates to NSC reserves (Landhäusser et al., 2012). In our experiment, the greenhouse (G) stock type had lower needle, stem, and root NSC concentrations—likely due to seedlings having a longer period of stem elongation and having reflushed under favorable conditions—whereas aboveground primary growth likely stopped earlier in outdoor seedlings due to environmental conditions, enabling them to accumulate higher NSC concentrations.

2.4.3 Conclusion

Greenhouse conditions produced large jack pine seedlings with low RSR while seedlings grown outside had high RSR. Drought affects seedlings differently depending on their initial morphological characteristics which in turn influences allocation and physiological responses—demonstrating the complexity of drought stress. Under drought, large greenhouse seedlings exhibited high root growth, low shoot water potential under severe drought, and retained greater size than other seedlings despite having low stomatal conductance per needle. These seedlings appear to be best suited for both well-watered and drought conditions, but seedlings with high RSR may still be beneficial for more intense drought conditions. It should be cautioned that large seedlings need to accumulate sufficient NSC reserves, and since these seedlings are evergreens, this is done by allowing seedlings adequate time to photosynthesize after bud set.

Table 2.1 Pre-drought average (± SE) height, root collar diameter, stem mass, needle mass, root mass, total mass, RSR, whole tree NSC content, whole tree NSC concentration, needle NSC concentration, stem NSC concentration, and root NSC concentration for jack pine stock types. Different letters signify statistical differences among means based on comparisons using Tukey-Kramer adjustment (n=24 with the exception of NSC content and concentrations: n=15)

	Stock type		
	G	O	OL
Height (cm)	11.0 ± 0.5 A	5.0 ± 0.3 C	6.8 ± 0.3 B
Root collar diameter (mm)	2.6 ± 0.05 A	2.3 ± 0.07 B	1.9 ± 0.06 C
Needle mass (g)	1.524 ± 0.056 A	0.666 ± 0.045 B	0.482 ± 0.046 C
Stem mass (g)	0.415 ± 0.022 A	0.177 ± 0.013 B	0.143 ± 0.009 B
Root mass (g)	1.142 ± 0.040 A	1.186 ± 0.067 A	0.661 ± 0.038 B
Total mass (g)	3.080 ± 0.111 A	2.030 ± 0.110 B	1.286 ± 0.078 C
RSR ($g\ g^{-1}$)	0.601 ± 0.022 C	1.458 ± 0.071 A	1.128 ± 0.061 B
Whole seedling NSC (g)	0.457 ± 0.032 A	0.330 ± 0.018 B	0.203 ± 0.016 C
Whole seedling NSC (%)	14.03 ± 0.33	14.47 ± 0.27	14.85 ± 0.21
Needle NSC (%)	16.80 ± 0.32 B	16.84 ± 0.30 B	18.34 ± 0.38 A
Stem NSC (%)	14.26 ± 0.58 B	16.92 ± 0.59 A	17.60 ± 0.44 A
Root NSC (%)	10.33 ± 0.57 B	12.14 ± 0.46 A	12.00 ± 0.39 A

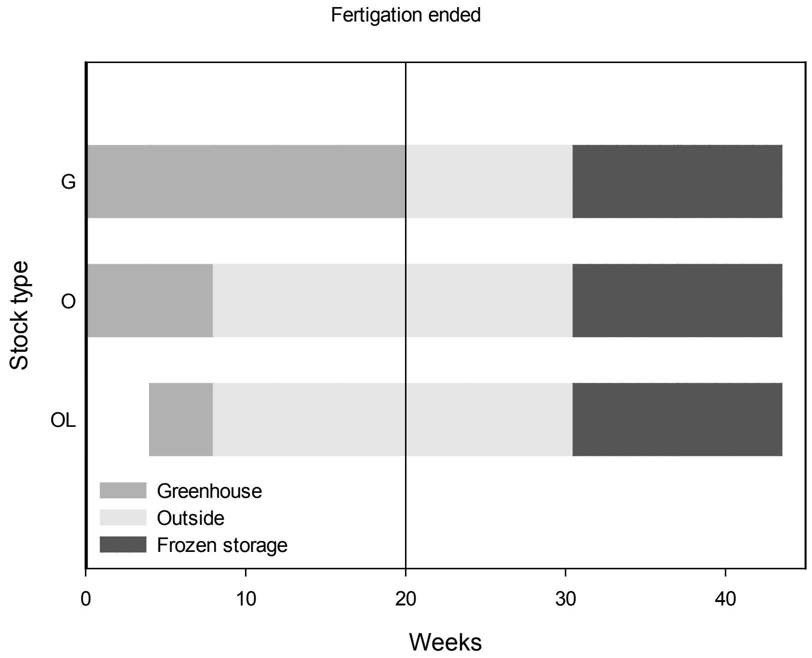

Figure 2.1 Growing conditions and growing season duration for each stock type before frozen storage.

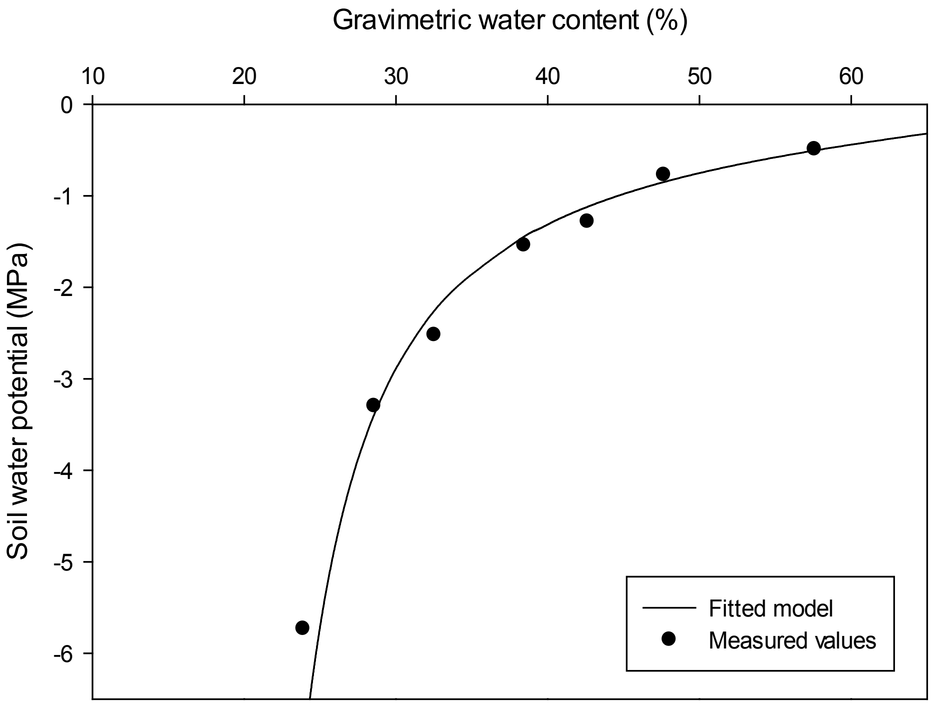

Figure 2.2 Soil moisture retention curve of the potting mixture used for the growth chamber experiment with measured values and fitted the van Genutchen model (n=2).

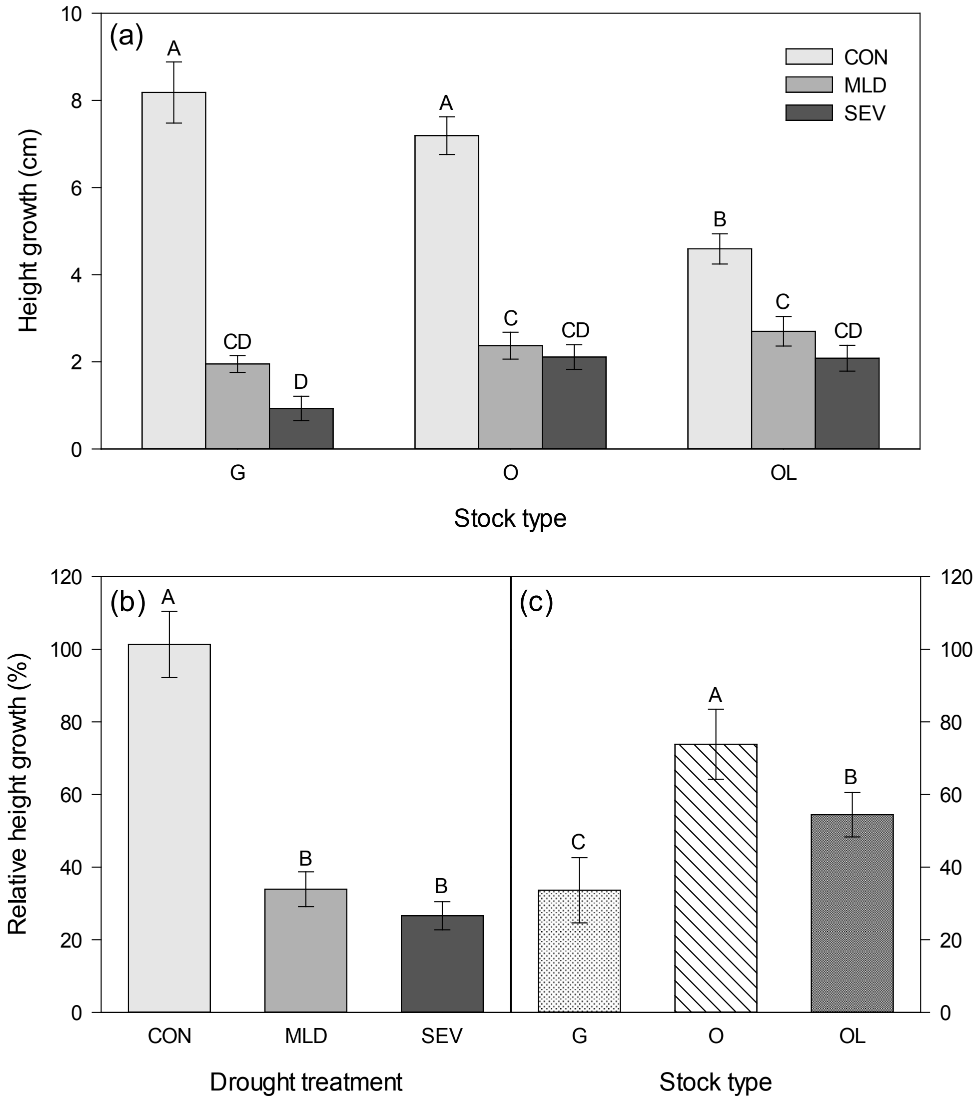

Figure 2.3 Average (a) height growth (n=10) and average height growth relative to initial height of (b) stock types and (c) drought treatments (n=30). Error bars represent one standard error, and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

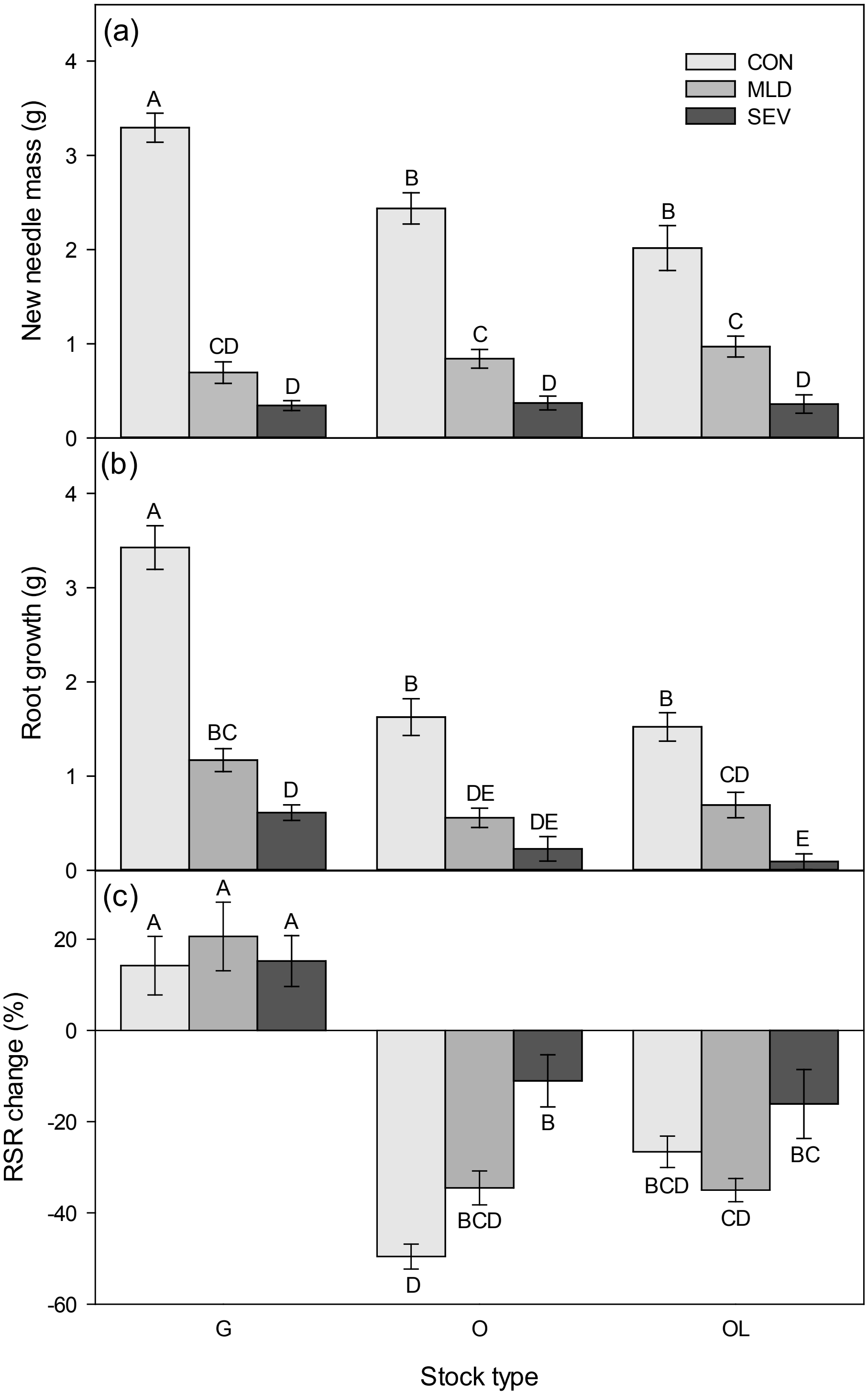

Figure 2.4 Average (a) new needle mass, (b) root growth, and (c) RSR change. Error bars represent one standard error (n=10), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

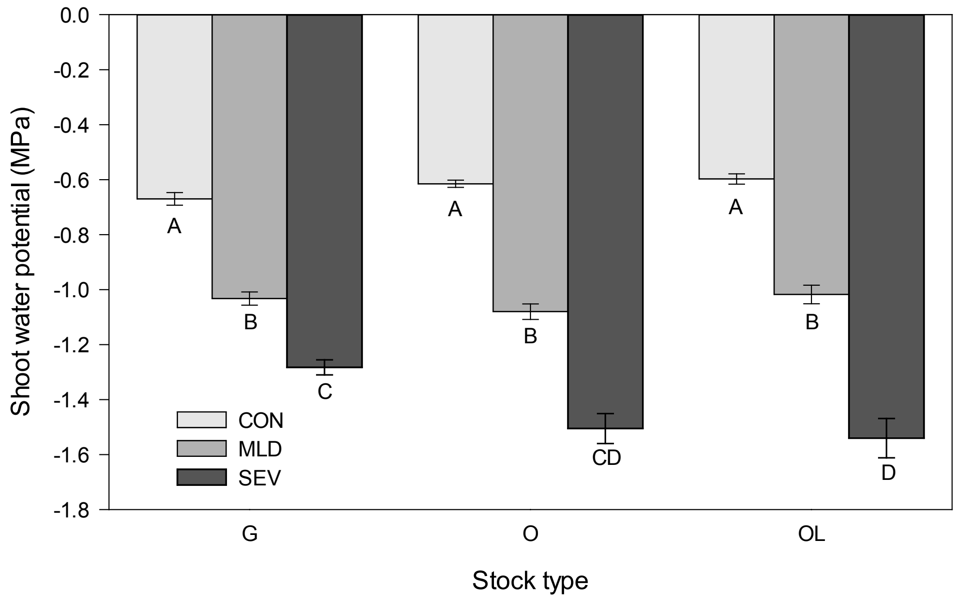

Figure 2.5 Average shoot water potential during and at the end of the experiment. The error bars represent one standard error (n=20), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

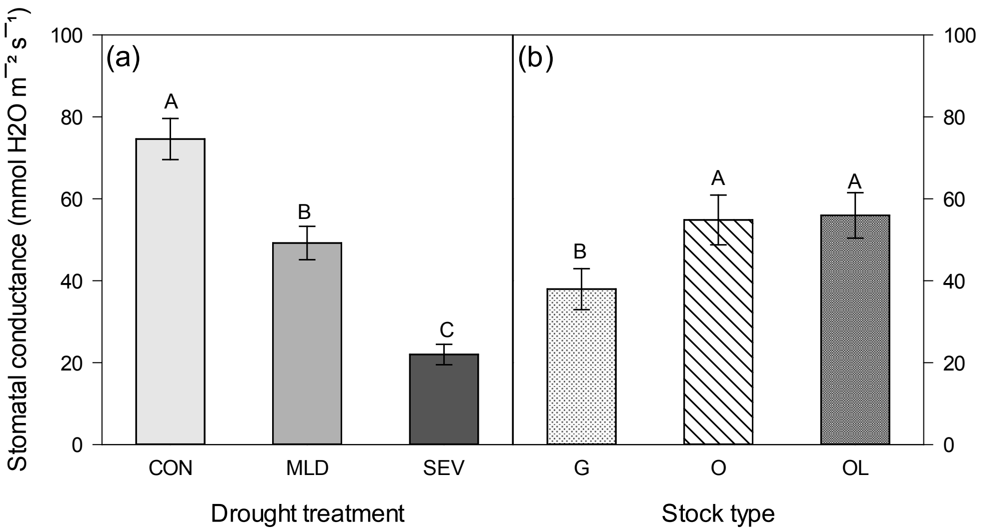

Figure 2.6 Average stomatal conductance of (a) drought treatments and (b) stock types at the end of the growth chamber experiment. The error bars represent one standard error (n=30), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

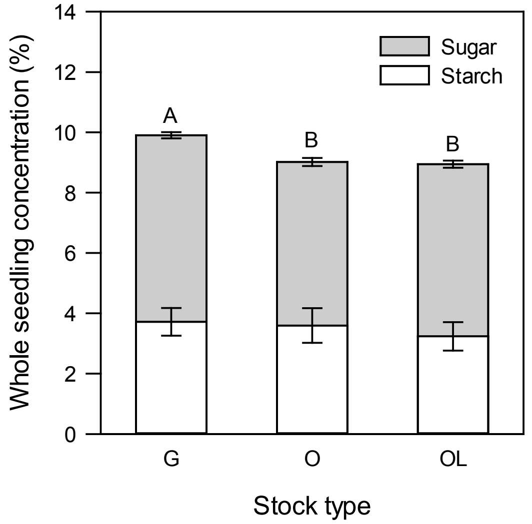

Figure 2.7 Average whole seedling soluble sugar and starch concentrations of stock types at the end of the experiment. The error bars represent one standard error (n=30), and different letters indicate statistical differences among means for sugar based on comparisons using Tukey-Kramer adjustment.

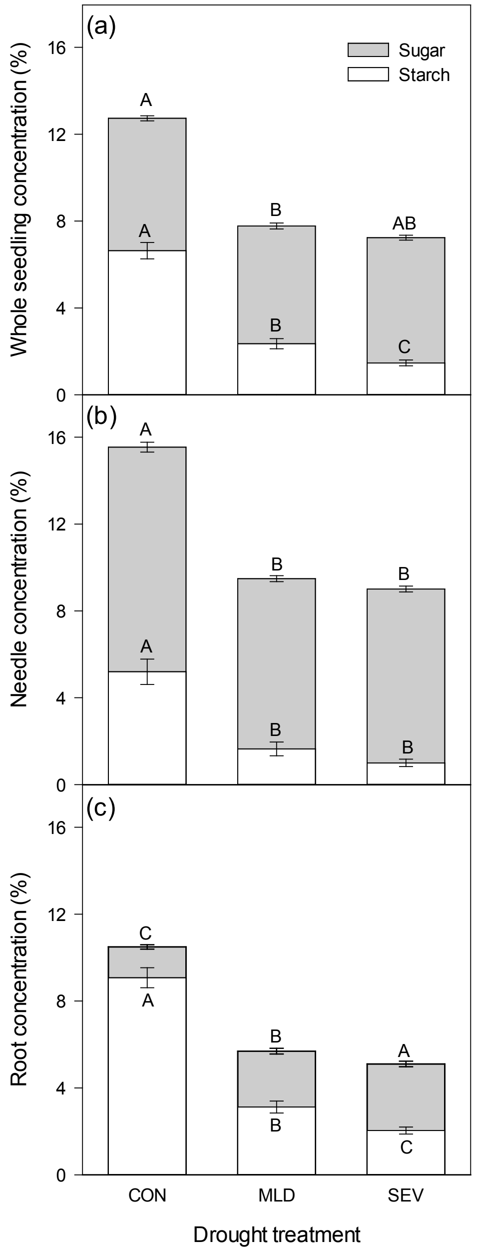

Figure 2.8 Average (a) whole seedling, (b) needle, and (c) root soluble sugar and starch concentrations of drought treatments. The error bars represent one standard error (n=30), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

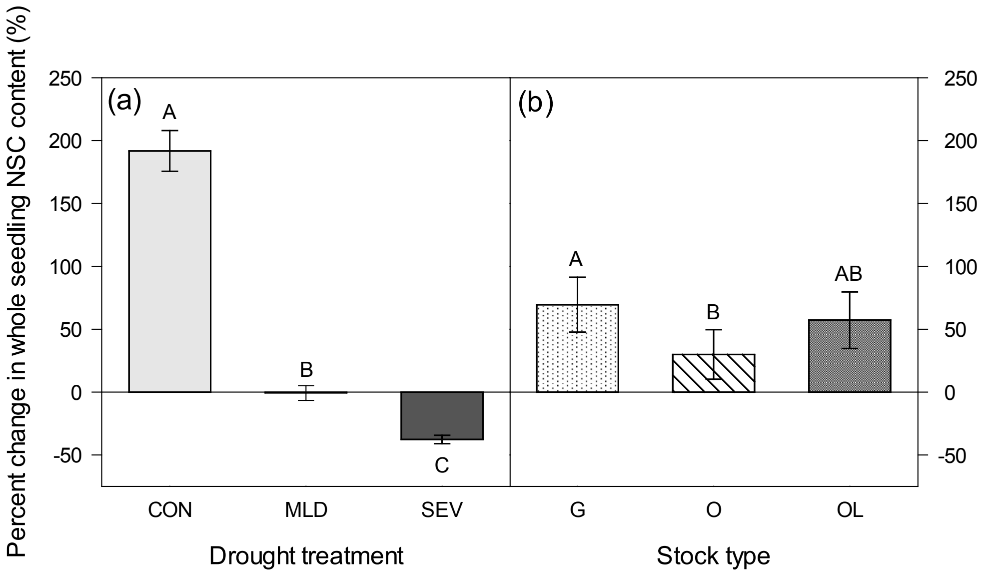

Figure 2.9 Average percent change in whole seedling NSC content from frozen storage to the end of the drought relative to frozen storage levels of (a) drought treatments and (b) stock types. The error bars represent one standard error (n=30), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

Chapter 3: Evaluating Jack Pine Seedling Characteristics in Response to Aspect and Hydrogel Amendment

3.1 Introduction

The boreal forest boasts some of the richest renewable and mineral resources worldwide, leading to a wide range of anthropogenic disturbances, from logging to surface mining. To initiate or accelerate forest reclamation on these severely disturbed sites, planting nursery-grown seedlings may often be necessary. However, because of cool temperatures, low precipitation, and poor nitrogen availability in the region (Näsholm et al., 1998; Baldocchi et al., 2000), successful seedling establishment on reclaimed land in the boreal forest can be challenging (Macdonald et al., 2012).

Changes in topography due to hill structures and landforms are often created to manage the excess overburden (overlaying material that needs to be removed to access ore), but these structures may pose both suitable or challenging growing conditions for seedlings on reclamation sites depending on slope and exposure. Slope and aspect influence solar insolation, which in turn affects air temperatures near the soil surface and soil temperatures (Daly et al., 2007; Letts et al., 2009). These energy driven variables affect water availability and physiological activity of plants (Leij et al., 2004). Aspects with higher energy inputs (or in the northern hemisphere, south-facing slopes) can exacerbate drought stress, reducing tree establishment and growth (Oberhuber and Kofler, 2000; Johnstone et al., 2010) and favoring more drought tolerant tree species (Johnstone et al., 2010). In contrast, tree invasion appears more successful on cooler, wetter north-facing slopes in the Colorado Front Range (Mast et al., 1997). Sunlight exposure in the subalpine was believed to influence survival of emergent Engelmann spruce (*Picea engelmannii* Parry) seedlings where survival was 48 % higher on north-facing slopes (Germino et al., 2002). In the Siberian glades, Scots pine (*Pinus sylvestris* L.) seedlings on the south side of parent trees had more exposure to photosynthetic photon flux making them prone to photoinhibition especially during cool mornings (Slot et al., 2005).

Challenges such as dry conditions inhibiting seedling establishment and growth can be overcome by using site preparation (Rietveld, 1989). Hydrogel can be used as a soil amendment to improve water availability for plants on xeric sites. Hydrogel is a polymer that forms an aqueous gel when in contact with water increasing water retention of the soil and making more water available for seedlings. Using hydrogel to improve soil water retention is more beneficial in coarser material such as drought prone sandy soils (Agaba et al., 2010), and studies have found that hydrogel-amended sandy soils can bear hydraulic characteristics closer to that of loam or even silty clay (Hüttermann et al., 1999; Al-Humaid and Moftah, 2007). Under drought, hydrogel amendments improve physiological responses including stomatal conductance and photosynthesis as well as seedling height and root growth (Hüttermann et al., 1999; Al-Humaid and Moftah, 2007; Beniwal et al., 2010; Chirino et al., 2011; Jamnická et al., 2013). However, one greenhouse study observed higher mortality of Aleppo pine (*Pinus halepensis* Mill.) seedlings in hydrogel-amended soil under drought (Del Campo et al., 2011). This was attributed to seedlings in hydrogel exhausting available soil moisture through high root growth and transpiration within pots that restrict rooting volume (Del Campo et al., 2011). It is not known whether hydrogel-amended soils would benefit seedling establishment on boreal forest reclamation sites.

In the previous research chapter, the performance of seedlings that varied in characteristics such as size, root:shoot ratio (RSR), and non-structural carbohydrates (NSC) content were assessed under drought in a growth chamber. However, it is not known if results from the growth chamber experiment would be replicated under field conditions. Non-structural carbohydrates are important sources of energy and higher concentrations may aid recently established seedlings in growing new tissues after outplanting (Puttonen, 1986). Larger seedling size increased growth and survival following outplanting In the Mediterranean (Puértolas et al., 2003; Villar-Salvador et al., 2004a; Cuesta et al., 2010). However, larger seedlings generally have greater transpiring surface which may lead to elevated stress under dry conditions. Alternatively, high RSR may improve seedling establishment on dry sites as it allows high water uptake capacity via large roots relative to aboveground water loss.

In this study we tested whether initial seedling characteristics, aspect, and hydrogel amendment influence the performance of jack pine (*Pinus banksiana* Lamb.) seedlings one growing season after outplanting. We evaluated outplanting performance by measuring growth and using physiological measurements to infer seedling water status on north and south-facing plots of the different stock types created in the previous chapter that differed in size, RSR, and NSC content. Then, because south-facing aspects are usually drier, we tested whether an amendment of hydrogel on these south-facing aspects would improve jack pine seedling performance.

3.2 Material and Methods

3.2.1 Initial Seedling Characteristics

Three different stock types were created as described in Chapter 2 by growing seedlings 1) in the greenhouse (G) 2) outside (O) or 3) outside with a truncated growing season (OL). The table below shows initial seedling characteristics of the different stock types (Table 3.1; same as Table 2.1 in previous chapter).

3.2.2 Aspect and Hydrogel

After 29 weeks of frozen storage at -3 ^{o}C, seedlings were outplanted on hummocks at the Sandhill Watershed reclamation site (Syncrude Canada Ltd.) near Fort McMurray, AB, Canada (57.0408 ^{o}N, 111.5957 ^{o}W). These hummocks were composed of tailings that were covered with 0.3 to 0.4 m of fluvial sand subsoil and then 0.1 m to 0.2 m of forest floor transferred from an upland site. The forest floor material had a sandy texture where sand constituted 89-94 % of the material's dry weight with the remaining weight mostly composed of silt (Appendix 4). Forest floor had negligible amounts of available NO_3^- while available NH_4^+ varied from <0.3 to 1 µg g^{-1}. Available P and K ranged from 8 to 18 and <25 to 56 µg g^{-1}, respectively. Total organic carbon varied from 1.3 to 2 % based on dry weight which translated to a C:N ratio of 17 to 20; pH ranged from 5.5 to 7.2 (Appendix 4). Daily weather data for this region was obtained from an Environment Canada weather station near Mildred Lake (57.0333 °N, 111.5667 °W) which was approximately 2 km away from the outplanting location (AgroClimatic Information Service, 2013).

A week prior to outplanting, seedlings were removed from frozen storage and thawed slowly. On June 1 and 2, 2012, seedlings were outplanted in 1m by 2 m plots cleared of all vegetation and equipped with soil water potential and moisture sensor probes. There were a combined total of 30 north-facing, south-facing, and hydrogel plots (10 plots each) distributed on four hummocks with slopes around 22.5 º (Appendix 4). Each south-facing plot was paired with a hydrogel plot, as we anticipated warmer temperatures and greater water limitations on the south-facing aspect. Hydrogel plots were amended with granular hydrogel (Stockosorb 660 XL, Stockhausen GmbH, Krefeld, Germany) that was composed of cross-linked potassium polyacrylate, and it varied from one to four millimeters in size. Hydrogel was mixed in the top 15 cm of soil with trowels at a rate of 4.8 kg m^{-3} of soil. Each plot had three rows of 10 pine seedlings, and each row was comprised of one stock type (G, O, and OL). Rows were separated by 30 cm, and seedlings within a row were 20 cm away from each other. A 5TM soil moisture sensor probe (Decagon, Pullman, WA, USA) and a MPS-2 dielectric water potential sensor probe (Decagon, Pullman, WA, USA) were installed at 10 cm depth to measure volumetric soil water content, soil water potential, and soil temperature in seven of the ten north-facing, south-facing, and hydrogel plots. Soil temperature, water content, and water potential were logged hourly and summarized as daily averages.

Plant Root Simulator (PRS) probes (Western Ag Innovations Inc., Saskatoon, SK, Canada)–ion exchange resin membranes used to measure bioavailable nutrients–were placed in each of the four corners of all plots on July 20, 2012. At each location a pair of PRS probes that captures anion and cation separately was pressed into the ground vertically while ensuring the membrane of the probes was below the soil surface. All probes were removed after 32 days (August 21), placed in Ziploc bags which were then placed in a cooler with icepacks, and transferred to a refrigerator. Probes were cleaned later that day with toothbrushes and de-ionized water to remove any remaining soil. Afterwards, probes were placed in new bags and shipped to Western Ag Innovation for nutrient analysis. For analyses, the four pairs of anion and cation probes from each plot were pooled. The membranes of the PRS probes were placed in a 0.5 N HCl solution for 1 hour before analysis. Nitrate (NO_3^-) and Ammonium (NH_4^+) was analyzed colormetrically with automated flow injection analysis system while total N was calculated by adding NO_3^- and NH_4^+ values together. Other nutrients including Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd were analyzed with inductively-coupled plasma spectrometry.

3.2.3 Seedling Measurements

3.2.3.1 Growth Variables

To determine initial seedling characteristics prior to cold storage and the outplanting experiment, 24 seedlings from each stock type were destructively sampled. Stem height from the root collar to the base of the terminal bud was measured for each seedling, and dry mass was determined for needles, stems, and roots by oven drying samples at 70 °C for at least 72 hours (table 3.1). Prior to drying and weighing, roots were carefully washed to remove growing substrate (Professional Growing Mix, Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) from being grown in 4-12A (cavities: 4 cm wide and 12 cm deep; 125 ml) styroblock containers (Beaver Plastics Ltd, Edmonton, AB, Canada). Thirteen weeks after outplanting (August 26 and 31, 2012), the same root and shoot measurements were taken on three randomly selected seedlings from each stock type within each plot.

Seedling growth performance was assessed in several ways. Height growth of each seedling was measured from the bud scar of the previous growing season to the base of the terminal bud. Relative height growth of each seedling was calculated by dividing height growth by the initial seedling height to measure how much a seedling grew relative to initial height over one growing season. Needle, stem, and root dry mass growth as well as change in RSR were calculated by subtracting the average initial dry mass or ratio for each stock type from the final dry mass or ratio of each outplanted seedling. RSR was calculated as root dry mass divided by needle and stem dry mass, and relative change in RSR was estimated by dividing RSR change by the average initial RSR. These growth measurements were then averaged for each stock type per plot.

3.2.3.2 Physiological Variables

Thirteen weeks after outplanting (August 26 and 31) and prior to destructive sampling, a leaf porometer (AP4, Delta-T Devices Ltd, Burwell, Cambridge, England) was used to measure stomatal conductance on six seedlings of each stock type growing in four north-facing, south-facing, and hydrogel plots each between 09:00 and 16:00 hours. The same seedlings were destructively sampled afterwards for growth measurements. A slotted cup on the sensory head was used for measuring stomatal conductance on recently formed needles situated near the terminal bud. All measured needles were marked with a permanent maker. Marked needles were cut after shoot water potentials were taken and placed into paper bags within a Ziploc bag. These bags were kept in a cooler with icepacks until transported to a freezer (-20 ^{o}C). The projected leaf area of the cut needles was calculated using winSEEDLE software (winSEEDLE 2006 Régent Instruments Inc., Sainte-Foy, QC, Canada) after being scanned. Stomatal conductance was scaled by needle surface area to account for differences in leaf area placed inside the cup of the porometer.

Just after measuring stomatal conductance, shoot water potential was measured in the field on two of the six seedlings measured for stomatal conductance. Shoot water potential was measured by cutting the seedlings just above the root collar and placing the stem in a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA). Both stomatal conductance and shoot water potential measurements among the treatment combinations were blocked by time, so that after measuring a G seedling within a plot, one seedling of each O and OL stock type was measured before proceeding to the next G seedling. In addition, after completing measurements on a north-facing plot, a south-facing and hydrogel plot was completed before proceeding to the next north-facing plot.

3.2.4 Data Analysis

One-way ANOVA was used to compare initial seedling measurements of the three stock types (G, O, and OL) and soil available nutrients between north and south-facing plots and between hydrogel and non-amended south-facing plots. Differences in soil temperature, water content, and water potential between north and south-facing plots and between hydrogel and south-facing plots were compared using repeated measures with plot included as a random variable. Stock type and aspect effects on growth and physiological variables were analyzed using a two-way ANOVA. Because growth variables were calculated as an average for each stock type per plot, a plot was not included as a variable in the analysis. However, as stomatal conductance and shoot water potential measurements were blocked by time, time block was included as a random variable. Hydrogel effects on growth and physiological responses were analyzed as a split-plot design comparing non-amended south-facing and hydrogel plots. PROC MIXED (SAS 9.2, SAS Institute, Cary, North Carolina, USA) was used for all analyses. Prior to all statistical analyses, tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and equal variance (Levene's) of the residuals were performed. Initial measurements such as height, stem mass, root mass, RSR, NSC content had unequal variance. Total N, S, new needle mass, root growth, RSR change, stomatal conductance, shoot water potential had unequal variance in the aspect analysis while K, relative height growth, shoot growth, new needle mass, and RSR change had unequal variance in the soil amendment analysis. For variables with unequal variance, ANOVA models that assume unequal variance were used, and the unequal variance model with the best fit was determined using Bayesian information criterion (BIC) (Littell et al., 2006). BIC was also used to determine which variance covariance structure provides the best fit to soil temperature, water content, and water potential data. Treatment and stock type averages were compared using Tukey-Kramer adjustment with $\alpha=0.05$ as the significance level.

3.3 Results

3.3.1 Weather Conditions after Outplanting

Weather conditions after outplanting were characterized by relatively warm temperatures with ample rainfall throughout most of the growing season. June 2012 was cooler compared to the rest of the growing season with average daily temperatures rarely above 20 °C until the end of the month (Figure 3.1a and b). June had an average temperature of 17 °C and accumulated 60 mm of precipitation compared to an average of 45 mm over the previous six year (AgroClimatic Information Service, 2013). The beginning of July was characterized by heavy rainfalls, followed by the warmest temperatures during the growing season. Through the rest of July and into August, average temperatures remained between 14 and 23 °C, and rain events were regular but smaller. July and August 2012 had 88 and 35 mm of precipitation and average temperatures of 20 and 18 °C, respectively. In the previous six years, July and August had 61 and 69 mm of precipitation and average temperatures of 19 and 16 °C, respectively (AgroClimatic Information Service, 2013). Relative humidity peaked during rain events and lower temperatures (Figure 3.1c).

3.3.2 Soil Moisture and Temperature Conditions

North-facing plots were cooler (aspect: *P*<0.001, Figure 3.2a; Appendix 5) and wetter (aspect: *P*<0.001, Figure 3.2b; Appendix 5) than south-facing plots at a 10 cm depth of soil. No difference in soil water potential was detected between aspects (aspect: *P*=0.210; Appendix 5), but there was a difference later in the growing season where south-facing plots had more negative soil water potentials compared to north-facing plots (aspect*day: *P*<0.001, Figure 3.2c; Appendix 5).

Hydrogel plots were generally cooler compared to non-amended plots (treatment: *P*<0.001; Appendix 5), although this difference in soil temperature was only apparent at warmer temperatures (Figure 3.2a). Hydrogel plots were also wetter throughout the growing season (treatment: *P*=0.032; Appendix 5), and water content tended to not fluctuate as much as non-amended plots (Figure 3.2b). There was no difference between hydrogel and non-amended plots (treatment: *P*=0.543; Appendix 5), but non-amended plots tended to have more negative water potentials than hydrogel plots at the end of the growing season (treatment*day: *P*<0.001, Figure 3.2c; Appendix 5).

3.3.3 Soil Nutrient Supply

North and south-facing plots had slightly different rates of available nutrients in the soil. Both north and south-facing plots had similar available total N, NO_3^-, NH_4^+, and Mg (Table 3.2). However, south-facing plots had twice as much K and P whereas north-facing plots had more than twice as much S and 1.7 times as much Ca (Table 3.2).

Hydrogel plots generally had more available soil nutrients than non-amended plots. Hydrogel did not affect available NH_4^+, Ca, or S (Table 3.2), but plots amended with hydrogel had 4.6 times more $N0_3^-$, resulting in roughly 2.7 times more total available N. In addition, hydrogel plots also had 7.8 and 1.7 times more K and P, respectively, than non-amended plots, while the non-amended south-facing plots had 1.4 times more Mg.

3.3.4 Growth and Physiological Response on North and South-Facing Plots

Stock type had a strong influence on height, relative height, and root growth (stock type: all *P*=0.001; Appendix 5) whereas aspect had a strong influence on new needle mass (aspect: *P*=0.003; Appendix 5). The O stock type had 21 % more height growth than both the G and OL stock types which were not different from each other (Figure 3.3a). The O stock type, which had initially the highest RSR, had the highest relative height growth while the G stock type had the least (Figure 3.3b). Root growth showed the opposite trend, G stock type with initially the smallest RSR had the most root growth (Figure 3.3c). In contrast, new needle mass was influenced by aspect rather than stock type, with south-facing plots having 47 % greater needle mass (Figure 3.3d).

Changes in RSR from initial conditions were influenced by both stock type and aspect, but stock types responded similarly to change in aspect (stock type*aspect: *P*=0.901; Appendix 5). The G stock type did not change in RSR from initial conditions whereas both the O and OL stock types reduced RSR by 47 % (Figure 3.4a). Across stock types, seedlings growing in north-facing plots had a smaller reduction in RSR from initial conditions compared to seedlings growing in south-facing plots (Figure 3.4b).

Unlike most of the growth responses, stomatal conductance was influenced by aspect rather than stock type (aspect: *P*=0.001; Appendix 5). Seedlings in north-facing plots had approximately 50 % lower stomatal conductance than seedlings in south-facing plots (Figure 3.5). Despite differences in stomatal conductance, there were marginal differences observed with shoot water potential (aspect: *P*=0.098; Appendix 5). Seedlings in north-facing plots had average shoot water potentials of -1.4 MPa whereas seedlings in south-facing plots had -1.2 MPa.

3.3.5 Growth Performance on South-Facing Plots after Hydrogel Amendment

Hydrogel amendment had no effect seedling height and relative height growth regardless of stock type (stock type: *P*=0.012 and *P*<0.001, respectively; Appendix 5). Trends were the same as height and relative height growth of north and south-facing plots (Figure 3.3a and b).

Stem growth had an interaction between stock type and hydrogel amendment (stock type*treatment: *P*=0.037; Appendix 5). While there was no effect of hydrogel on stem growth for O and OL seedlings, hydrogel did increase stem growth of G seedlings so that they had greater growth than the other stock types (Figure 3.6a).

Similar to height and relative height growth, hydrogel had no influence on root growth (stock type: *P*<0.001; Appendix 5). Trends were similar to root growth of north and south-facing plots (Figure 3.3c), but O and OL stock types did not differ significantly (Figure 3.6b). Since there was no differences in needle production, root growth drove differences in RSR (Appendix 5). The G stock type experienced no change, but the O and OL stock types had approximately 50 % smaller RSR than their initial levels (Figure 3.6c).

3.4 Discussion

There was no difference in physiological responses among stock types during the first year in the field, and none of the stock types clearly demonstrated better growth performance. Instead, stock types differed in their allocation of growth to organs. The smaller O seedlings with initially high RSR had similar needle growth to large seedlings (G), but less root growth, therefore, smaller seedlings allocated relatively more to needles. Since seedlings will generally partition growth to organs responsible for the acquisition of a limiting resource (Chapin et al., 1987), this suggests that O seedlings were carbon limited. Greater water and nutrient supply via large root system relative to aboveground mass may have supported greater height growth and relatively higher needle production than large seedlings. On the contrary, large seedlings with low initial RSR did not demonstrate a clear resource limitation. These seedlings had greater root growth without changing RSR. Negligible change in RSR indicates the large seedlings' initial ratio between below and aboveground dry mass was adequate for the given growing conditions.

One of the main objectives of this experiment was to determine whether a stock type bearing specific characteristics would outperform other stock types after outplanting, but determining which stock type performed the best in this study is circumstantial. Large seedlings were expected to be water limited due to their relatively small root systems, and they would therefore allocate more growth to roots to increase RSR. Furthermore, large seedlings were anticipated to grow more under less-stressful conditions on north-facing slopes since greater leaf area of large seedlings, and thus greater photosynthesizing capacity, may fuel more growth. In the field, large seedlings did not differ in water stress or growth between north and south-facing slopes. Instead, large seedlings on both slopes allocated growth to roots. If having greater root growth is an indication of good seedling performance in reclamation, large seedlings would be good candidates. Root growth may be advantageous as recently planted seedlings need to have adequate contact between the roots and the surrounding soil in order to obtain sufficient moisture and nutrients (Radoglou and Raftoyannis, 2002; Seifert et al., 2006). It was expected that smaller seedlings with high RSR would have greater growth or experience less water stress than large seedlings under drier conditions on south-facing slopes. After outplanting, seedlings with high RSR did not experience less water stress or grow more than large seedlings. However, smaller seedlings had relatively more shoot growth despite their smaller size. If growth performance is based on relative growth, smaller seedlings grew better than large seedlings.

Seedlings on south-facing plots were expected to experience less growth and greater water stress than seedlings on north-facing plots. Instead, seedlings on south-facing plots had more growth, specifically needles, and they had higher stomatal conductance. Seedlings received ample precipitation in June and July, and soil water potentials in these plots did not decline until August. Seedlings only had an average shoot water potential of -1.3 MPa, and jack pine do not experience significant losses of stem conductivity until water potential declines below -2 MPa (Schoonmaker et al. 2010). Since seedlings were not stressed by drought, other variables such as temperature may have determined seedling performance.

Differences in soil temperatures may have impacted P and K availability on south-facing plots, but some nutrients did not differ between aspects. Studies have indicated that nutrient availability, specifically N, increases with warmer soil temperatures (Van Cleve et al., 1990; Chapin et al., 1995; Lükewille and Wright, 1997; Rustad and Fernandez, 1998). Of these studies, Chapin et al. (1995) and Van Cleve et al. (1990) also found P to increase in warmer soils. Curiously, we did not detect differences in N between warm south-facing and cool north-facing plots. Available N was not found to differ between European beech (*Fagus sylvatica* L.) stands occupying a warm, dry and a cool, wet aspect (Dannenmann et al., 2007). Since net N mineralization is sensitive to water availability and higher temperatures increases N mineralization and nitrification (Hart and Perry, 1999; Emmett et al., 2004; Domisch et al., 2006), it was concluded that the benefit and drawback of increasing soil temperature and dryness on N availability counterbalanced each other (Rennenberg et al., 2009). In our experiment, drying soils could have inhibited increased N availability due to warming soil temperatures on south-facing plots.

Higher P and K availability on south-facing plots could have increased stomatal conductance and therefore, needle production. Soil and leaf P is positively correlated with maximal photosynthesis rates among maritime pine (*Pinus pinaster* Ait.) (Ben Brahim et al., 1996; Loustau et al., 1999), Monterey pine (*P. radiata* D. Don) (Sheriff et al., 1986; Conroy et al., 1990), and eastern white pine (*P. strobus* L.) (Reich and Schoettle, 1988). Maximal photosynthesis rates were also found to increase with K in Norway spruce (*Picea abies* (L.) Karst) (Barnes et al., 1995). In addition, K deficiency was observed to decrease photosynthesis in cotton (*Gossypium hirsutum* L.) mostly due to reduced stomatal conductance (Bednarz et al., 1998).

In boreal reclamation sites, jack pine is ideal for planting on dry and warm south-facing slopes, and better performance on these slopes is consistent with where jack pine is generally found under natural circumstances. Less growth on north-facing slopes could be attributed to cooler temperatures which inhibit water flow and reduce shoot water potential in seedlings (Wan et al., 1999). Pine use isohydric regulation whereby reductions in stomatal conductance prevented seedlings from experiencing low plant water potential (Tardieu and Simonneau, 1998), and boreal tree species such as trembling aspen (*Populus tremuloides* Michx.) have been observed to utilize isohydric regulation in response to cool soil temperatures (Wan et al., 2004). This may explain why no difference in shoot water potential was detected between north and south-facing plots, and low stomatal conductance may have hindered growth of pine seedlings on north-facing plots. Higher solar insolation likely drove higher soil temperatures and lower soil water content on south-facing plots which suggests that air temperatures near the soil surface were higher as well (Leij et al., 2004; Daly et al., 2007; Letts et al., 2009). A study on limber pine (*Pinus flexilis* James) in the subalpine found that despite the fact that trees on a south-facing aspect were exposed to greater drought stress and warmer temperatures near the soil surface, they had longer photosynthetically active day length which facilitated greater branch length increments (Letts et al., 2009).

Furthermore, a meta-analysis by Way and Oren (2010) found that seedlings under warmer air and soil temperatures allocate more growth to leaf production and thus decrease RSR.

Moist soil conditions prevented hydrogel from improving seedling water status. There was no difference in soil water potential between hydrogel-amended and non-amended plots although soil water potential declined more in the non-amended plots than hydrogel plots near the end of the growing season. Furthermore, no difference in stem water potential or stomatal conductance was detected between hydrogel plots and non-amended plots (data not shown).

Unexpectedly, hydrogel increased the bioavailability of total N, P, and K, though it generally did not affect growth. One study observed the influence of amending soil with a hydrogel composed of potassium polyacrylate (similar to what was used in our experiment) on changing nutrient availability under wetting and drying cycles, and they found soil available P and K increased with the first drying cycle compared to a control (Bai et al., 2010). However, this trend disappeared after conditions became drier and then wetter (Bai et al., 2010), and it was not determined why this happened. Alternatively, there are many studies that show fertilizer in addition to hydrogel slows the leaching of nutrients (Smith and Harrison, 1991; Mikkelsen, 1994; Syvertsen and Dunlop, 2004; Rowe et al., 2005), but the rate of leaching depends on the nutrient in question. For example, one study found hydrogels to retain more NH_4^+ compared to NO_3^- (Bres and Weston, 1993). Even though hydrogel appeared to increase the availability of nutrients, it only had a subtle influence on stem growth of large seedlings. Large seedlings have lower nutrient and water supply relative aboveground mass compared to other stock types, and therefore, they are likely more responsive in terms of growth to increased water and/or nutrient availability.

Overall, site conditions determined which stock type would demonstrate better outplanting performance. In our study, large jack pine seedlings were best for these ideal growing conditions after outplanting since they have the advantage of larger initial size and more root growth which builds a better connection with the surrounding soil. However, seedlings with high RSR grew more relative to their initial size especially in aboveground organs, and they may potentially perform better under drier conditions when root growth is severely hindered. Even though this experiment did not experience dry conditions, pine is still better suited for south-facing aspects under drought compared to other boreal tree species, and less growth on north-facing slopes suggests that an alternative tree species more adapt to growing under cooler temperatures should be planted on such sites.

For site conditions similar to those experienced in this experiment, using hydrogel for site preparation to enhance outplanting performance of jack pine seedlings is not necessary. Although hydrogel increased nutrient availability, it did not improve seedling water status or growth. Perhaps a more appropriate application for hydrogel is to be used for species more sensitive to drought or improve nutrient availability of nutrient deficient sites.

Table 3.1 Pre-outplanting average (± SE) of morphological and carbon reserve characteristics of seedling stock types (G, O, OL). RSR is root:shoot ratio and NSC is nonstructural carbohydrates. Different letters signify statistical differences among means based on comparisons using Tukey-Kramer adjustment (n=24 with the exception of NSC content and concentrations: n=15)

	Stock type		
	G	O	OL
Height (cm)	11.0 ± 0.5 A	5.0 ± 0.3 C	6.8 ± 0.3 B
Root collar diameter (mm)	2.6 ± 0.05 A	2.3 ± 0.07 B	1.9 ± 0.06 C
Needle mass (g)	1.524 ± 0.056 A	0.666 ± 0.045 B	0.482 ± 0.046 C
Stem mass (g)	0.415 ± 0.022 A	0.177 ± 0.013 B	0.143 ± 0.009 B
Root mass (g)	1.142 ± 0.040 A	1.186 ± 0.067 A	0.661 ± 0.038 B
Total mass (g)	3.080 ± 0.111 A	2.030 ± 0.110 B	1.286 ± 0.078 C
RSR (g g^{-1})	0.601 ± 0.022 C	1.458 ± 0.071 A	1.128 ± 0.061 B
Whole seedling NSC (g)	0.457 ± 0.032 A	0.330 ± 0.018 B	0.203 ± 0.016 C
Whole seedling NSC (%)	14.03 ± 0.33	14.47 ± 0.27	14.85 ± 0.21
Needle NSC (%)	16.80 ± 0.32 B	16.84 ± 0.30 B	18.34 ± 0.38 A
Stem NSC (%)	14.26 ± 0.58 B	16.92 ± 0.59 A	17.60 ± 0.44 A
Root NSC (%)	10.33 ± 0.57 B	12.14 ± 0.46 A	12.00 ± 0.39 A

Table 3.2 Average available nutrients (± SE) of north-facing, south-facing, and hydrogel plots. Units are expressed as µg 10 cm^{-2} 32 days^{-1} and different letters signify statistical differences between means based on Tukey-Kramer adjustment (n=10)

	Aspect		Treatment
	North-facing	South-facing	Hydrogel
Total N	14.39 ± 1.25	14.59 ± 0.80 **Y**	38.58 ± 6.86 **Z**
NO$_3^-$	9.23 ± 1.18	6.90 ± 1.04 **Y**	31.52 ± 6.77 **Z**
NH$_4^+$	7.06 ± 1.08	7.70 ± 1.01	7.06 ± 1.08
P	3.66 ± 0.42 **B**	6.32 ± 0.83 A **Y**	11.09 ± 0.99 **Z**
K	125.25 ± 17.84 **B**	251.52 ± 29.35 **A Y**	1972.45 ± 213.30 **Z**
Ca	2168.18 ± 115.29 **A**	1279.44 ± 118.96 **B**	1056.51 ± 141.15
Mg	253.37 ± 21.02	272.40 ± 14.78 **Z**	189.42 ± 8.72 **Y**
S	608.05 ± 113.72 **A**	264.82 ± 34.39 **B**	381.69 ± 71.71

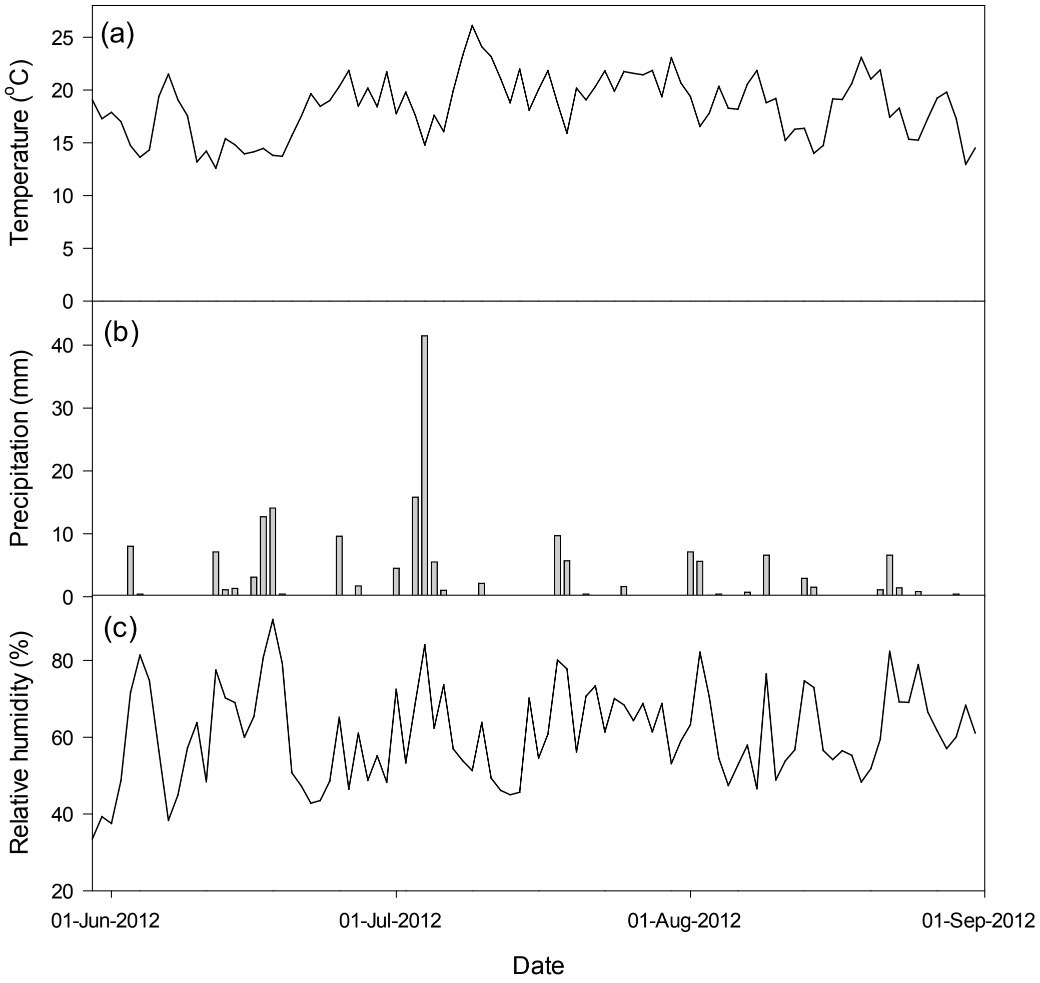

Figure 3.1 Daily (a) average temperature, (b) precipitation, and (c) average relative humidity during the growing season at Mildred Lake (AgroClimatic Information Service 2013).

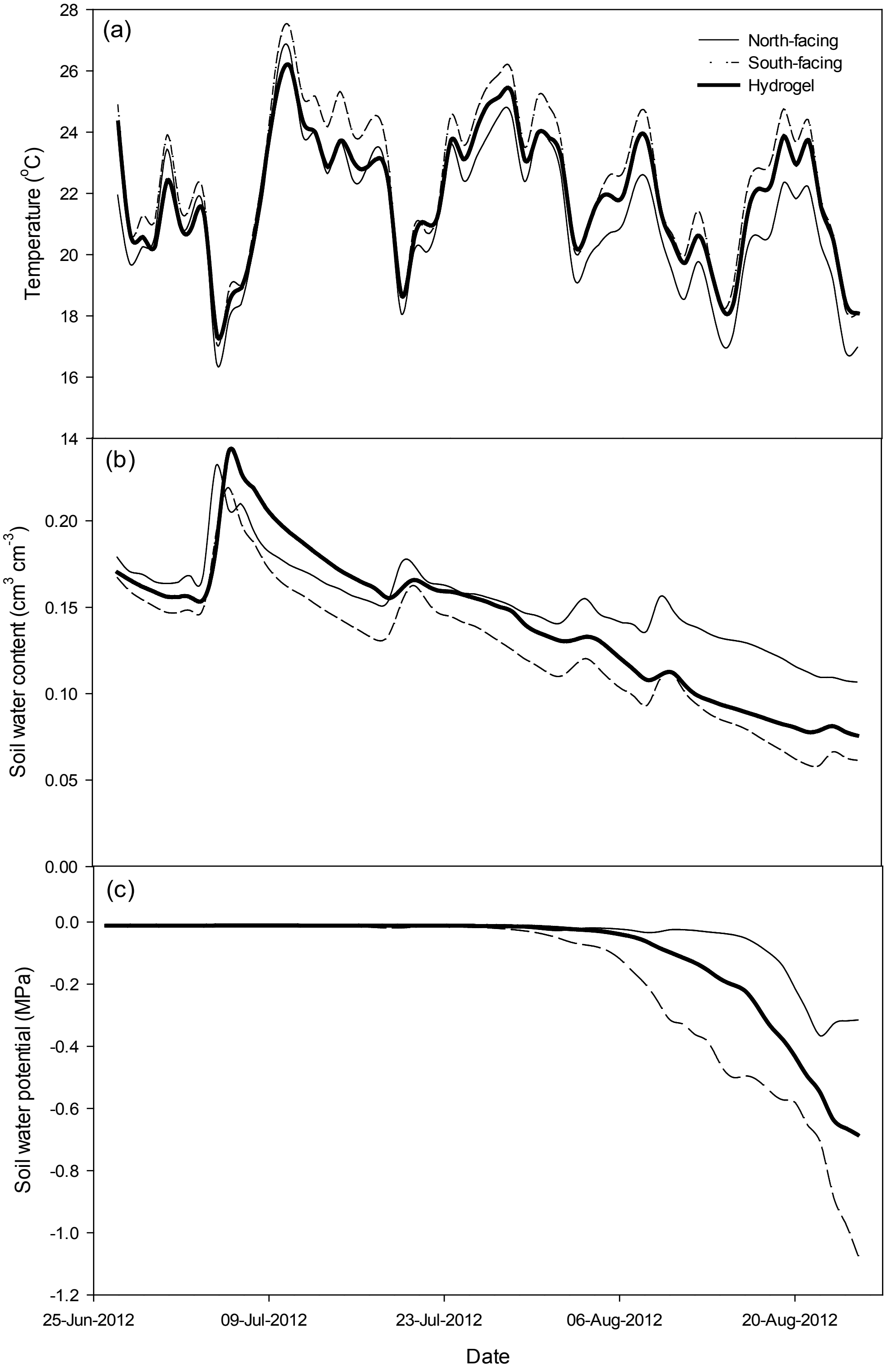

Figure 3.2 Daily average (a) temperature, (b) soil water content, and (c) soil water potential of north-facing, south-facing, and hydrogel plots at 10 cm depth (n=7).

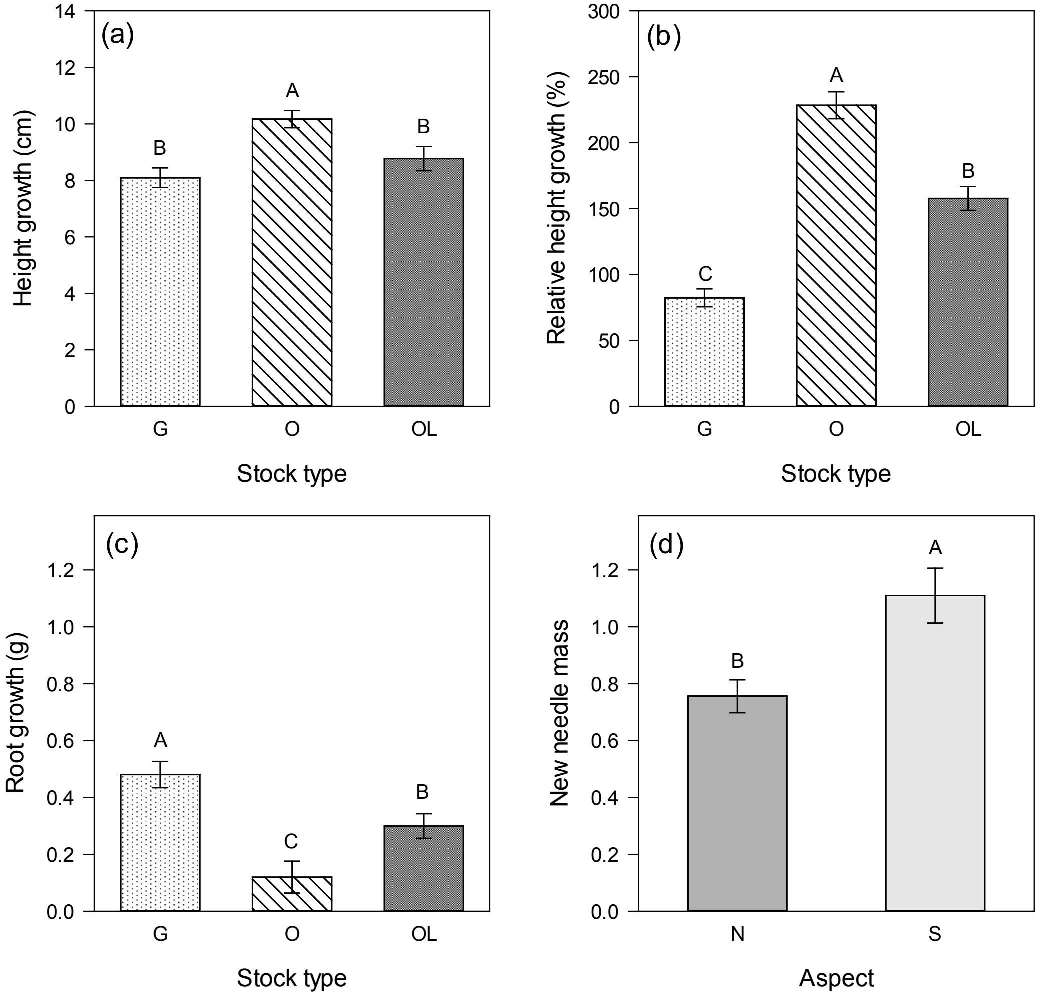

Figure 3.3 Average (a) height growth, (b) height growth relative to initial height, and (c) root growth of different stock types(n=20) as well as average (d) new needle mass of north and south-facing aspects(n=30) over one growing season. Error bars represent one standard error, and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

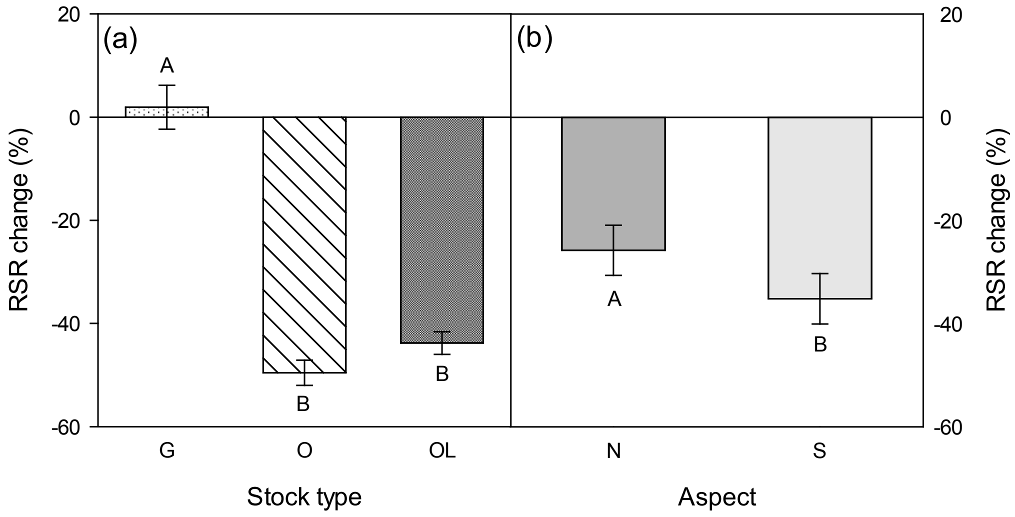

Figure 3.4 Average RSR percent change of (a) stock types (n=20) and (b) north and south-facing aspects (n=30) over one growing season. Error bars represent one standard error, and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

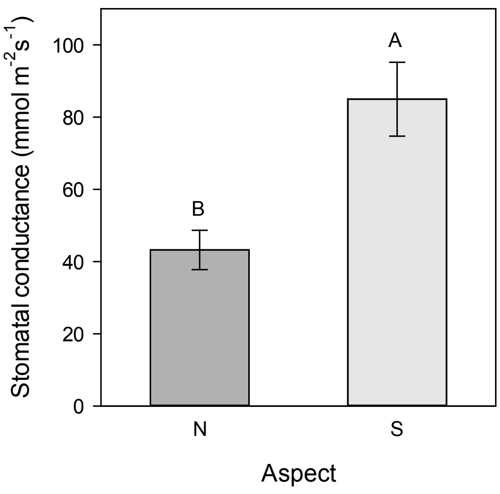

Figure 3.5 Average stomatal conductance of north and south-facing aspects. Error bars represent one standard error (n=30), and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

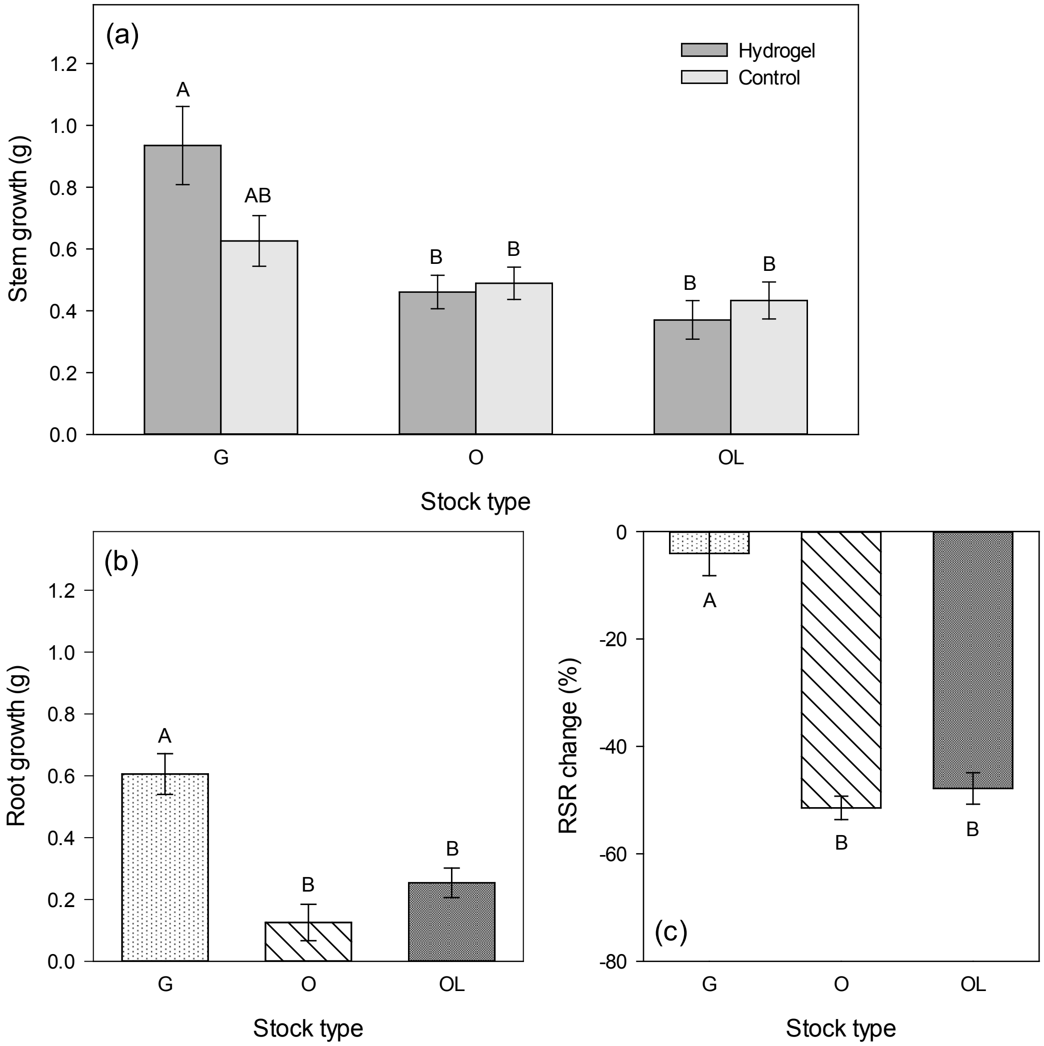

Figure 3.6 Average (a) stem growth (n=10) as well as average (b) root growth and (c) RSR change s (n=20) of hydrogel-amended and non-amended plots on a south-facing aspect over one growing season. Error bars represent one standard error, and different letters indicate statistical differences among means based on comparisons using Tukey-Kramer adjustment.

Chapter 4: Summary and Implication

4.1 Research Summary

The objective of the first experiment was to assess the performance of jack pine seedlings of varied size, root:shoot ratio (RSR), and non-structural carbohydrate (NSC) content under a well-watered, mild drought, or severe drought treatment in a growth chamber. Results from this experiment indicated that drought reduced all growth and physiological responses, but the degree to which growth of specific organs or physiological responses declined depended on initial stock type characteristics. Smaller seedlings with high RSR allocated relatively more growth to shoots under drought. In contrast, larger seedlings with initially low RSR experienced the opposite trend where relatively more growth was allocated to roots. In terms of physiological responses, both stomatal conductance and shoot water potential decreased with increasing drought intensity among all seedling types. However, large seedlings had more leaf area which restricted the supply of water per needle and thus suppressed stomatal conductance whereas smaller seedlings with high RSR had higher stomatal conductance. All seedlings shared similar shoot water potential except under the severe drought treatment where large seedlings had less negative stem water potential indicating that they were under less water stress which may be attributed to greater water uptake due to root growth.

The negative effects of drought on physiological responses led to reductions in whole seedling NSC content and concentration. Jack pine displayed a water conserving strategy where seedlings limited water loss by closing stomata, which also restricted photosynthesis and reduced NSC concentrations. Starch concentrations decreased with increasing drought intensity. In contrast, root sugar concentrations increased with increasing drought intensity; this was likely due to osmotic adjustment (Koppenaal et al., 1991). Despite small seedlings with high RSR and large seedlings having different growth strategies and physiological responses due to distinct initial characteristics, this had little influence on NSC concentrations since little difference was seen among different seedling types at the end of the growth chamber experiment. This may suggest that drought or the response to drought has a strong influence over seedling NSC.

The results from the first experiment led to testing the performance of the same stock types one growing season after outplanting on a boreal reclamation site. The influence of site conditions on north and south-facing aspects and soil amendment with hydrogel on seedling performance was also assessed. It was assumed that the south-facing aspect would have warmer temperatures and greater water constraints, so each south-facing plot was paired with a hydrogel plot. Hydrogel was hypothesized to improve seedling water status and growth performance under dry conditions.

Both the field and growth chamber experiment revealed similarities; specifically, growth of certain organs was heavily influenced by initial seedling characteristics and resource limitation. Smaller seedlings with initially high RSR grew more in height and increase needle production to intercept more light and increase carbon uptake which implies these seedlings were carbon limited. Comparatively, large seedlings with initially small RSR had more root growth. This suggests that water was more of a limiting resource and increased root growth may have helped large seedlings increase their water uptake capacity.

Unlike the growth chamber experiment, seedlings were not under continuous drought conditions in the field. Both June and July were wet, and reductions in soil water potential were not seen until August towards the end of the growing season. Growth of outplanted seedlings also demonstrated that there was little influence of drought stress. Large seedlings with relatively small root systems had high root growth but did not change their RSR to substantially increase their water uptake capacity. Smaller seedlings with relatively large root systems allocated considerable growth to aboveground organs which resulted in large reductions in RSR. In comparison to the growth chamber experiment, these seedlings had small reductions in RSR under drought.

Since seedlings were not drought stressed, other variables influenced growth such as temperature. Seedlings on south-facing plots experienced high stomatal conductance and needle growth which may have been driven by temperature in a couple different ways. High solar insolation likely drove warmer soil temperatures on south-facing plots, suggesting that air temperatures near the soil surface were warmer as well. Warm air and soil temperature could have prompted seedlings to allocate more growth to photosynthesizing tissue (Way and Oren, 2010). Warmer soil temperatures on south-facing plots increased P and K availability which may have improved photosynthesis and as a result, fuelled growth. Alternatively, seedlings on north-facing plots decreased stomatal conductance as a preventative measure to protect the stem from experiencing exceedingly low water potential likely driven by cool soil temperatures. In turn, low stomatal conductance hindered growth of these seedlings.

4.2 Management Implication

Site conditions determine which seedling characteristics translate to better performance in terms of growth and stress tolerance, and large seedlings are suitable for most planting sites. Results show that large seedlings maintained their large size and developed a better connection with the surrounding soil through root growth. Furthermore, root growth reduced stress among large seedlings under severe drought conditions. These seedlings may have facilitated growth by having higher photosynthesis per plant due to larger needle area which may be beneficial when photosynthesis rates per needle are low. More needle area also indicates a bigger reservoir for NSC reserves in the needles. However, it should be cautioned that not all large seedlings will have similar NSC concentrations. Seedlings need to have sufficient time to accumulate NSC reserves by setting bud. Otherwise, low NSC may result in poor growth performance or survival after outplanting (Puttonen, 1986). Overall, large seedlings are suitable for most sites which experience both good growing conditions and occasionally drought. Current measurements of seedling quality such as stem height and root collar diameter would be adequate for selecting seedlings of large size.

Even though large seedlings would suffice for sites with adequate water availability, seedlings with high RSR may be suitable for particularly xeric sites. Studies have found that pine with greater root area relative to needle area are less susceptible to drought stress (Sperry et al., 1998; Ewers et al., 2000; Hacke et al., 2000; Addington et al., 2006). High RSR may be advantageous on sites with greater constraints on growth, particularly root growth, due to high drought severity. Measuring RSR requires a less conventional and convenient form of assessing seedling quality whereby destructive harvesting of a subsample of seedlings is required.

After determining a suitable stock type for a particular site, it is necessary to have appropriate nursery conditions to produce jack pine seedlings with desired characteristics. Transferring seedlings outside minimized shoot elongation due to moisture constraints driven by large fluctuations in vapor pressure deficit. More growth was therefore allocated to roots which increased RSR. Growing seedlings under greenhouse growing conditions permitted shoot elongation which produced large seedlings with low RSR.

4.3 Research Limitations and Future Research

Testing the performance of pine seedlings with different NSC concentration under drought requires further investigation. Whole seedling NSC concentrations did not differ between stock types. As well, both concentration and content among different stock types followed similar trends under different drought treatments. This makes it difficult to determine if NSC content or concentration is a better predictor of growth performance and /or stress tolerance under drought. Being an evergreen tree species may prevent pine stock types from having differences in whole seedling NSC concentrations due to a longer period of photosynthesis throughout the year, longer retention of photosynthesizing tissue, and needles facilitating storage for NSC (Landhäusser and Lieffers, 2012). Therefore, alternative growing conditions that change NSC concentration may need to be explored, and this would allow for a comprehensive study to test NSC content or concentration as predictors for pine seedling performance under drought.

Shoot water potentials verified that distinct drought treatments were established in the drought experiment, but maintaining these drought treatments proved problematic. Pots were weighed daily to measure water loss from the previous day. However, growth was not accounted for, so seedlings that grew more received less water and were more drought stressed. Seedlings under drought, especially severe drought, received small quantities of water which made it difficult for uniform distribution of moisture over the surface of the potting material. In addition, conditions were variable in the growth chamber which created variability in plant water loss.

Outplanting conditions were not dry enough to truly test the effectiveness of certain seedling characteristics under drought stress, but it did emphasize the importance of aspect and the potential influence of warmer temperatures on seedling growth and physiological responses. Seedlings on the warmer south-facing aspect allocated more growth to aboveground organs, specifically needles. This may make seedlings more susceptible to drought stress by promoting larger transpiring surface area. Studies have observed that warmer air and soil temperatures alter physiological responses of seedlings. For example, a study by Way et al. (2013) explored the influence of growing trembling aspen (*Populus tremuloides* Michx.) at 5 °C above ambient temperatures, and they observed that slightly higher temperatures can alter hydraulic resistance of different organs. The findings from this study suggest that seedlings grown under warmer temperatures are more likely to encounter drought stress. It is unlikely that temperature differences between aspects in our experiment were enough to promote such changes, but it is not known whether such changes seen in aspen would occur in boreal conifers grown in warmer temperatures. Research on aspect and temperature would give a better understanding of how and why some tree species may persist on certain sites under natural conditions. Consequently, this may translate to better planting practices by optimizing the suitability of specific species on certain reforestation or forest reclamation sites.

The mechanism behind hydrogel increasing nutrient availability is not fully understood and requires further investigation. Research intended to better understand how hydrogel increases nutrient availability may have interesting implications in improving specific planting sites that experience nutrient deficiencies.

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Appendix 1

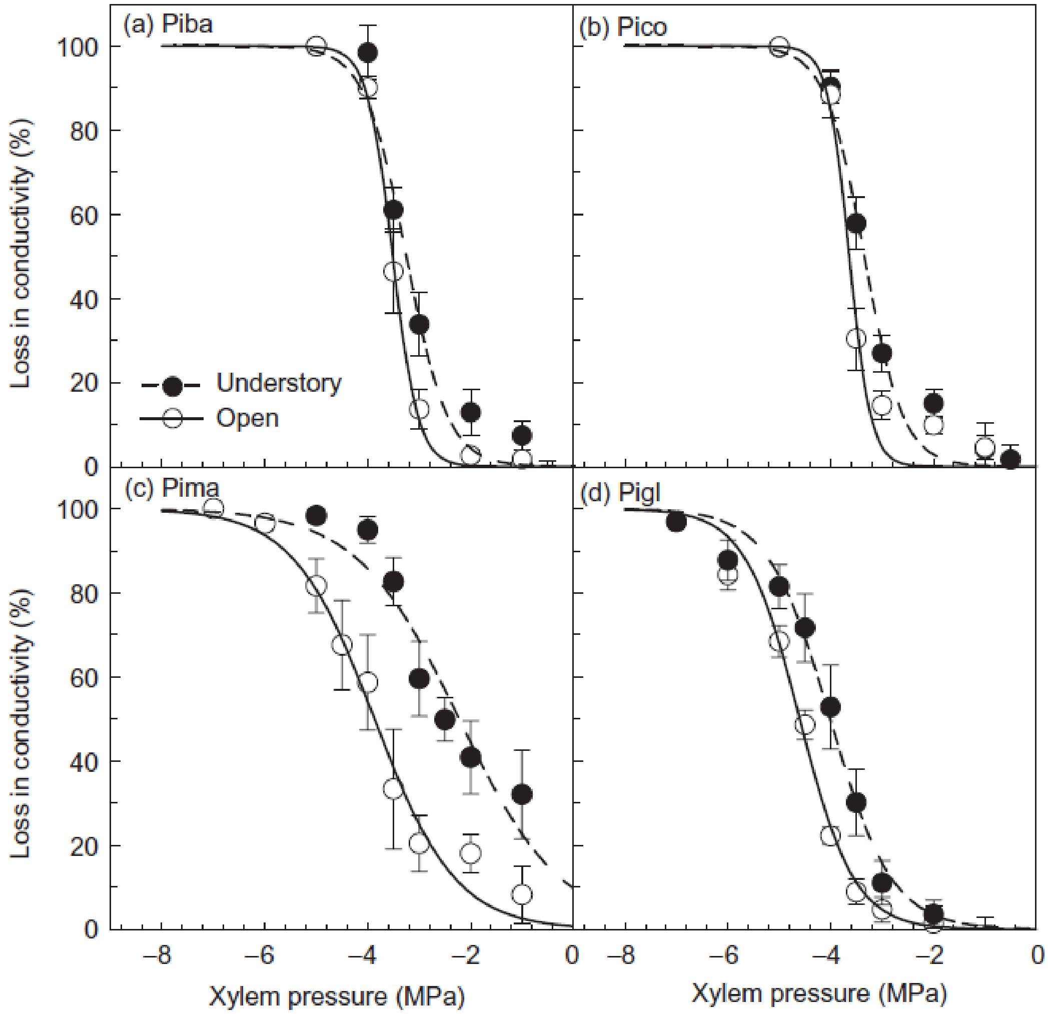

Figure A1.1 Vulnerability curves of the stem xylem tissue of four tree species: (a) Piba (*Pinus banksiana*), (b) Pico (*Pinus contorta*), (c) Pima (*Picea mariana*) and (d) Pigl (*Picea glauca*). These trees were either grown in an understory (understory) or open field (open) conditions. This graph was taken from Schoonmaker et al. (2010).

The van Genutchen model that was used as follows:

$$\theta g = \theta r + \frac{\theta s - \theta r}{[1 + (\alpha|\varphi|)^n]^{1-\frac{1}{n}}}$$

Where

θg is the soil moisture retention curve (g g^{-1})

φ is the soil water potential (cm H_2O)

θs is the saturated gravimetric water content (g g^{-1})

θr is the residual gravimetric water content (g g^{-1})

$\propto$ is the inverse of air entry water potential (cm^{-1} H_2O)

n is the pore-size distribution

All soil water potential values were converted from MPa to cm H_2O, and all gravimetric water contents that were generated from the model were converted to a percentage

$\propto$ = -0.000218 cm^{-1} H_2O

n = 1.85

Appendix 2

Table A2.1 ANOVA results for initial characteristics and the level of significance used is α = 0.05

		Variable	**DF**	**Den DF**	**F**	**P**
Source of Variation	**Stock**	Height (cm)*	2	69	44.38	<0.0001
		Root collar diameter (mm)	2	69	53.78	<0.0001
		Stem mass (g)*	2	69	64.64	<0.0001
		Needle mass (g)	2	67	126.09	<0.0001
		Root mass (g)*	2	69	46.37	<0.0001
		Total mass (g)	2	67	79.56	<0.0001
		RSR (g g^{-1})*	2	67	91.30	<0.0001
		NSC content (g)*	2	42	30.70	<0.0001
		NSC (%)	2	42	2.21	0.1222
		Needle NSC (%)	2	42	6.84	0.0027
		Stem NSC (%)	2	42	10.65	0.0002
		Root NSC (%)	2	42	4.43	0.0180

*ANOVA model that assumes unequal variance was used

Appendix 3

Table A3.1 ANOVA results for growth responses, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Stock	Height growth (cm)*	2	81	3.29	0.0424
		Relative height growth (cm)*	2	81	10.47	<0.0001
		New needle mass (g)*	2	81	4.77	0.0110
		Root growth (g)*	2	79	42.65	<0.0001
		RSR change (g g^{-1})	2	79	72.97	<0.0001
	Drought	Height growth (cm)*	2	81	108.00	<0.0001
		Relative height growth (cm)*	2	81	35.29	<0.0001
		New needle mass (g)*	2	81	180.03	<0.0001
		Root growth (g)*	2	79	110.36	<0.0001
		RSR change (g g^{-1})	2	79	8.05	0.0007
	Stock*Drought	Height growth (cm)*	4	81	8.92	<0.0001
		Relative height growth (cm)*	4	81	1.37	0.2517
		New needle mass (g)*	4	81	6.69	0.0001
		Root growth (g)*	4	79	7.45	<0.0001
		RSR change (g g^{-1})	4	79	4.53	0.0024

*ANOVA model that assumes unequal variance was used

Table A3.2 ANOVA results for shoot water potential, and the level of significance used is α = 0.05

		DF	Den DF	F	P
Source of Variation	**Stock**	2	162	3.39	0.0360
	Drought	2	162	472.70	<0.0001
	Stock*Drought	4	162	5.86	0.0002
	Time	1	162	6.90	0.0095
	Stock*Time	2	162	1.09	0.3384
	Drought*Time	2	162	6.64	0.0017
	Stock*Drought*Time	4	162	0.66	0.6236

*ANOVA model that assumes unequal variance was used

Table A3.3 ANOVA results for stomatal conductance, and the level of significance used is α = 0.05

		DF	Den DF	F	P
Source of Variation	**Stock**	2	77	5.82	0.0044
	Drought	2	77	51.26	<0.0001
	Stock*Drought	4	77	0.95	0.4381

Table A3.4 ANOVA results for final NSC, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	**Stock**	Whole tree soluble sugar (%)	2	79	13.03	<0.0001
		Whole tree starch (%)*	2	79	0.74	0.4798
		Needle soluble sugar (%)*	2	81	3.01	0.0551
		Needle starch (%)*	2	81	2.88	0.0619
		Root soluble sugar (%)	2	79	1.16	0.3177
		Root starch (%)*	2	79	0.88	0.4203
		Change in whole tree NSC content (%)*	2	79	60.50	0.0167
	Drought	Whole tree soluble sugar (%)	2	79	9.43	0.0002
		Whole tree starch (%)*	2	79	79.99	<0.0001
		Needle soluble sugar (%)*	2	81	49.41	<0.0001
		Needle starch (%)*	2	81	24.74	<0.0001
		Root soluble sugar (%)	2	79	53.71	<0.0001
		Root starch (%)*	2	79	105.9	<0.0001
		Change in whole tree NSC content (%)*	2	79	127.5	<0.0001
	Stock*Drought	Whole tree soluble sugar (%)	4	79	1.46	0.2223
		Whole tree starch (%)*	4	79	0.35	0.8467
		Needle soluble sugar (%)*	4	81	1.17	0.3313
		Needle starch (%)*	4	81	0.77	0.5467
		Root soluble sugar (%)	4	79	2.47	0.0511
		Root starch (%)*	4	79	1.36	0.2538
		Change in whole tree NSC content (%)*	4	79	7.97	0.3878

*ANOVA model that assumes unequal variance was used

Appendix 4

Table A4.1 Range of soil measures of forest floor material taken from north and south-facing hummocks in close proximity to plots. Soil samples were taken by NorthWind Land Resources Inc. and sent to Exova Laboratories in Edmonton, AB, Canada for analysis

		Aspect	
Variable	**Unit**	North-facing	South-facing
Available NO_3^-	µg g^{-1}	<2	<2
Available NH_4^+	µg g^{-1}	<0.3-0.6	<0.3-1
Available P	µg g^{-1}	9-10	8-18
Available K	µg g^{-1}	32-56	<25-55
C:N		20	17-20
Total organic C	% dry weight	1.53-1.76	1.3-2
Total N	% dry weight	0.07-0.08	0.07-1
Texture		sand	sand
Sand	% dry weight	88.8-90.8	89.6-94
Silt	% dry weight	5.4-7.2	4.6-9
Clay	% dry weight	3.8-4	<0.1-2.6
pH		6.4-7.2	5.5-6.9

Table A4.2 Aspect, slope, and location of different hydrogel (H), North-facing (N), and South-facing (S) plots

Plot	Aspect (°)	Slope (°)	UTM N	UTM W
H1*	192	32	57 02.503	111 35.355
H2*	177	19	57 02.477	111 35.562
H3*	210	19	57 02.478	111 35.583
H4	221	22	57 02.480	111 35.590
H5*	139	25	57 02.412	111 35.883
H6	144	26	57 02.410	111 35.889
H7*	150	22	57 02.407	111 35.896
H8*	160	23	57 02.401	111 35.912
H9	150	24	57 02.399	111 35.918
H10*	147	20	57 02.397	111 35.922
N1*	33	22	57 02.405	111 35.712
N2	33	23	57 02.404	111 35.712
N3*	33	23	57 02.400	111 35.710
N4*	32	24	57 02.400	111 35.705
N5*	32	24	57 02.400	111 35.706
N6*	37	22	57 02.399	111 35.702
N7	38	23	57 02.397	111 35.697
N8*	38	21	57 02.396	111 35.694
N9	39	26	57 02.395	111 35.692
N10*	39	22	57 02.392	111 35.689
S1*	188	27	57 02.504	111 35.351
S2*	171	15	57 02.475	111 35.560
S3*	208	18	57 02.479	111 35.581
S4	210	23	57 02.479	111 35.587
S5*	135	24	57 02.414	111 35.880
S6	140	21	57 02.411	111 35.885
S7*	149	24	57 02.409	111 35.892
S8*	158	20	57 02.402	111 35.908
S9	151	22	57 02.399	111 35.914
S10*	149	19	57 02.398	111 35.921

*Plots that had 5TM soil moisture and MPS-2 dielectric water potential sensor probes

Appendix 5

Initial Characteristics

Table A5.1 ANOVA results for initial characteristics and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Stock	Height (cm)*	2	69	44.38	<0.0001
		Root collar diameter (mm)	2	69	53.78	<0.0001
		Stem mass (g)*	2	69	64.64	<0.0001
		Needle mass (g)	2	67	126.09	<0.0001
		Root mass (g)*	2	69	46.37	<0.0001
		Total mass (g)	2	67	79.56	<0.0001
		RSR (g g^{-1})*	2	67	91.30	<0.0001
		NSC content (g)*	2	42	30.70	<0.0001
		NSC (%)	2	42	2.21	0.1222
		Needle NSC (%)	2	42	6.84	0.0027
		Stem NSC (%)	2	42	10.65	0.0002
		Root NSC (%)	2	42	4.43	0.0180

*ANOVA model that assumes unequal variance was used

Aspect Effects on Soil

Table A5.2 ANOVA results for soil available nutrients of the aspect analysis, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Aspect	Total N*	1	18	0.02	0.8911
		NO_3^-	1	18	2.21	0.1543
		NH_4^+	1	18	2.79	0.1121
		P	1	18	8.20	0.0103
		K	1	18	13.51	0.0017
		Ca	1	18	28.78	<0.0001
		Mg	1	18	0.55	0.4683
		S*	1	18	8.35	0.0098

*ANOVA model that assumes unequal variance was used

Table A5.3 ANOVA results for soil conditions of the aspect analysis, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Aspect	Soil temperature (°C)	1	663	815.97	<0.0001
		Soil water content (cm^3cm^{-3})	1	663	24.45	<0.0001
		Soil water potential (MPa)	1	603	1.58	0.2096
	Day	Soil temperature (°C)	60	663	324.63	<0.0001
		Soil water content (cm^3cm^{-3})	60	663	9.58	<0.0001
		Soil water potential (MPa)	60	603	5.52	<0.0001
	Aspect* Day	Soil temperature (°C)	60	663	4.74	<0.0001
		Soil water content (cm^3cm^{-3})	60	663	1.71	0.0010
		Soil water potential (MPa)	60	603	4.02	<0.0001

Aspect Effects on Growth and Physiological Responses

Table A5.4 ANOVA results for growth responses of the aspect analysis, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Stock	Height growth (cm)	2	54	8.01	0.0009
		Relative height growth (cm)	2	54	66.20	<0.0001
		New needle mass (g)*	2	54	1.68	0.1961
		Root growth (g)*	2	54	12.23	<0.0001
		RSR change ($g\ g^{-1}$)*	2	54	60.21	<0.0001
	Aspect	Height growth (cm)	1	54	0.02	0.8921
		Relative height growth (cm)	1	54	0.01	0.9222
		New needle mass (g)*	1	54	9.76	0.0029
		Root growth (g)*	1	54	0.19	0.6611
		RSR change ($g\ g^{-1}$)*	1	54	7.46	0.0085
	Stock*Aspect	Height growth (cm)	2	54	0.02	0.9802
		Relative height growth (cm)	2	54	0.20	0.8176
		New needle mass (g)*	2	54	0.07	0.9355
		Root growth (g)*	2	54	0.53	0.5905
		RSR change ($g\ g^{-1}$)*	2	54	0.10	0.9012

*ANOVA model that assumes unequal variance was used

Table A5.5 ANOVA results for stomatal conductance of the aspect analysis, and the level of significance used is α = 0.05

		DF	Den DF	F	P
Source of Variation	**Stock***	2	113	1.38	0.2553
	Aspect*	1	113	14.10	0.0003
	Stock*Aspect*	2	113	0.45	0.6410

*ANOVA model that assumes unequal variance was used

Table A5.6 ANOVA results for shoot water potential of the aspect analysis, and the level of significance used is α = 0.05

		DF	Den DF	F	P
Source of Variation	**Stock***	2	35	0.23	0.7920
	Aspect*	1	35	2.89	0.0982
	Stock*Aspect*	2	35	0.22	0.8031

*ANOVA model that assumes unequal variance was used

Hydrogel Effects on Soil

Table A5.7 ANOVA results for soil available nutrients of the soil amendment analysis, and the level of significance used is α = 0.05

		Variable	DF	Den DF	F	P
Source of Variation	Aspect	Total N	1	9	12.06	0.0070
		NO_3^-	1	9	13.54	0.0052
		NH_4^+	1	9	0.19	0.6763
		P	1	17	13.72	0.0018
		K*	1	17	63.89	<0.0001
		Ca	1	9	2.90	0.1227
		Mg	1	9	23.39	0.0009
		S	1	17	3.83	0.0671

*ANOVA model that assumes unequal variance was used

Table A5.8 ANOVA results for soil conditions of the soil amendment analysis, and the level of significance used is $\alpha = 0.05$

		Variable	DF	Den DF	F	P
Source of Variation	**Treatment**	Soil temperature (°C)	1	665	56.66	<0.0001
		Soil water content (cm^3cm^{-3})	1	665	4.62	0.0320
		Soil water potential (MPa)	1	544	0.37	0.5431
	Day	Soil temperature (°C)	60	665	198.39	<0.0001
		Soil water content (cm^3cm^{-3})	60	665	7.02	<0.0001
		Soil water potential (MPa)	60	544	14.08	<0.0001
	Treatment *Day	Soil temperature (°C)	60	665	3.09	<0.0001
		Soil water content (cm^3cm^{-3})	60	665	0.84	0.7916
		Soil water potential (MPa)	60	544	2.27	<0.0001

Hydrogel Effects on Growth and Physiological Responses

Table A5.9 ANOVA results for growth responses of the soil amendment analysis, and the level of significance used is α = 0.05

		Variable	**DF**	**Den DF**	**F**	**P**
Source of Variation	**Stock**	Height growth (cm)	2	36	5.03	0.0118
		Relative height growth (cm)*	2	36	38.98	<0.0001
		Stem growth (g)*	2	36	15.02	<0.0001
		New needle mass (g)*	2	36	0.45	0.6415
		Root growth (g)	2	36	29.62	<0.0001
		RSR change (g g^{-1})*	2	36	78.58	<0.0001
	Treatment	Height growth (cm)	1	9	1.70	0.2241
		Relative height growth (cm)*	1	9	1.02	0.3396
		Stem growth (g)*	1	9	3.61	0.2290
		New needle mass (g)*	1	9	0.59	0.4605
		Root growth (g)	1	9	0.30	0.5986
		RSR change (g g^{-1})*	1	9	0.18	0.6792
	Stock*Treatment	Height growth (cm)	2	36	1.14	0.3315
		Relative height growth (cm)*	2	36	0.31	0.7385
		Stem growth (g)*	2	36	3.61	0.0374
		New needle mass (g)*	2	36	1.61	0.2140
		Root growth (g)	2	36	1.77	0.1856
		RSR change (g g^{-1})*	2	36	1.72	0.1936

*ANOVA model that assumes unequal variance was used

Table A5. 10 ANOVA results for stomatal conductance of the soil amendment analysis, and the level of significance used is α = 0.05

		DF	**Den DF**	**F**	**P**
Source of Variation	**Stock**	2	90	0.97	0.3838
	Treatment	1	23	0.04	0.8414
	Stock*Treatment	2	90	0.79	0.4554

Table A5.11 ANOVA results for shoot water potential of the soil amendment analysis, and the level of significance used is α = 0.05

		DF	Den DF	F	P
Source of Variation	**Stock**	2	28	2.22	0.1270
	Treatment	1	7	0.02	0.8857
	Stock*Treatment	2	28	0.30	0.7436