Evaluating trembling aspen (*Populus tremuloides* Michx.) seedling stock characteristics in response to drought and out-planting on a reclamation site

by

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Abstract

Trembling aspen (*Populus tremuloides* Michx.) seedlings often display reduced growth, or transplant shock, following out-planting largely due to moisture constraints. This thesis explores the influence of seedling size (root volume), root to stem ratio (RSR) and non-structural carbohydrate (NSC) reserves on the growth performance and physiological status of aspen seedlings under varying levels of drought stress in a controlled growth chamber and following out-planting on a reclamation site. These characteristics have been found to improve out-planting success in aspen and may also increase drought tolerance. In the growth chamber study, stem growth and foliar development was reduced under drought, but the degree growth decreased was greatly influenced by initial seedling characteristics. Small seedlings with high RSR displayed the greatest stem growth and leaf area under drought stress, while large seedlings with low RSR had the poorest shoot growth. Similarly, high initial RSR resulted in the greatest above-ground growth performance in seedlings after out-planting. Root growth was sensitive to environmental factors in the growth chamber and on the reclamation site, but was not influenced by initial characteristics.

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Chapter 1: Introduction

1.1 The boreal forest and trembling aspen

The boreal forest biome represents the largest in tact forested area within Canada, and covers approximately one third of its total land area (Brandt 2009). The climate of this region is defined by its long, cold winters and short, cool summers (Bonan and Shugart 1989). Plant growth in this region is predominantly limited by cold air temperatures; cold temperatures also contribute to slow decomposition rates and nutrient cycling, resulting in low nutrient availability, particularly nitrogen, across much of this region (Vitousek and Howarth 1991; Lupi et al. 2013).

Trembling aspen (*Populus tremuloides* Michx.) is perhaps the most common and wide-spread deciduous tree species in the boreal biome, particularly within the boreal mixedwood forest zone of central Alberta, Canada (Rowe 1972). Aspen is considered a pioneer species, and it is typically found in early-successional stands mixed with other deciduous species such as paper birch (*Betula papyrifera* Marsh) and balsam poplar (*Populus balsamifera* L.), as well as interspersed with conifer species such as white spruce (*Picea glauca* Moench) and *Pinus* sp. (Rowe 1972). Relative to coniferous species and other deciduous species native to this region, aspen leaves decompose at a faster rate under cool climatic conditions, which can greatly influence nutrient turnover in soils and improve site conditions for plant establishment (Bockhheim et al. 1991; Prescott et al. 2004). While this species is able to naturally reproduce by seed (Landhäusser et al. 2010), it predominantly reproduces vegetatively via root suckering after severe disturbance events such as fire or drought have killed the above-ground portion of the tree (Lieffers et al. 2001; Frey et al. 2003). Aspen is considered relatively tolerant to moisture stress in relation to other native boreal tree species, and as such is well-adapted to persist across a large breadth of harsh sites with disturbance legacies that include periodic fires and drought (Lieffers et al. 2001; Frey et al. 2003). Because of this species' high resilience to disturbances, relatively high drought tolerance and fast early growth rates, aspen can be considered an ideal candidate for reforestation on harsh, disturbance-prone reclamation sites within this region (Macdonald et al. 2012).

1.2 Boreal forest reclamation

The boreal mixedwood zone of central Alberta is host to a richness of natural resources, including wood, coal and oil. Because of this, this region has recently experienced unprecedented growth in resource extraction, which has resulted in heavy anthropogenic disturbance across much of the landscape. As some of these resources, such as oil and coal, are housed below-ground, open pit surface mining is necessary to excavate them. In the past, natural fires shaped this landscape creating a mosaic of different forest assemblages in this region and allowed for species to develop adaptations to this disturbance regime; however, surface mining entails the stripping of all vegetation, soil and subsoil from the landscape and the mixture of soil horizons during the soil salvaging process, which may be more characteristic of natural disturbances such as landslides or flooding events. As boreal tree species have not evolved and adapted to these types of large-scale disturbance events, the reestablishment of previously existing forest ecosystems can be challenging.

In Alberta, legal regulations require all operators to reclaim mined lands with the goal of "returning it to an equivalent land capability" using naturally occurring vegetation (Cumulative Environmental Management Association 2009). Under this framework, the land use of a specific reclamation site must be similar to the ability that existed prior to disturbance, though it may not necessarily be identical. Overall, the goal of reclamation is to achieve a self-supporting ecosystem similar to the state that existed pre-disturbance.

The reclamation of mine sites involves many lengthy, challenging steps, the first of which is the creation of landform structures. Overburden generated during the mining process is first deposited, which results in the creation of hill slope landforms that vary in exposure, slope and hydrology (Leatherdale et al. 2012). Overburden is then capped with subsoil to aid in plant rooting, with the depth of subsoil used being dependent on the salinity and pH of the overburden material (Rowland et al. 2009). The final step in soil reconstruction involves the placement of salvaged organic topsoil, such as peat mineral mix (PMM) or forest floor material (FFM), over the subsoil material to improve soil water holding capacity and nutrient availability. PMM is salvaged from lowland forest, bog and fen donor sites, while FFM contains a mixture of salvaged organic forest floor topsoil and the underlying mineral soil (A and B soil horizons).

The next step following soil reconstruction is the re-establishment of native vegetation, which often includes the re-introduction of prominent tree species such as trembling aspen. While aspen is able to establish from seed on reclamation areas (Schott et al. 2014), certain sites may not be conducive to germination and growth due to nutrient limitations or unfavorable microsite conditions (Wolken et al. 2010; Pinno et al. 2012; Schott et al. 2014). As such, nursery-grown, containerized seedlings are typically favored for out-planting on reclamation sites to initiate the reforestation process (Macdonald et al. 2012). Aspen seedling stock can be created several ways, including from seed and from root cuttings; however, seedlings germinated from seed typically exhibit greater genetic diversity, are better able to establish new root systems and are more easily grown at a large scale than from cuttings (Snedden et al. 2010). Thus, aspen stock produced from seed may be preferred for use in reclamation.

However, harsh site conditions can induce several years of slow growth, or transplant shock, in out-planted aspen seedlings (Van den Driessche et al. 2003; Martens et al. 2007; Landhäusser et al. 2012b). In particular, drought stress is widely considered one of the main factors driving transplant shock following out-planting (Burdett 1990; Haase and Rose 1993; Close et al. 2005). Moisture deficits are often a product of low root permeability and poor soil-root contact following planting (Sands 1984; Radoglou and Raftoyannis 2002; Seifert et al. 2006), which can severely inhibit a seedling's ability to uptake water to satiate the hydraulic demands of the above-ground portion of the plant (Thompson and Schultz 1995; Jacobs et al. 2009). Drought stress may be also be exacerbated on reclamation sites, as the lack of vegetative cover during the early stages of reclamation can result in high vapor pressure deficits (VPD), which can lead to transpirational water loss in planted seedlings (Groot et al. 1997). Much like low soil moisture availability, high VPD can result in internal water deficits in out-planted seedlings.

As such, it is important to produce high quality seedlings that possess morphological and physiological characteristics conducive to growth and establishment success on harsh, drought-prone sites. While there is a large amount of literature assessing seedling quality in conifer (Rose 1990; Mattsson 1996; Grossnickle 2005) and Eastern hardwood (Thompson and Schultz 1995; Jacobs et al. 2005; Davis and Jacobs 2005) seedlings, relatively little research has been conducted in identifying aspen stock characteristics that improve seedling quality and growth following out-planting, and to our knowledge none has assessed aspen stock performance during periods of drought stress. Therefore, it is important to investigate characteristics and traits that may improve the quality and growth performance of seedlings out-planted on droughted reclamation areas.

1.3 Physiological responses to drought

1.3.1 Stomatal Regulation

In order to maintain a proper internal water balance and to prevent hydraulic failure, trees often limit water loss via stomatal regulation. Most trees exhibit either a drought tolerance (anisohydric) or avoidance (isohydric) strategy of stomatal regulation (Turner 1986; Chaves et al. 2003; McDowell et al. 2008). Species with an anisohydric drought response strategy are able to maintain high stomatal conductance rates during both severe and prolonged periods of drought stress, and are able to withstand greater hydraulic tensions in stem and leaf tissues (more negative water potentials) when exposed to low soil water potentials and high evapotranspirational demands (McDowell et al. 2008). In contrast, species exhibiting an isohydric drought response strategy, such as aspen, have high levels of stomatal regulation. When soil water potential is low, isohydric species will close their stomata in order to minimize transpirational water loss, as well as to maintain adequate tissue hydration to prevent root and shoot organ dessication (Tardieu and Simonneau 1998; Chaves et al. 2003; Galvez et al. 2011). As a result, species with tight stomatal regulation exhibit relatively stable shoot water potentials, despite decreases in soil water potential and moisture availability. However, stomatal closure comes at the cost of reduced photosynthesis (Cowan and Farquhar 1977; Farquhar and Sharkey 1982).

1.3.2 Osmotic adjustment

Soluble sugars may also aid in tree survival under drought stress through osmotic adjustment. During this process, solutes are accumulated in tissues to maintain cellular turgor, which may prevent tissue desiccation and allow proper metabolic functioning to persist at low water potentials (Hsiao et al. 1976; Chaves 1991; Chaves et al. 2002; Close et al. 2005). While osmotic adjustment is not observed in all species, it has been observed in other poplars (*Populus* sp.) (Gebre et al. 1998) and suggested in aspen (Galvez et al. 2013).

1.4 Morphological responses to drought

1.4.1 Leaf and branch abscission

In addition to stomatal regulation, trees may reduce transpirational demands and water loss by altering their shoot morphology. Short-term responses to severe or prolonged periods of drought stress include curling or wilting of leaves, reduced leaf size, and decreased crown leaf area (Struve and Joly 1992; Chaves et al. 2003; McDowell et al. 2008). Galvez et al. (2011) observed that aspen seedlings began to shed mature leaves after only two weeks of severe drought stress, and crown leaf area decreased by 53 %. By reducing leaf area, plants limit the amount of water lost via transpiration, which can lessen tissue dehydration and xylem cavitation at low water potentials (Chaves et al. 1991; Tardieu and Simonneau 1998; McDowell et al. 2008; Lu et al. 2010). In more extreme cases of severe and/or prolonged drought, some poplars (*Populus deltoides* W. Batram ex Marshall and *Populus fremontii* S. Wats.) have been known to sacrifice full branches to further limit transpirational water loss (Rood et al. 2000). While these responses may severely inhibit photosynthesis and carbon assimilation, they have been found to significantly improve the water status of the remaining root and shoot organs (Tyree and Sperry 1989; McDowell et al. 2008). As aspen readily suckers following above-ground disturbances, this species is able to reestablish following full shoot abscission, provided that the parent root system remains intact (Lieffers et al. 2001; Frey et al. 2003). Therefore, aspen may be able to “sacrifice” shoot tissues during severe moisture deficits in order to preserve their root systems for future clonal ramet growth.

1.4.2 Root growth

Another adaptation that plants have evolved to mitigate drought stress is increasing growth allocation to root tissues relative to shoot tissues, which can thus result in an increased root to shoot ratio (RSR) (Jacobs et al. 2009). RSR represents the balance between water absorbing area (roots) to transpirational area (shoots, including leaves) (Haase et al. 2008), and it has been well documented that seedlings with high RSR are better able to persist on droughted sites due to higher soil to root contact and greater access to soil water resources (Lloret et al. 1999; Chaves et al. 2002; Grossnickle 2005). High RSR can improve hydraulic conductivity in root and shoot tissues and overall water status in seedlings (Sperry et al. 2002), which in turn may allow for greater shoot growth and foliar development.

Many studies have indicated that high RSR and root growth allocation play a large role in reducing transplant shock in droughted seedlings. For example, red oak (*Quercus rubra* L.) seedlings that experienced high root growth allocation had higher stomatal conductance and photosynthetic assimilation and had less negative shoot water potentials when grown under drought conditions in a glasshouse study (Jacobs et al. 2009), while in a Mediterranean field study holm oak (*Quercus ilex* subsp. *Ballota* Desf) seedlings with high RSR had higher stomatal conductance and tissue water content (Leiva and Fernandez –Ales 1998). In separate field studies conducted by Martens et al. (2007) and Landhäusser et al. (2012b), it was found that aspen seedlings with high initial RSR out-planted onto reclamation sites in Fort McMurray had greater root, height and leaf growth and survivorship than other seedling stock types, though conditions were not moisture-limited. The results of these studies suggest that high root growth allocation and RSR may be effective drought avoidance mechanisms in planted seedlings.

1.5 Other characteristics influencing drought tolerance

1.5.1 Non-structural carbohydrates

Due to decreased carbon acquisition following exposure to drought stress, seedlings may have to rely on carbon reserves such as non-structural carbohydrates (NSC), which are composed of starch and water-soluble sugars, for seedling growth, respiration and tissue maintenance. NSC can act as a readily available carbon pool for growth and metabolic functions during periods of moisture limitation when photosynthesis becomes limited (Kozlowski 1992), and may be used to initiate growth and foliar development following bud break (Struve and Joly 1992; Landhäusser and Lieffers 2002). For example, recent research has indicated that seedlings out-planted with high tissue NSC concentrations were able to develop a greater number of fine and lateral roots in a number of species, including Douglas fir (*Pseudotsuga menziesii* Mirb.)

(Grossnickle 2005) and red oak (Davis and Jacobs 2005), while a study conducted by Canham et al. (1999) observed greater seedling survivorship in red oak seedlings with high root NSC content following a severe disturbance that limited photosynthesis (Canham et al. 1999). Similar results have been observed in aspen seedlings. For example, height growth and root expansion was greater in aspen seedlings that had accumulated greater NSC reserves in the previous growing season (Martens et al. 2007; Snedden et al. 2010; Landhäusser et al. 2012b). Based on these findings, it is possible that high NSC reserves may improve aspen seedling performance under drought stress.

1.5.2 Seedling size

There has been a long-held belief that seedling size (tall with high root collar diameter, RCD) is the best predictor of out-planting success under stressful conditions in boreal tree species (Thompson 1985; Mexal and Landis 1990; Pinto et al. 2011). Large seedlings (particularly with high RCD) typically possess greater root volume (Sands 1984; Mexal and Landis 1990) and have high root growth capacity (Ritchie and Dunlap 1980; Rose et al. 1990; Haase and Rose 1993; Villar-Salvador et al. 2012), resulting in an increased ability to access soil water resources. High root volume has been correlated with increased shoot growth and foliar development across a number of tree species (Burdett 1990; Haase 2008), including Douglas fir (Haase and Rose 1993; Haase 2008), loblolly pine (*Pinus taeda* L.) (Rose et al. 1990), and red oak (Thompson and Schultz 1995; Jacobs et al. 2005). However, transpirational demands may be high in these seedlings, as seedlings with large root systems often possess large shoots with high leaf area (Sands 1984); as such, it is possible that transpirational water loss associated with large shoot size may offset these seedling’s relatively high water uptake ability under severe drought or high VPD, resulting in internal water deficits (Sands 1984). For example, in a glasshouse study, Jacobs et al. (2009) found that tall red oak seedlings produced more leaf area and subsequently had lower stomatal conductance and more severe leaf water deficits than smaller seedlings when exposed to severe drought, despite possessing root systems with much larger volume. As such, high root volume alone may not be able to sufficiently alleviate transplant shock in droughted seedlings.

1.6 Seedling quality

In order to reduce transplant shock in aspen seedlings out-planted on reclamation sites, it is important to develop high quality seedling stock types with characteristics that are suitable for establishment under moisture limitation (Rose et al. 1990; Rose and Haase 1995; Landis 2003). Seedling quality can best be defined as “fitness for purpose” (Ritchie 1984). Under this definition, a high quality seedling can be considered such if it possesses physiological or morphological characteristics that result in high growth rates and survivorship following out-planting (Mexal and Landis 1990; Landis and Dumroese 2006); therefore, a seedling stock type cannot be described as “high quality” until its performance has been quantitatively evaluated following out-planting. Over the last several decades, research into developing seedling stock types specific to different reforestation prescriptions has expanded under the “Target Seedling Concept” (TSC) (Rose et al. 1990; Rose and Haase 1995; Landis 2003; Landis and Dumroese 2006). Using the TSC framework, seedling characteristics are quantitatively linked to reforestation success on specific target sites. The TSC dictates that there is no all-purpose seedling stock type, but rather specific stock types with unique characteristics that may be better suited for growth on specific sites (Rose et al.

1990; Rose and Haase 1995; Landis 2003; Landis and Dumroese 2006). As drought stress is considered the main factor influencing poor seedling growth and establishment on boreal reclamation sites, it is important to evaluate and field test different seedling stocks in the context of drought stress in order to determine which characteristics are most beneficial in improving seedling quality.

Current nursery practices for aspen stock types focus on the production of seedlings with characteristics traditionally used to assess conifer quality, such as height and root collar diameter (Chavasse 1980; Thompson 1985). However, out-planting success on stressful sites has been mixed, which suggests that these characteristics may not be beneficial for assessing quality under drought stress. Recent studies suggest that aspen seedling stock with characteristics such as high RSR and NSC reserves may be better predictors of quality and growth performance on dry reclamation sites in North-central Alberta (Martens et al. 2007; Snedden et al. 2010; Landhäusser et al. 2012b). However, little is known about the role of RSR and NSC in relation to the growth and physiological performance of aspen seedlings under severe drought conditions.

1.7 Research outline/objectives

The aim of my research was to determine how initial seedling characteristics (RSR, NSC content and concentration, and seedling size (root volume and shoot size)) influence aspen seedling growth performance and water status under drought stress. In Chapter 2, I explored this topic by exposing three different aspen seedling stocks with different characteristics to varying levels of simulated drought stress in a controlled growth chamber study. In Chapter 3, I explored the influence of hill slope and aspect on drought conditions on a reclaimed mine site. Similarly, I aimed to determine which seedling stock was best suited for out-planting onto drought-prone reclamation sites by examining growth and physiological responses of seedlings planted on different aspects in a field study. In a separate study, I also investigated whether soil amendments such as hydrogels were beneficial to seedling establishment and growth when applied to droughted reclamation sites.

Chapter 2: Evaluating *Populus tremuloides* Michx. seedling stock characteristics in response to drought stress

2.1 Introduction

Reforestation projects require the production of high quality seedling stock with characteristics that are correlated with high survivorship and growth rates following planting (Rose et al. 1990; Rose and Haase 1995; Landis 2003). However, it is often difficult to determine seedling quality, as it is species specific and can be greatly influenced by planting site conditions (Ritchie 1984; Burdett 1990; Rose et al. 1990). Site characteristics at the time of planting play a pivotal role in assessing seedling quality, as stressful conditions often cause depressed root, shoot and foliar growth rates, or transplant shock, in planted seedlings (Haase and Rose 1993; Struve and Joly 1992; Davis and Jacobs 2005). Drought stress is thought to be one of the most common causes of transplant shock, and is perhaps the main factor driving seedling quality following planting (Burdett 1990; Haase and Rose 1993; Jacobs et al. 2009; Close et al. 2005). Drought stress often is a result of poor soil root contact (Sands 1984; Radoglou and Raftoyannis 2002; Seifert et al. 2006), which can severely inhibit a seedling's ability to uptake water to satiate the hydraulic demands of the above-ground portion of the plant (Thompson and Schultz 1995; Jacobs et al. 2009). Therefore, it is important to determine which characteristics improve drought tolerance and seedling quality on harsh, droughted sites.

Current research suggests that characteristics such as high root volume, root to shoot ratio (RSR) and non-structural carbohydrate (NSC) reserves improve seedling performance under drought stress (Jacobs et al. 2005; Martens et al. 2007; Jacobs et al.

2009; Snedden et al. 2010; Landhäusser et al. 2012b). Seedlings with large volume have greater root growth potential and have more surface area to access soil water (Ritchie and Dunlap 1980; Rose et al. 1990; Haase and Rose 1993), which has been correlated with improved shoot growth and foliar development in a number of species, including: loblolly pine (*Pinus taeda* L.) (Rose et al. 1990), Douglas fir (*Pseudotsuga menziesii* Mirb.) (Haase and Rose 1993; Haase 2008), and red oak (*Quercus rubra* L.) (Thompson and Schultz 1995; Jacobs et al. 2005). Similarly, seedlings with high RSR are better able to balance transpirational demands with water uptake (Chaves et al. 2002; Grossnickle 2005; Haase 2008), and have greater hydraulic conductivity in tissues (Sperry et al. 2002); thus, high RSR may improve seedling water status under drought stress, and may allow for greater growth and foliar development. High NSC reserves may also improve seedling quality under moisture stress by acting as a carbon pool to initiate growth when photosynthesis is limited by drought (Struve and Joly 1992; Landhäusser and Lieffers 2002). For example, conifer and red oak seedlings with high NSC reserves were able to develop more lateral roots, which subsequently improved water uptake following planting and reduced transplant shock (Davis and Jacobs 2005; Grossnickle 2005). A study by Canham et al. (1999) also found that red oak seedlings with high root NSC content had greater survivorship following disturbance events that limited photosynthesis, which could correlate to greater seedling survival and establishment following drought.

Trembling aspen (*Populus tremuloides* Michx.) is an early-successional deciduous tree species that is widely distributed across the boreal forest region of North America (Rowe1972). Aspen is considered fast-growing and relatively drought tolerant compared to other boreal tree species (Lieffers et al. 2001), making it an ideal candidate species for reforestation on harsh, disturbed sites in this region (Macdonald et al. 2012). However, the establishment success of planted aspen seedlings has been limited and seedlings often display depressed growth rates for several years following out-planting on stressful, moisture-limited sites (Van den Driessche et al. 2003; Martens et al. 2007). This indicates that characteristics currently used to evaluate aspen planting stock quality, such as height and root collar diameter (Hallman et al. 1978; Chavasse 1980; Thompson 1985), are not beneficial for assessing performance under drought stress.

Currently, research into characteristics that improve seedling performance, including root volume, is generally centred on coniferous species (Rose 1990; Mattsson 1996; Grossnickle 2005) and Eastern hardwoods (Thompson and Schultz 1995; Jacobs et al. 2005; Davis and Jacobs 2005), and no research has been conducted linking root system size to aspen seedling quality under drought stress. Despite this bias, recent research has indicated that characteristics such as high root to shoot ratio (RSR) and non-structural carbohydrate reserves (NSC) improve aspen seedling quality and growth performance following planting in non-drought stressed conditions (Martens et al. 2007; Snedden et al. 2010; Landhäusser et al. 2012b). However, further study is necessary to test whether these characteristics (high RSR and high NSC) improve growth performance in aspen during periods of drought.

In a controlled growth chamber study, I explored how characteristics such as tissue NSC concentration/content, root to stem ratio, and seedling size (height, root system) of dormant aspen seedlings influence growth and drought tolerance.

2.2 Material and Methods

2.2.1 Seedling stock type production

The trembling aspen seedling stock used for the drought experiment was grown at the Crop Diversification Centre North in Edmonton, Alberta (53° 64’ 28.27”, -113° 36’ 13.19”) from an open-pollinated seed source collected in the Fort McMurray, Alberta area (56° 73’ 50.25”, -111° 38’ 01.91”). 192 aspen seedlings were grown in 615A styroblocks (5 total; Beaver Plastics Ltd., Alberta, Canada), which housed 45 cavities containing one seedling each. Each cavity measured 60 mm in diameter and 150 mm depth (340 mL volume). Cavities were filled with a mixture of 10 % perlite, 90 % *Sphagnum* sp. peat moss (Rich Grow, Sungro Horticulture Ltd., British Columbia, Canada) and treated with lime and soap to increase pH levels and water absorption capacity. Cavities were watered to field capacity, seeded and covered with plastic sheathing. Germination occurred after one week. Germinants were regularly misted and kept at soil field capacity (approximately 0.6 g g^{-1} gravimetric soil water content). After two weeks, when the first mature pair of leaves was expanded, seedlings were given a single fertilization of 2 g L^{-1} 5-15-5 (N-P-K) containing chelated micronutrients (Plants Products Co. Ltd., Ontario, Canada). Similarly, a single fertilization event occurred during the third week using a 1 g L^{-1} 10-52-10 NPK fertilizer (Plants Products Co. Ltd., Ontario, Canada) in order to stimulate root growth. After initial establishment (four weeks), seedlings were fertilized twice weekly using a nursery blend of liquid 91-77-161 N-P-K water soluble fertilizer with chelated micronutrients (Smoky Lake Nursery Ltd., Alberta, Canada).

After 5 weeks, when seedlings had reached approximately 20 cm height, seedlings were separated into three groups and exposed to different growing conditions known to create different seedling characteristics (Landhäusser et al. 2012a) (Figure 1). These stock types included: (1) a seedling with *highNSC* reserves*,* (2) a seedling with *highRSR,* and (3) a *large* seedling. To produce the first stock type (*highNSC*), seedlings were grown under greenhouse conditions (50 % relative humidity, 16-17 hours of light, ambient greenhouse temperature) for a total of 15 weeks following germination and subjected to a 5 g L^{-1} application of a paclobutrazol-based shoot growth inhibitor ("Bonzi," 0.4 % paclobutrazol; Evergro Canada Inc., British Columbia, Canada) during the seventh growing week. These seedlings were subsequently transferred outdoors for hardening (Figure 1). The second stock type (*highRSR*) was established under the aforementioned greenhouse conditions, but was transferred outdoors after five growing weeks. The final seedling stock used in the experiment, *large*, was established under the same greenhouse conditions as the *highNSC* stock type, but was not treated with a stem growth inhibitor and was grown under greenhouse conditions for 17 weeks. All three stock types were lifted 27 weeks after germination and packaged into plastic bags and waxed cardboard boxes, whereupon they were placed into frozen storage (-2 °C) until the commencement of the experiment in February, 2012.

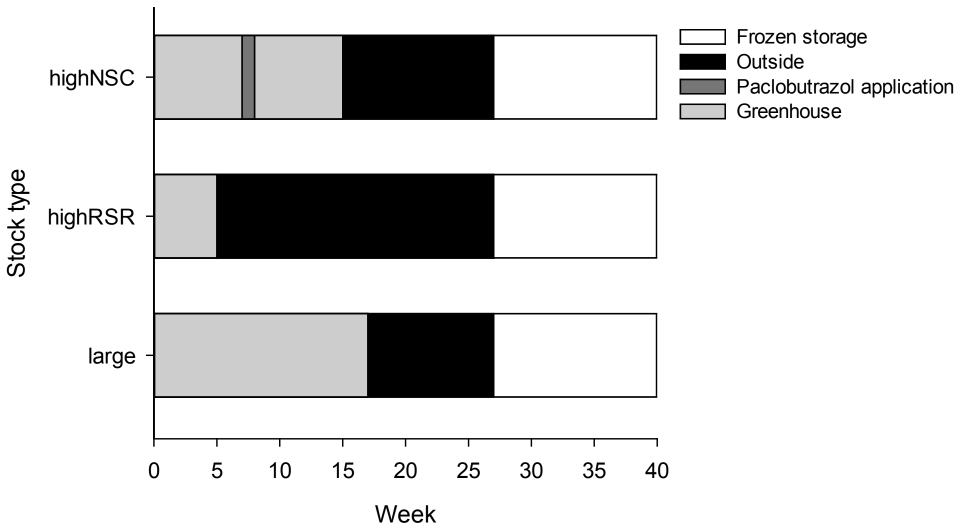

Figure 1. Timeline of growing conditions and treatment applications for aspen seedling stock types used in the drought study.

2.2.1.1 Initial seedling characteristics

To characterize the three stock types, 24 seedlings of each stock type were randomly collected and measured to determine initial seedling characteristics. Stem height and root collar diameter (RCD) were measured; stems were then cut at the root collar and placed in the oven at 70 °C for 72 hours in order to determine dry stem mass. To remove the soil from the root plug, the roots were gently washed in cold water. After removing excess moisture from the root systems, root volume (mL^3) was measured on the digital balance using the water displacement method (Archimedes' principle) (Burdett 1979). Root tissues were also placed in a drying oven at 70 °C for 72 hours to measure dry root mass. Root to stem ratio (RSR, without leaves) was then calculated from dry root and stem mass values for each seedling.

In addition to morphological measurements, initial non-structural carbohydrate (NSC) reserve status in the stems and roots of 10 randomly chosen seedlings of each stock type were determined. Dried stems and roots were ground to pass through #40 mesh (0.4 mm) using a Wiley Mini-Mill (Thomas Scientific, New Jersey, U.S.A.). Starch and soluble sugar concentrations were determined for each organ following the method described in Chow and Landhäusser (2004). Briefly, this method entailed the following: ground stems and roots were placed in an 80% ethanol solution at 95 ^{o}C, and water soluble sugars were extracted three times. Soluble sugar concentration was then measured colorimetrically after the extract was mixed into phenol-sulfuric acid. The remaining pellet was then digested using α-amylase and amyloglucosidase enzymes, and starch concentrations were colorimetrically measured after the reagent peroxidase-glucose oxidase/*o*-dianisidine was added to the solution. Based on organ dry mass and starch and sugar concentrations, total NSC concentration and NSC content were calculated for each tissue type, as well as at the whole seedling level.

Relative to the other stock types, initial measurements indicated that the *highNSC* stock type was characterized by being medium sized in height, total mass and RSR (32.3 cm, 3.9 g and 3.2 g g^{-1}, respectively); however, it had a high root volume with both the highest total seedling NSC content (1.20 g) and concentration (29.6 %) of the three stock types (Table 1). The *highRSR* stock type was differentiated from the two other stock types by having the highest RSR (4.9 g g^{-1}), despite being the smallest in terms of height and mass. This stock type also had high NSC concentration (27.0 %), even though it had the lowest NSC content overall (Table 1). The *large* stock type was characteristically tall with high root and stem mass, high root volume, low RSR, and low total NSC reserves (concentration) in relation to the other two stock types (Table 1).

Table 1. Initial morphological (height, tissue masses and RSR; n=24) and carbohydrate reserve (n=10) characteristics (average ± SE) of the three trembling aspen seedling stock types prior to the drought experiment. Different letters indicate differences among the three stock types.

Initial seedling conditions	**Stock type**		
	highNSC	*highRSR*	*large*
Height (cm)	32.3 ± 0.8 **b**	15.9 ± 0.6 **c**	51.8 ± 1.3 **a**
Root collar diameter (mm)	3.9 ± 0.1 **b**	3.0 ± 0.1 **c**	4.8 ± 0.1 **a**
Stem mass (g)	0.9 ± 0.04 **b**	0.3 ± 0.01 **c**	1.9± 0.1 **a**
Root volume (mL^3)	7.8 ± 0.4 **a**	4.9 ± 0.4 **b**	8.0 ± 0.6 **a**
Root mass (g)	2.9 ± 0.1 **a**	1.6 ± 0.1 **b**	2.9 ± 0.2 **a**
Total mass (g)	3.9 ± 0.1 **b**	2.0 ± 0.1 **c**	4.8 ± 0.2 **a**
RSR (g g^{-1})	3.2 ± 0.1 **b**	4.9 ± 0.2 **a**	1.6 ± 0.1 **c**
Total seedling NSC (g)	1.20 ± 0.15 **a**	0.54 ± 0.20 **c**	0.90 ± 0.17 **b**
Total seedling NSC (%)	29.6 ± 0.8 **a**	27.0 ± 1.2 **a**	19.7 ± 1.1 **b**

2.2.2 Growth chamber conditions

The drought experiment was initiated on February 8, 2012 and lasted for 16 weeks. Seedling were removed from frozen storage one week prior to the experiment and slowly thawed in a refrigerator (4 °C). Seedlings were then planted in square pots (13.7 cm x 13.7 cm; 15.6 cm deep; 2 L volume). The planting medium was a 2:1:1 mixture by volume of peat moss (Pro-Moss, Premier Tech Horticulture, Québec, Canada), vermiculite (Grace Specialty Vermiculite, Grace Construction Products, British

Columbia, Canada), and turface clay (MVP, Profile Products LLC, Illinois, U.S.A.). Water was added to the substrate to bring its gravimetric soil water content to 74 %. Prior to adding the water, it had been blended with a 2 g L^{-1} of 10-52-10 N-P-K fertilizer mix with chelated micronutrients to minimize nutrient limitations during the experimental period. In order to accurately assess the water status and to assure similar soil bulk density of each individual pot, the same mass (1000 g ± 11.9 SD) of substrate was added to each pot and compacted to the same volume.

During the drought, experimental seedlings were transferred into a growth chamber where growing conditions were kept constant throughout the course of the experiment; the growth chamber had an average daytime/nighttime temperature of 20.5 °C (± 1.6 SD)/18°C (± 1.4 SD), an average relative humidity of 41.4 % (± 7.1 SD) and 18 hours of light (fluorescent bulbs producing 300 µmol m^{-2} s^{-1} PAR at the pot level). To account for subtle temperature and humidity differences throughout the growth chamber, all pots were re-randomized every four weeks within the growth chamber.

2.2.3 Drought treatments

Seedlings were randomly assigned to one of three drought treatments: control no-drought treatment (*Con*), mild-drought treatment (*Ml*), and severe-drought treatment (*Sev*) (30 seedlings/ stock type). Prior to assigning seedlings to their respective drought treatments, initial measurements of seedling height and RCD were used to ensure even distributions of seedling sizes within stock types and among drought treatments. This was tested using a two-way mixed ANOVA (PROC MIXED, SAS 9.2, SAS Institute, North Carolina, U.S.A.). Within each stock type there were no significant differences in initial seedling height (P= 0.638) or RCD (P= 0.244) amongst the drought treatments.

For the two drought treatments, the target shoot water potentials were based on a published vulnerability curve for trembling aspen seedlings, which indicated a 50 % loss of hydraulic conductivity in stem tissues at tensions of -2.4 MPa (Cai and Tyree 2010). To correlate the shoot water potentials with pot weight (e.g. soil water content), a soil water retention curve (SWRC) was constructed for the planting medium using a WP4C dewpoint potentiameter (Decagon Devices Ltd, Washington, U.S.A.) (Figure 2). Because catastrophic embolisms (run-away cavitation) is expected when loss of stem hydraulic conductivity is greater than 50 % (P50), care was taken to ensure that aspen seedlings did not reach a shoot water potential of less than -2.4 MPa. The resulting shoot water potential targets for *Ml* and *Sev* treatments were -1.5 MPa and -2.1 MPa, with an associated gravimetric soil water content of 45.1 % and 41.6 %, respectively. The corresponding soil water potentials for these treatments were -1.09 MPa and -1.37 MPa respectively, which was above the permanent wilting point of -1.5 MPa. Seedlings in the *Con* treatment were well-watered twice weekly, resulting in gravimetric water content near field capacity and a corresponding soil water potential of 0 MPa.

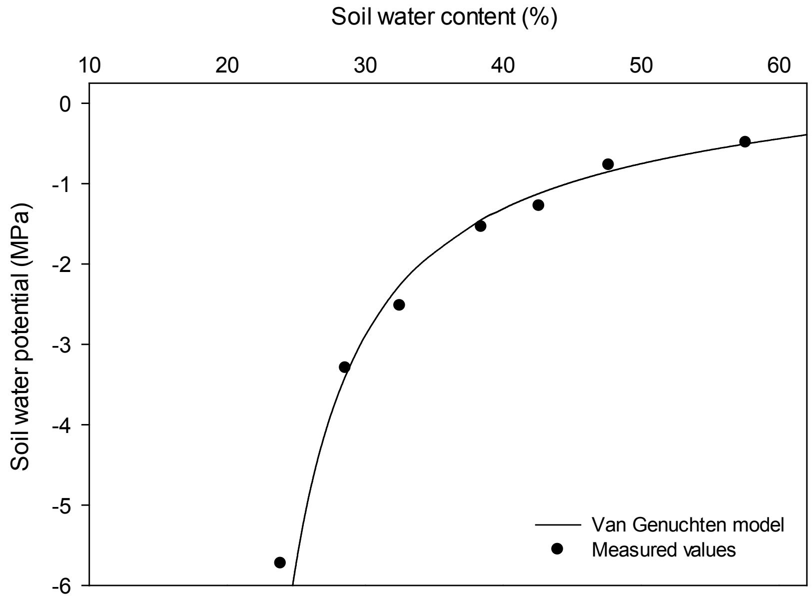

Figure 2. Soil water retention curve for the soil mixture used in the growth chamber drought study. Measured values were obtained using a WP4C dewpoint potentiometer, and the Van Genuchten model (Van Genuchten 1980) was used for curve fitting.

A subsample of *Ml* and *Sev* pots (24 per treatment) across representative seedling sizes were weighed daily using a digital balance (Adam Equipment, Connecticut, U.S.A.) to determine the average gravimetric water content within each drought treatment, and to ensure uniform pot weights amongst seedlings of different stock types. Pots in the *Ml* and *Sev* drought treatments were left unwatered until the pots reached a mass corresponding to the target gravimetric soil water contents calculated from the SWRC for each specific drought treatment; The target pot mass was reached after 14 days and 20 days for the *Ml* and *Sev* drought treatments, respectively . Daily water loss for pots in each treatment was then calculated. Pots were watered with the equivalent mass of water lost from the prior day in order to maintain constant soil water content for each treatment. *Con* treatment seedlings were watered to field capacity twice weekly and weighed weekly to confirm their target weights.

2.2.4 Seedling measurements

The drought experiment was terminated after 16 weeks because all seedlings had set bud. Just prior to the final harvest, stomatal conductance (g_s) and net photosynthesis (A_{net}) were measured on 10 seedlings in each treatment combination (total 90 seedlings) using an infrared gas analyzer with a broad leaf cuvette (LI-6400, LI-COR, Nebraska, U.S.A.). Light intensity in the cuvette was set at 1000 µmol m^{-2} s^{-1}. The cuvette temperature was held constant at 20 ^{o}C. Measurements were taken on the first fully expanded mature leaf of each selected seedling. To account for the effects of sampling time, one seedling of each treatment combination was randomly assigned to 10 time blocks. Measurements were taken over two days; between 08:00-15:00, five time blocks (45 seedlings total) were measured each day. Since growth chamber conditions remained constant throughout the day, no mid-day stomatal depression was observed. Immediately after these physiological measurements, seedlings were cut at the root collar using a razorblade and placed into a pressure bomb (Soilmoisture Equipment Corp., California, U.S.A.) to determine shoot water potential.

To assess growth variables, current height growth was measured from the bud scar to the terminal bud tip of the longest shoot. New stems (terminal leader and branches), leaves and roots were separated for each seedling and their dry mass was determined. Prior to measuring root dry mass, root volume was determined using the methods described above. Prior to measuring leaf dry mass, total leaf area for each seedling was measured using a Leaf Area Meter (LI-3100C, LI-COR, Nebraska, U.S.A.). Dry mass of new stem growth was measured as the total mass of all newly formed branches and the portion of the terminal leader grown during the experimental period, and did not include leaf tissues. Root mass growth for each seedling was calculated by subtracting the dry root mass of each seedlings at the end of the experiment from the average initial dry root mass for each corresponding stock type; because initial dry root mass could not be collected on our experimental seedlings without destructively sampling, the average initial dry root mass was used in the calculations. This was repeated to calculate root volume growth. After dry mass determination, the stem and root tissues analyzed for NSC reserves using the methods previously described.

2.2.5 Data analysis

All data from both the initial and final harvest periods were analyzed using the PROC MIXED function in SAS (SAS 9.2, SAS Institute, North Carolina, U.S.A.). Because climatic conditions were well controlled for in the growth chamber and little variation occurred at different bench positions, no random effects were used in the models for the final harvest period. Prior to any statistical analysis, the residuals of each response variable were analyzed to test for the assumptions of ANOVA. Residuals were plotted graphically using scatter plots to examine normality. Residuals were analyzed for homogeneity of variance using Levene's test. In the case of non-equal variance, the residual error for each main effect with unequal variance and the interaction were separately included in the PROC MIXED model using the REPEATED/GROUP= statement; the model with the best fit, based on having the lowest Akaike Information Criterion (AIC) value, was selected for statistical analysis.

Morphological data from the initial sample period was analyzed using a two-way ANOVA with a 3 x 3 factorial design using stock type (*large*, *highNSC* and *highRSR*) and drought treatment (*Con*, *Ml*, and *Sev*). Stem length, stem mass, and RSR response variables had unequal variances and were analyzed using the REPEATED/GROUP= statement. This statement was not included for the root collar diameter, root volume, and root mass response variables due to homogeneity of variance. Differences of least square means were compared using an LSD test with initial significance set at α= 0.05 (Bonferroni adjustment: α=0.0167; Appendix 1); this test was used as there were no significant interactions and only main effects existed.

Because stock types were not assigned to specific drought treatments before initial carbohydrate analysis, NSC, starch and sugar content/concentration for each tissue type were analyzed using a one-way ANOVA with stock type only. Normality and homogeneity of variance existed for all response variables. Least square means were compared using an LSD with significance set at α=0.0167 after a Bonferroni adjustment (Appendix 1).

Growth and physiological responses at the end of the experiment were analyzed using a two-way ANOVA with a 3 x 3 factorial design using stock type and drought treatment. A simple linear regression was performed to assess the relationship between leaf area and shoot water potential in each drought treatment using leaf area as a dependent variable and shoot water potential as an independent variable. Height growth, stem growth, leaf area, leaf and root mass, RSR, change in RSR, and g_s had unequal variance and were analyzed using the REPEATED/GROUP= statement. A_{net} and shoot water potential did not require this analysis due to homogeneity of variance. The least square means for response variables with significant interactions were compared using a Tukey-Kramer adjustment with significance set at α=0.05. Least square means for response variables with only significant main effects were compared using an LSD test with a Bonferroni adjustment and significance set at α=0.0167.

2.3 Results

2.3.1 Morphological responses in stems, leaves and roots

Average height growth and the corresponding new stem mass growth (data not shown) declined sharply with increasing drought severity in the *highRSR* and *large* stock types, whereas in the *highNSC* stock type height growth and stem mass growth were not reduced by drought stress. This resulted in a significant stock type and drought interaction effect for both response variables (both $P < 0.004$) (Figure 3A). Overall, the *highRSR* stock type had the greatest height and stem mass growth in all drought treatments (Figure 3A), while *large* seedlings had the least height and stem mass growth under *Ml* (2.5 cm and 0.2 g) and *Sev* (2.0 cm and 0.1 g) stress (Figure 3A). Well-watered height and stem mass growth in the *highNSC* and *large* stock types (both 10.5 cm and 0.35 g) was half that of the *highRSR* stock type (23.2 cm and 0.7 g) (all $P < 0.001$). Stem growth in all stock types did not differ between the *Ml* and *Sev* drought treatments, though growth tended to be greater under *Ml* stress (Figure 3A).

Under well-watered conditions, the *highRSR* stock type had the highest total leaf area (398 cm^2), followed by the *large* stock type (285 cm^2) and the *highNSC* stock type (189 cm^2) ($P < 0.004$). When exposed to drought, the *highRSR* and *highNSC* stock types experienced an average 48 % reduction in leaf area (Figure 3B) and a 53 % reduction in leaf mass (data not shown) compared to the control seedlings. However, leaf area and mass decreased by 69 % and 71 %, respectively in the *large* seedling stock type, which resulted in a significant interaction effect for both variables (both $P < 0.001$)

(Figure 3B). Under both drought treatments, the *highRSR* seedlings maintained greater leaf area than the *highNSC* and *large* seedling stock types (Figure 3B), while leaf area did not differ between the *large* and *highNSC* stock types under drought stress (Figure 3B).

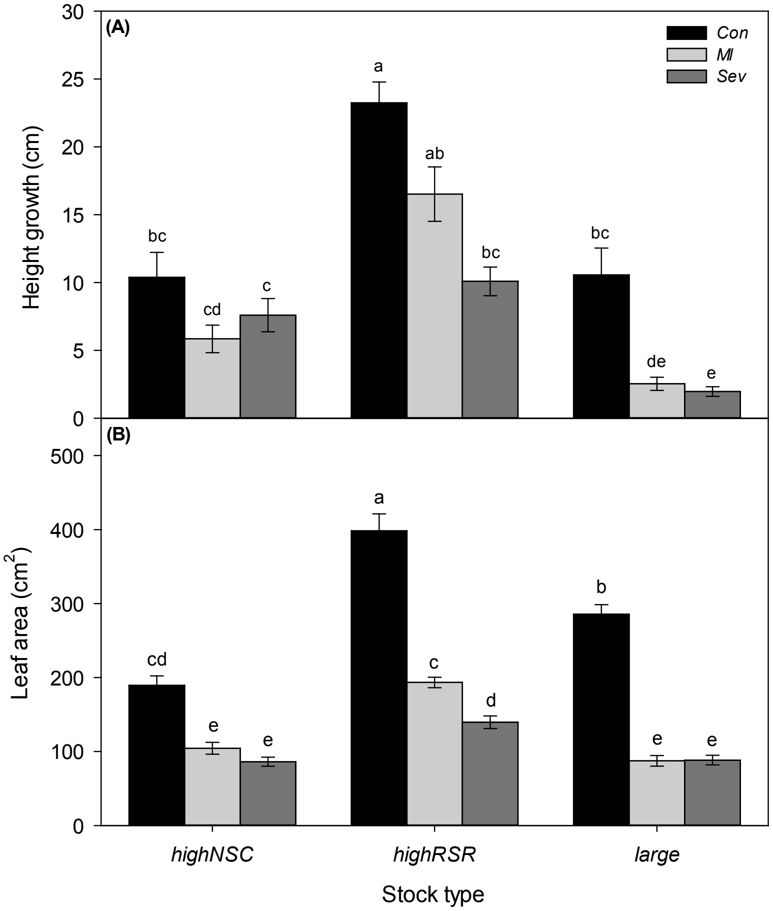

Figure 3. Average height growth (A), and leaf area development (B) of three aspen seedling stock types exposed to three different levels of drought. Error bars represent one standard error of the mean (n=10) and different letters indicate significant differences among means.

Root volume growth (Figure 4A) and root mass growth (data not shown) decreased linearly as drought severity increased, which resulted in droughted seedlings possessing smaller root systems (both P< 0.001) (Figure 4B). However, while root volume and root mass of all seedlings increased from initial conditions, all stock types added similar amounts of new root volume and mass to their root systems (P= 0.101 and P= 0.241, respectively) (data not shown).

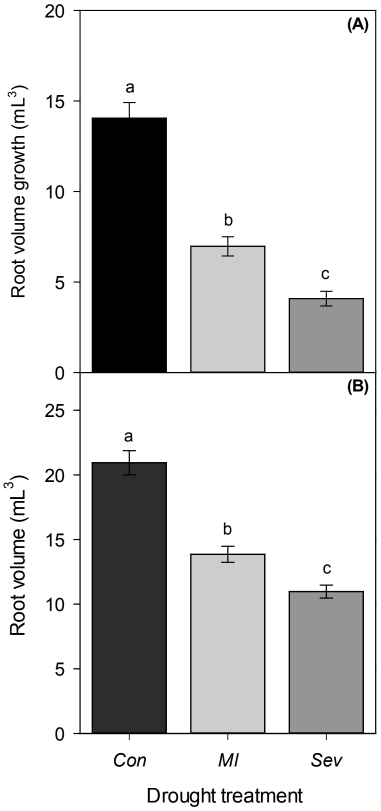

Figure 4. Average root volume growth (A), and total root volume (B) of aspen seedlings exposed to different drought treatments. Error bars represent one standard error of the mean (n=30) and different letters indicate differences among means.

Root to stem ratio (RSR) in *highRSR* seedlings decreased from 4.9 g g^{-1} to 3.5 g g^{-1} (30%), compared to increases of 22 % and 39 % in *highNSC* (from 3.2 g g^{-1} to 3.9 g g^{-1}) and in *large* (from 1.6 g g^{-1} to 2.3 g g^{-1}) seedlings, respectively (all $P< 0.001$) (Table 1 and Figure 5). This response can largely be attributed to the increases in stem growth under all levels of drought stress in the *highRSR* stock type, compared to the modest increases in stem growth observed in the *highNSC* and *large* stock types (Figure 3A).

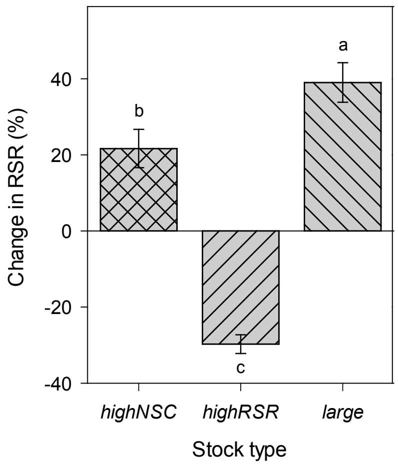

Figure 5. Average change in root to stem ratio (RSR, without leaves) of three aspen seedling stock types. Error bars represent one standard error of the mean (n=30) and different letters indicate differences among means.

2.3.2 Physiological responses of aspen seedling stock types

Drought stress reduced shoot water potential in all stock types (P< 0.001) (Figure 6). Shoot water potential decreased by 61 % and 78 % in *large* and *highNSC* seedlings, respectively, when exposed to drought conditions. However, shoot water potential decreased more in *highRSR* seedlings (111%) than the other stock types from well-watered conditions to *Ml* drought, which resulted in a borderline significant interaction effect (P= 0.052) (Figure 6). All stock types displayed similar shoot water potentials within watering treatments with the exception of the *Sev* treatment, where *Sev* drought stress elicited a more negative shoot water potential in the *highRSR* stock type (-1.71 MPa) than in the *highNSC* (-1.37 MPa) and *large* (-1.20 MPa) stock types (Figure 6).

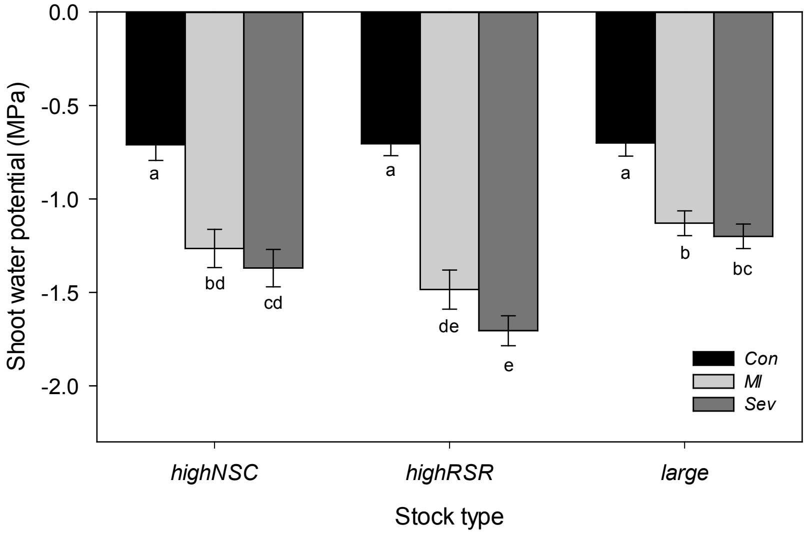

Figure 6. Average shoot water potential of three aspen seedling stock types exposed to different levels of drought. Error bars represent one standard error of the mean (n=10) and different letters indicate differences among means.

Overall, there was a negative linear relationship between leaf area and shoot water potential in seedlings exposed to *Sev* drought, where shoot water potential became more negative as leaf area increased ($P< 0.001$; $R^2= 0.461$) (Figure 7B). Similar results were observed in the *Ml* treatment, though the relationship was weaker ($P=0.018$; $R^2= 0.183$) (Figure 7A). In contrast, no observable relationship between these variables was found in the *Con* treatment ($P= 0.504$) (data not shown).

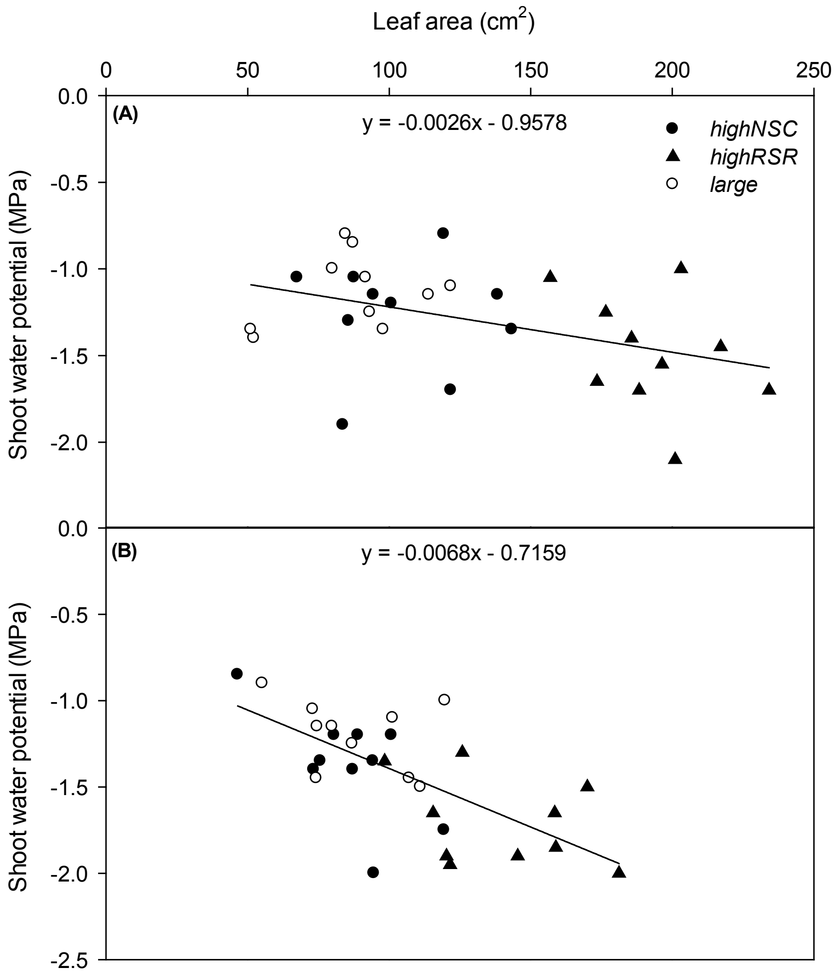

Figure 7. The relationship between shoot water potential and leaf area three aspen seedling stock types exposed to mild drought (A), and severe drought (B). All seedling stock types were analyzed together within each drought treatment (n= 30).

Net assimilation (A_{net}) declined with increasing drought severity (P= 0.001) (Figure 8A). Well watered seedlings had higher net photosynthesis (5.2 µmol m^{-2} s^{-1}) than those grown under *Ml* (3.9 µmol m^{-2} s^{-1}) or *Sev* drought (3.4 µmol m^{-2} s^{-1}), though there was no difference in A_{net} between the *Ml* and *Sev* treatments (P= 0.306). These responses were closely mirrored by stomatal conductance (g_s) (Figure 8B). Net photosynthesis (A_{net}) and g_s did not differ among stock types (P= 0.105 and P= 0.235, respectively).

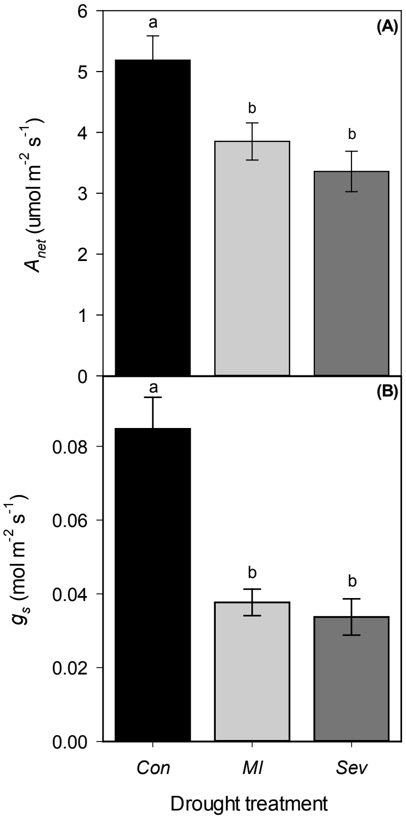

Figure 8. Average net photosynthesis (A_{net}) (A), and stomatal conductance (g_s) (B), of aspen seedlings exposed to different drought treatments. Error bars represent one standard error of the mean (n=30) and different letters indicate differences among means.

2.3.3 NSC reserves

After 90 days of growth, total NSC (soluble sugars and starch combined) concentration was highest in the *highNSC* stock type across all drought treatments (Figure 9A). This difference was mostly driven by the sugar concentration in the stem (Figure 9C) and the starch concentrations in the roots (Figure 9E). Generally the *highRSR* and *large* seedling stock type had similar NSC tissue concentrations (Figure 9A, 9E); however, the NSC concentrations in the stem tissues were higher in the *large* stock type compared to the *highRSR* stock type (Figure 9C), which was caused by higher starch concentrations in the stem.

Across all stock types, NSC concentrations at the seedling level decreased with drought; however, the NSC concentrations were not different between the *Ml* and *Sev* treatments (Figure 9B). This response was driven by the starch concentrations in the root system, which were higher in the control seedlings than in the *Ml* and *Sev* treatments (Figure 9F). In the stem, NSC concentrations did not change with drought; however, the concentration of soluble sugars increased with drought, while starch concentration decreased (Figure 9D).

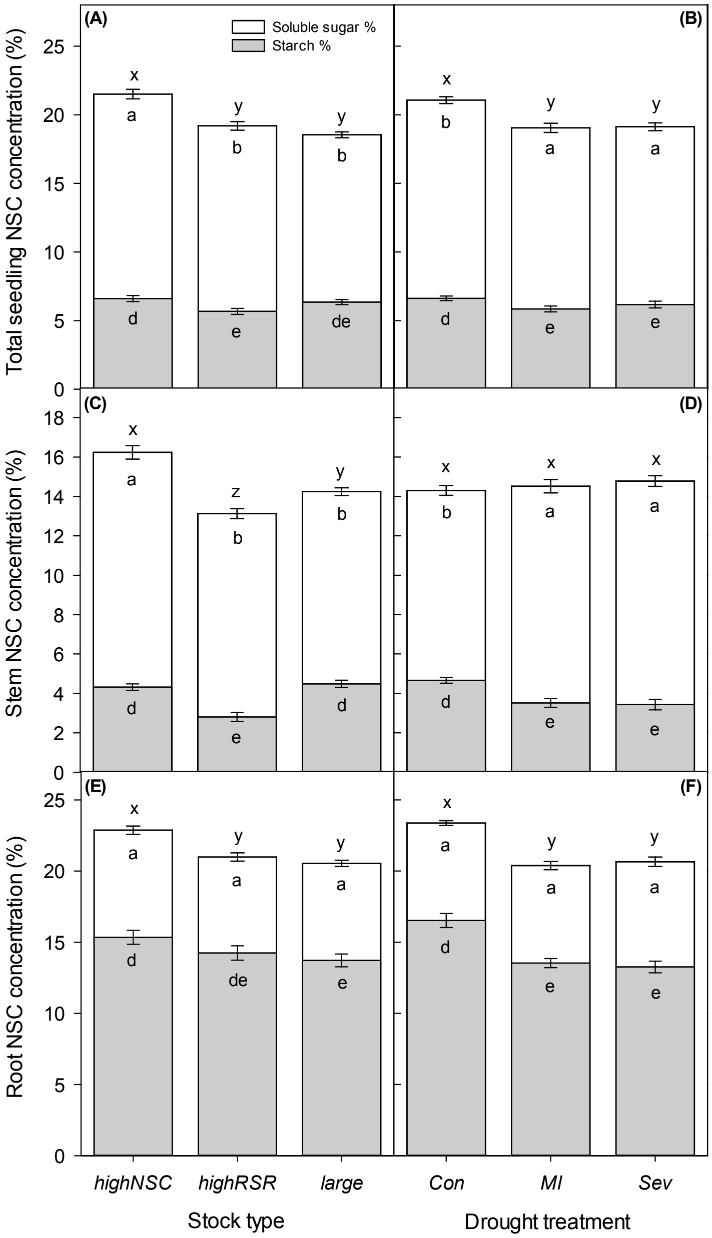

Figure 9. Average total seedling NSC concentration (%) (A, B), stem NSC % (C, D), and root NSC % (E, F) of aspen seedlings exposed to three different levels of drought. (A), (C), and (E) represent NSC % of stock types, and (B), (D), and (F) represent NSC % of all seedlings in response to drought treatments. Total NSC values are the sum of soluble sugar and starch fractions, with significant differences among means denoted as letters x-z. Significant differences in soluble sugar % (white portion) among treatments are denoted with the letters a-c, and significant differences in starch % (grey portion) among treatments are denoted with the letters d-f. Error bars represent one standard error of the mean (n=30).

2.4 Discussion

2.4.1 Growth performance of stock types

Drought stress reduced growth in all stock types and in all parts of the seedlings, though the degree it was reduced was greatly influenced by initial stock type characteristics. Under drought stress the *highRSR* seedlings had greater height and stem mass growth and leaf area development than the *large* and *highNSC* stock types. Since this stock type had a larger root systems relative to it shoot (it was overall the smallest seedling stock), it likely increased water and nutrient uptake relative to the initially developing leaf areas during leaf flush and increased the capacity to supply water to above-ground organs and allowed for greater leaf and shoot expansion (Hilbert 1990; Ibrahim 1997; Poorter and Nagel 2000). Several studies have observed greater growth performance in planted aspen seedlings with high RSR (Martens et al. 2007; Landhäusser et al. 2012a; Landhäusser et al. 2012b), even when site conditions were not moisture-limiting; our results suggests that seedlings with high RSR are well-adapted for growth on not only sites with ideal growing conditions, but sites with low moisture availability as well. In contrast, *large* seedlings, which had the lowest initial RSR, had the poorest shoot growth under drought stress, which indicates that low root mass allocation may be a detrimental characteristic for growth performance in seedlings exposed to drought.

While high root mass allocation appears to facilitate greater growth of above-ground tissues under well-watered and drought conditions, it is important to note that *highRSR* was also characterized by its relatively high initial NSC concentration, which was only marginally lower than the *highNSC* stock type. Therefore it is difficult to disentangle the relationship between RSR and root NSC reserves and the ability of a seedling to cope with drought stress in this study. It is likely that high NSC concentration also played a role in facilitating the greater above-ground growth observed in *highRSR*. NSC reserves provide an easily accessible carbon source for shoot growth and foliar development prior to the commencement of photosynthesis (Landhäusser and Lieffers 2002; Grossnickle 2005; Jacobs et al. 2005; Oberhuber et al. 2011). Landhäusser and Lieffers (2002) found that aspen saplings with high root starch concentrations had greater sucker initiation and higher shoot and leaf growth. Like the previous works of Martens et al. (2007) and Landhäusser et al. (2012b), we were unable to completely decouple the effects of RSR and NSC characteristics on seedling growth. However, despite similarities in NSC characteristics, the *highRSR* stock type outperformed the *highNSC* stock type in every above-ground growth response, regardless of moisture regime. This suggests that RSR rather than the NSC concentrations was the main characteristic driving above-ground performance and seedling quality during drought.

Overall, there was a large reduction in new root growth (root volume) within all seedlings as drought severity increased. However, root growth did not differ among the stock types, and all stock types displayed similar decreases in root growth within drought treatments. This suggests that initial RSR, root size, height and NSC reserve (content and concentration) characteristics did not influence root development under drought. Rather, it appears that root growth was dictated by the drought stress alone. As root growth did not differ among stock types, differences in stem growth allocation dictated changes in RSR among seedling stocks. Seedlings with high RSR (*highRSR* stock type) allocated more growth to above-ground organs, which resulted in a marked decrease in RSR at the conclusion of the experiment. In contrast, *highNSC* and *large* seedlings with relatively lower initial RSR incurred a positive increase in RSR under both well-watered and droughted conditions due to their considerably lower stem growth. Shoot growth is thought to be functionally related to root mass allocation (Borchert 1975; Hilbert 1990; Ibrahim et al. 1997), and plants will disproportionally allocate carbon to growth of a specific organ to reach an ideal balance between carbon acquisition (shoot tissue) and soil resource uptake (root tissue) (Chapin et al. 1987). In a controlled glasshouse study, Borchert (1975) observed that pin oak (*Quercus palustris* Muenchh.) seedlings ceased shoot growth under favorable environmental conditions when RSR began to decrease, which he postulated was to allow for sufficient water uptake to meet shoot water demands in order to prevent internal moisture deficits, and did not display indeterminate shoot growth until shoot mass allocation was reduced. It is possible that *highNSC* and *large* seedlings experienced limited shoot growth in order to obtain a more balanced RSR.

2.4.2 Physiological responses to drought

Stomatal conductance (g_s) was similar among stock types, though it decreased under drought stress in general. However, there was no discernable difference in g_s between *Ml* and *Sev* drought; in response to drought, aspen displays isohydric regulation where stomata close at the onset of drought to limit transpirational demands and water loss and to maintain stable water potentials within seedlings (Tardieu and Simonneau 1998; McDowell et al. 2008). This may explain why shoot water potential was similar between *Ml* and *Sev* drought treatments.

However, differences in growth responses among stock types elicited differences in shoot water status under *Sev* drought. Severe moisture stress produced more negative shoot water potentials in the *highRSR* stock type than the *highNSC* and *large* stock types, indicating that *highRSR* seedlings were under greater hydraulic strain. This was largely driven by the *highRSR* stock type's high foliar development. Under *Sev* drought, shoot water potential became more negative in seedlings as leaf area increased (Figure 7). Within this treatment, the *highRSR* stock type had the highest leaf area, which also represents more area for transpirational water loss (Haase and Rose 1993). In a glasshouse study conducted by Newton et al. (1992), cuttings of *Terminalia spinosa* (Engl.) with high leaf area had higher water deficits within leaf and petiole tissues than cuttings with relatively less leaf area, while a glasshouse study conducted by Jacobs et al. (2009) observed more negative leaf water potentials in red oak seedlings with high leaf area when exposed to drought. Sperry et al. (2002) posits that seedlings with high leaf area relative to root area have steeper hydraulic gradients due to high transpirational demands, which can subsequently reduce hydraulic conductivity within tissues (Tyree et al. 1993; Ewers et al. 2000; Ewers et al. 2005). In this experiment, seedlings were potted in soils with water contents near field capacity, and it took 20 days for our *Sev* treatment to reach its target soil water content. It is possible that the *highRSR* stock type allocated higher growth to leaves before and during the early onset of *Sev* drought, which subsequently led to greater transpirational water loss and more negative shoot water potentials as drought intensified.

Differences in growth responses among stock types also influenced seedling NSC concentration. *HighNSC* seedlings continued to have higher NSC concentrations than the other stock types, while *highRSR* seedling NSC concentration decreased to levels similar to *large* seedlings. There is a direct tradeoff between photosynthate allocation to growth and NSC reservation in plants (Chapin et al. 1990), and it is possible that the *highRSR* stock type's high shoot growth came at the expense of NSC reserve accumulation (Galvez et al. 2011). However, despite differences in growth allocation patterns, no interaction terms existed between drought and stock type treatments, and NSC concentration responded similarly among stock types in response to drought. This suggests that NSC reserves at the termination of the study were not influenced by initial NSC characteristics or growth responses under drought.

In this experiment, it is likely that NSC concentrations decreased under drought stress due to reductions in photosynthesis. In order to limit transpirational water loss, aspen displayed tight stomatal regulation; however, stomatal closure also limits CO_2 assimilation (Cowan and Farquhar 1977; Farquhar and Sharkey 1982), and in turn reduces photosynthesis. Consequently, stomatal regulation and the rate of photosynthetic reduction can profoundly influence NSC reserves. McDowell (2011) postulated that NSC reserves increase shortly after the onset of drought because growth decreases at a faster rate than photosynthetic activity, which has been demonstrated in numerous studies (Körner 2003; Sala and Hoch 2009; Anderegg 2012; Anderegg et al. 2012); however, persistent drought conditions may further reduce photosynthesis, resulting in reduced NSC. The depletion of NSC reserves following prolonged periods of drought stress is well-documented (Körner 2003; McDowell 2011) and has been observed in a number of species, including pinyon pine (*Pinus edulis* Engelm.) (Adams et al. 2013), radiata pine (*Pinus radiata* D. Don.) (Mitchell et al. 2013), longleaf pine (*Pinus* *palustris* P. Mill.) (Sayer and Haywood 2006), Jack pine (*Pinus banksiana* Lamb.) (Goeppel 2014), and aspen (Galvez et al. 2013).

In the study by Galvez et al. (2013), this pattern of NSC decline was displayed in droughted aspen seedlings, though previous research on aspen (Galvez et al. 2011) observed an increase in NSC concentrations under drought due to early growth termination which allowed for NSC reserve accumulation. This response was not observed in this study; starch decreased in the tissues of all droughted seedlings, and only an increase in shoot soluble sugar concentration was observed, though this response may have been for osmotic adjustment (Chaves 1991; Chaves et al. 2002; Galvez et al. 2013). In our study, drought stress did not induce earlier bud set than in the *Con* treatment and seedlings displayed continuous growth until the termination of the experiment, when NSC was measured. Our seedlings may have continued to allocate photosynthates to structural growth over storage, which combined with decreased carbon assimilation delayed the replenishment of NSC reserves (Kays and Canham 1991; Landhäusser et al. 2012a).

2.5 Conclusions

This study suggests that high RSR is the main characteristic driving growth and overall performance in aspen seedlings under drought, regardless of absolute root system size (mass or volume). Although all seedling stock types performed similarly in terms of root growth, seedlings with high initial RSR (*highRSR*) had significantly greater growth allocation to stems, as well as greater foliar development, under drought stress compared to seedlings with high NSC content, shoot and root mass and root volume, indicating that RSR is the best predictor of seedling quality under drought; however, high NSC concentration may be also be a beneficial characteristic under drought conditions. Similar growth responses were also observed under well-watered conditions. As such, aspen seedling stock manipulated to have high RSR appears to be beneficial for planting under both well-watered and drought conditions. In contrast, seedling stock with high initial shoot mass allocation (low RSR, *large* stock type) had the poorest above-ground growth performance, and it appears that low RSR is disadvantageous for aspen seedling growth under drought conditions.

Chapter 3: Evaluating *Populus tremuloides* Michx. seedling stock characteristics in response to aspect and hydrogel amendment

3.1 Introduction

The southern extend of the boreal forest biome of Northern Alberta, Canada is comprised of mixed-wood stands that consist of mixtures of trembling aspen (*Populus tremuloides* Michx.) and other deciduous species interspersed with conifer species such as white spruce (*Picea glauca* Moench) and *Pinus* sp. (Rowe 1972). The mixedwood region of Alberta is rich in natural resources such as wood, coal, and oil, which has recently led to unprecedented growth in resource extraction. Parts of the below-ground resources are extracted via open-pit surface mining, which entails the stripping of all vegetation, soil and subsoil layers from the landscape. In order to facilitate the reclamation of these heavily disturbed sites, nursery grown seedlings are often planted. Trembling aspen seedlings are often used for planting on reclamation sites (Macdonald et al. 2012), as this species exhibits fast growth rates and, relative to most boreal tree species, a high drought tolerance (Lieffers et al. 2001). However, successfully establishing seedlings in these reclaimed areas is often difficult (Macdonald et al. 2012), due to cool growing season temperatures (Bonan and Shugart 1989), low precipitation (Rumney 1968) and high evapotranspirational (ET) demands (Devito et al. 2005).

Upland reclamation areas are often dominated by hill formations, created to deposit excess overburden and tailings materials which are generated during the mining process (Leatherdale et al. 2012). Due to the varied structure of these landscapes, seedling growing conditions, such as soil and air temperature and moisture availability, can vary greatly depending on slope and aspect (Leij et al. 2004; Fisher et al. 2005; Letts et al. 2009). In the Northern hemisphere, South-facing slopes receive greater solar insolation than North-facing slopes (Holland and Steyn 1975; Astrom et al. 2007; Daly et al. 2007) which influence soil temperatures, vapour pressure deficits (VPD), and soil water availability for plant growth (Hasler 1982; Leij et al. 2004; Fisher et al. 2005). As such, moisture stress can be amplified on South aspects during periods of drought (Fekedulegn et al. 2002; Letts et al. 2009), leading to reduced stomatal conductance and photosynthesis (Slot et al. 2005; Letts et al. 2009), tree growth (Oberhuber and Kolfer 2000; Fekedulegn et al. 2002) and seedling mortality. Most aspect studies occur over a wide geographic range, and intra-site heterogeneity due to soil substrates, tree density and canopy closure can profoundly influence tree growth (Clinton and Boring 1993; Ferrio et al. 2003). Alternatively, new reclamation areas are composed of homogeneous soil substrates collected regardless of aspect and therefore have no edaphic legacy effects that could influence seedling performance. As such, hill slopes in mine reclamation areas represent a unique opportunity to evaluate the influence of aspect on tree growth simply driven by climatic variables.

Newly planted seedlings in reforestation areas often experience reduced growth, or transplant shock, for several years following out-planting due to drought stress (Rietveld 1989; Close et al. 2005), which will be exacerbated on harsh and exposed reclamation sites, particularly those with Southern exposures. In order to overcome these harsh growing conditions, it is important to have access to high quality seedlings with characteristics specifically catered to drought-prone sites (Rose et al. 1990; Rose and Haase 1995; Landis 2003). Seedling characteristics such as high root to shoot ratios (RSR) and non-structural carbohydrate (NSC) reserves have been found to improve aspen seedling growth performance and quality following planting (Martens et al. 2007; Landhäusser et al. 2012b). NSC reserves may act as a carbon pool to increase tissue growth following out-planting (Struve and Joly 1992; Landhäusser and Lieffers 2002), and height and root growth were greater in trembling aspen seedlings with high NSC reserves following planting (Martens et al. 2007; Snedden et al. 2010; Landhäusser et al. 2012b). As well, seedlings with high RSR have larger root systems relative to above-ground tissues, which can improve soil water uptake due to more root mass relative to leaf area (see Chapter 2). However, it is not yet known whether NSC reserves or RSR are better predictors of aspen seedling quality on reclamation sites prone to drought.

Alternatively, transplant shock can also be alleviated through site preparation, including the use of soil amendments such as hydrogels. Hydrogels are coarse-textured, petroleum based polymers that increase plant water availability by improving the retention of soil water (Bhardwaj et al. 2007; Chirino et al. 2011) and nutrients (Magalhaes et al. 1987; Bres and Weston 1993). Significant improvements in water content were shown on coarse-textured soils when hydrogels were mixed within soils at volumetric concentrations as low as 0.4 % (Arbona et al. 2005; Al-Humaid and Moftah 2007). Hydrogels have been found to increase radial growth, height growth and leaf area, as well as reduce hydraulic strain, in a wide variety of species including *Citrus* sp. (Arbona et al. 2005), grey poplar (*Populus* x *canescens* Aiton.) (Beniwal et al. 2010), and beech (*Fagus sylvatica* L.) (Beniwal et al. 2011; Jamnicka et al. 2013). However, most research has focused on hydrogel application under greenhouse conditions using soilless media rather than natural soils, and no research has been conducted on the effects of hydrogel use on drought prone reclamation sites to reduce transplant shock in boreal tree species.

Two field-experiments were conducted to (1) explore the first-year out-planting performance (establishment and growth) of trembling aspen seedling stock types with different reserve status, root to shoot ratios and seedlings size on slopes with different exposure, and (2) to determine the impact of a hydrogel amendment on the first-year performance of aspen seedlings planted on a South-facing slope.

3.2 Material and Methods

3.2.1 Seedling stock type production

Three trembling aspen seedling stock types (*highNSC*, *highRSR* and *large*, see below and Chapter 2) were used in the aspect and soil amendment experiments. The unique seedling stock types (see Chapter 2 for information on the stock type development) were produced at the Crop Diversification Centre North in Edmonton, Alberta (53^{o} 64’ 28.27”, -113^{o} 36’ 13.19”). All seedling stock types were grown as outlined in Chapter 2 (Refer to Section 2.2.1 for additional details on seedling stock type growing conditions and initial seedling measurement protocols, and section 2.2.4 for data analysis of initial seedling characteristics). Following 27 weeks of growth, all seedlings were lifted and packaged into bags and waxed boxes, and were placed into frozen storage (-2 °C) until the commencement of the experiments in May, 2012 (approximately 55 weeks) (Figure 10). On May 25, 2012, one week prior to out-planting, seedlings were lifted from frozen storage and were slowly thawed in a refrigerator at 4˚C.

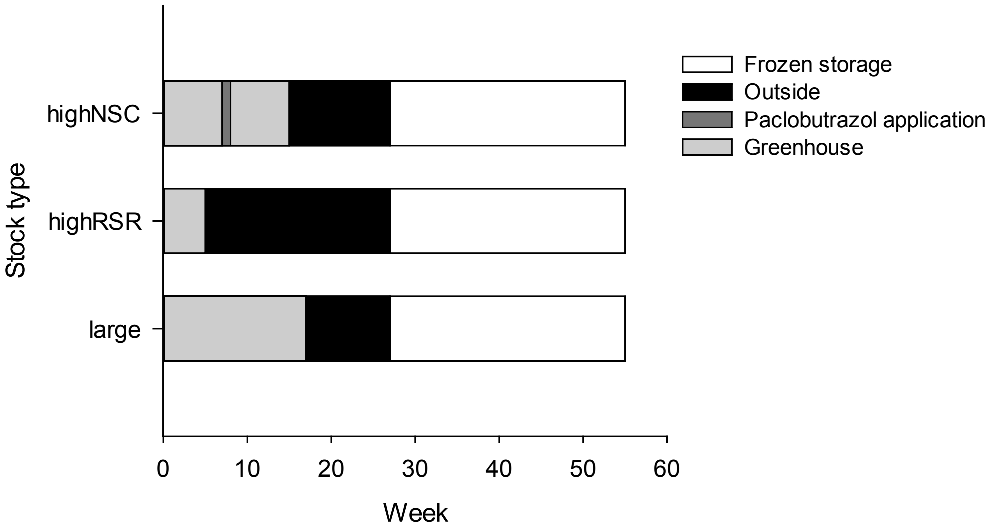

Figure 10. Timeline of growing conditions and treatment applications for aspen seedling stock types used in the aspect and soil amendment studies (see also Chapter 2).

3.2.1.1 Initial seedling characteristics

Relative to the other two stock types produced for this study, the *highNSC* stock type was characterized by being medium sized in height, total mass and root to stem ratio (RSR, without leaves) (32.3 cm, 3.9 g and 3.2 g g^{-1}, respectively); however, it had high root volume and mass with both the highest NSC content (1.20 g) and concentration (29.6 %) of the three stock types (Table 2). The *highRSR* stock type differed from the two other stock types by having the highest RSR (4.9 g g^{-1}), despite being the smallest in terms of height and mass. This stock type also had high NSC concentration (27.0 %); however, it had the lowest NSC content overall (Table 1). The *large* stock type was characteristically tall with high root and stem mass, high root volume, low RSR, and low total NSC reserves (concentration) in relation to the other two stock types (Table 2).

Table 2. Initial morphological (height, tissue masses and RSR; n=24) and carbohydrate reserve (n=8) characteristics (average ± SE) of the three trembling aspen seedling stock types prior to the drought experiment. Different letters indicate differences among means (see also Chapter 2).

Initial seedling conditions	**Stock type**		
	highNSC	*highRSR*	*large*
Height (cm)	32.3 ± 0.8 **b**	15.9 ± 0.6 **c**	51.8 ± 1.3 **a**
Root collar diameter (mm)	3.9 ± 0.1 **b**	3.0 ± 0.1 **c**	4.8 ± 0.1 **a**
Shoot mass (g)	0.9 ± 0.04 **b**	0.3 ± 0.01 **c**	1.9± 0.1 **a**
Root volume (mL^3)	7.8 ± 0.4 **a**	4.9 ± 0.4 **b**	8.0 ± 0.6 **a**
Root mass (g)	2.9 ± 0.1 **a**	1.6 ± 0.1 **b**	2.9 ± 0.2 **a**
Total mass (g)	3.9 ± 0.1 **b**	2.0 ± 0.1 **c**	4.8 ± 0.2 **a**
RSR (g g^{-1})	3.2 ± 0.1 **b**	4.9 ± 0.2 **a**	1.6 ± 0.1 **c**
Total seedling NSC (g)	1.20 ± 0.15 **a**	0.54 ± 0.20 **c**	0.90 ± 0.17 **b**
Total seedling NSC (%)	29.6 ± 0.8 **a**	27.0 ± 1.2 **a**	19.7 ± 1.1 **b**

3.2.2 Planting area

To assess the short-term establishment of these stock types, seedlings were out-planted on four different upland hill structures spread across a 50 Ha reclamation area (Sandhill watershed), located at the Syncrude East-in pit mine near Fort McMurray, Alberta (57^o 04’ 01.58”, -111^o 59’ 22.80”). One year prior to the start of the experiment, the upland hill structures were constructed by directly placing 40 cm of fluvial-sand subsoil over approximately 10 m of tailings sand. The subsoil was then capped with a 20 cm soil prescription of forest floor material (FFM) salvaged from a Jack pine (*Pinus banksiana* Lamb.) dominated forest ecotype (A/B ecosites; Beckingham and Archibald 1996). Daily weather data for the experimental period was collected from Agroclimate Canada’s Mildred Lake meteorological station (57^o 02’ 28.00”. -111^o 33’ 32.00”), which was located close to the planting area.

3.2.3 Plot establishment and planting time

3.2.3.1 Aspect and stock type selection

To assess the impact of aspect and seedling characteristics on first year seedling establishment and growth, ten 1 m x 2 m plots were established mid-slope on North and South-facing aspects (*North* and *South* treatments, respectively) across four hill structures in May, 2012. A minimum 1 m buffer was given to each neighbouring plot to reduce seedling shading and root competition effects. All ten *North* plots were situated on a single hill structure (32-39˚N, slope 23 % ± 0.44 SE), while *South* plots were situated on 3 separate hill structures (135-210 °S, slope 15-27 % ± 1.10 SE) due to spatial limitations. Each individual plot was manually cleared of coarse woody debris, and manual weeding of all existing vegetation occurred prior to seedling out-planting and was redone every two weeks until the conclusion of the experiment. From June 1-2, 2012, 6 seedlings of each stock type (subsamples) were planted in each plot by hand. As this was only a short-term establishment study, the seedlings were planted at a spacing of 20 cm x 30 cm.

3.2.3.2 Hydrogel amendment and stock type selection

To determine the impact of a hydrogel amendment on the first-year performance of aspen seedlings, ten additional plots were amended with hydrogel (*Hydrogel*) and paired with the *South* plots on the South-facing slopes. The *Hydrogel* amendment (Stockosorb 660 XL, Evonik Stockhaussen LLC, North Carolina, U.S.A.) was mixed within the FFM cap of these plots on May 30, 2012. Soil amendments with a hydrogel application involved the following: 2.904 kg of dry hydrogel was evenly poured along the topsoil of each *Hydrogel* plot and hand mixed to a depth of 15 cm in order to create an equivalent 0.4 % gravimetric concentration of hydrogel within the FFM. The mass of applied hydrogel used to achieve this concentration was based on a soil bulk density of 1.21 g m^{-3}. The concentration of hydrogel that was applied was based on studies conducted by Arbona et al. (2005) and Al-Humaid and Moftah (2007), who observed maximum tree growth at 0.4 %. The non-amended *South* plots were considered a control in this experiment (*Control*). As with the *Control* plots, the *Hydrogel* plots were cleared of coarse woody debris and weeded. From June 1-2, 2012, 6 seedlings of each stock type (subsamples) were planted in each of the 10 *Hydrogel* plots at the same spacing as the *Control* plots.

3.2.4 Soil bioavailable nutrients and soil moisture measurements

Plant Root Simulator (PRS) probes (Western Ag Innovations Inc., Saskatchewan, Canada) were installed in all plots (*North*, *South/Control* and *Hydrogel*) to estimate soil bioavailable nutrients. Each PRS probe consisted of a 17.5 cm^2 ion exchange resin membrane and a plastic frame. On July 20, 2012, four pairs of probes, consisting of one anion and one cation probe, were installed in each plot (120 total pairs); one pair was placed in each corner of the plot in order to account for any variations in soil nutrients within the plot. Each group of four anion and cation probes were pooled together for one sample analysis per plot (30 total samples). Each individual probe was inserted by hand into the soil at a 90^o angle in order to capture the vertical distribution of bioavailable nutrients. They were buried up to the base of the plastic handle in order to ensure complete resin membrane contact with the surrounding soil. Probes were left in the ground for 32 days. On August 21, 2012, all PRS probe pairs were removed from the soil, placed in sealed plastic bags and stored in a cooler for 9 hours until cleaned. The plastic casing and resin membrane were hand cleaned using de-ionized water and a toothbrush and were subsequently transferred into new, clean, sealed plastic bags. Cleaned PRS probes were then shipped in an insulated box to Western Ag Innovations in Saskatoon, where they were analyzed using the specific protocol listed below.

Ions were first desorbed off the ion resin membrane using 0.5 M HCl. The resulting eluate was then colorimetrically analyzed to determine the supply rate (µg-10cm^2-4 weeks^{-1}) of NO_3^-, NH_4^+ and P using an automated flow injection analysis system. Total plant available N was calculated by adding the supply rates for NO_3^- and NH_4^+, and P was calculated by adding the supply rates for $H_2PO_4^-$ and HPO_4^{2+}. The supply rate for K^+ remaining in the eluate was measured using inductively-coupled plasma spectrometry.

In addition to soil bioavailable nutrient analysis, soil temperature, volumetric water content and water potential measurements were recorded. Seven plots within every *North*, *South*/*Control* and *Hydrogel* treatment were randomly selected for measurements (7 per aspect/ soil amendment treatment, 21 total plots). On May 30-31, 2012, prior to seedling out-planting, a shallow pit 20 cm in depth from the soil surface was dug at the centre of every sampled plot. At 10 cm soil depth, one volumetric soil moisture sensor (5TM, Decagon Devices Inc., Washington, U.S.A.) was installed horizontally into the soil column to measure volumetric soil water content and soil temperature concurrently. One dielectric water potential sensor (MPS-2, Decagon Devices Inc., Washington, U.S.A.) was installed directly adjacent to the volumetric soil moisture sensor. This was also repeated at 20 cm depth.

Hourly values were recorded using an analog data logger (EM50, Decagon Devices Ltd., Washington, U.S.A.) and were then averaged daily to calculate daily soil volumetric water content, temperature and water potential for each individual plot. Subsequently, daily averages for each plot were pooled together within each aspect/amendment treatment to calculate daily values for the *North*, *South*/*Control* and *Hydrogel* treatments. However, values recorded before June 26, 2012 were not included in the daily average calculations, as it took approximately one month for the sensors to equilibrate to the soil.

3.2.5 Seedling measurements

3.2.5.1 Physiological responses

Prior to final measurements at the end of August, seedlings in 4 plots each of the *North*, *South*/*Control* and *Hydrogel* treatments were selected for physiological measurements. In each plot, 6 seedlings of each stock type (subsamples) were measured for stomatal conductance (g_s) using a leaf porometer (AP4 Leaf Porometer, Delta-T Devices Ltd., Cambridge, United Kingdom). Measurements were taken on the newest fully expanded mature leaf of each seedling. Immediately after measuring g_s, shoot water potential was measured on two of the six seedlings of each stock type (subsamples) in each plot that was measured for g_s. Seedlings were cut at the root collar using a razorblade and placed into a pressure bomb (Soilmoisture Equipment Corp., California, U.S.A.) to determine shoot water potential. To account for the effects of date and sampling time on physiological measurements, plots were sampled in blocks over four times between 09:00 and 16:00. Each block consisted of one *North*, *South*/*Control* and *Hydrogel* plot. On the first day of physiological sampling, three time blocks were measured, while one time block was measured on the second day. Immediately following physiological measurements, root systems and above-ground tissues (leaves and stems) of these seedlings were separated and placed in coolers and transported to a laboratory for growth measurements.

3.2.5.2 Morphological responses and growth

Seedling mortality was determined in all aspect/soil amendment treatments. Height growth of every seedling was measured from the bud scar to the terminal bud tip. Terminal dieback was also measured, and total seedling height was calculated by subtracting the length of dieback along the stem. Dry mass of new stem growth was measured as the total mass of all newly formed shoots, branches and the portion of the shoot grown during the experimental period (bud scar to terminal leader) without leaves. Total seedling leaf area was measured using a Leaf Area Meter (LI-3100C, LI-COR, Nebraska, U.S.A). Leaves were then dried in an oven at 30 °C for 72 hours to measure dry leaf mass production. Root volume and root mass growth, as well as change in RSR (without leaves), were calculated by subtracting the initial root volume, dry mass and RSR of each stock type from the final root volume, mass and RSR of each planted seedling. All physiological and morphological measurements were then averaged for each stock type per plot for the statistical analysis of each experiment.

3.2.6 Data Analysis

All data collected during the final harvest period was analyzed using the PROC MIXED function in SAS (SAS 9.2, SAS Institute, North Carolina, U.S.A.). A one-way analysis of variance (ANOVA) was used to compare bioavailable soil nutrients among *North*, *South*/*Control* and *Hydrogel* plots. Differences in soil temperature, water content and soil water potential between aspect treatments and soil amendment treatments were analyzed using repeated measures with plot as a random effect. Physiological and morphological growth responses in the aspect study were analyzed using a two-way ANOVA with a 3 x 2 factorial design. In the soil amendment study, physiological and morphological growth responses were analyzed using a split plot design to compare *Hydrogel* and *Control* treatments. Time blocks were used as random variables for the analysis of g_s and shoot water potential in both experiments.

Prior to any statistical analysis, the residuals of each response variable were tested for normality and homogeneity of variance. Residuals were visually examined for normality, while homogeneity of variance was analyzed using Levene's test. In the analysis of nutrient availability, total N, NO_3^-, and K^+ had unequal variance. Height growth, stem mass growth, leaf area, leaf mass, g_s and shoot water potential had unequal variance in the aspect experiment, while in the soil amendment experiment height growth, stem mass growth, leaf area, leaf mass, root mass, root mass growth, and total seedling mass had unequal variance. Variables with unequal variance were not transformed, and were instead analyzed using ANOVA models with covariance structures that account for unequal variance. The ANOVA model with the best fit was then selected based on Akaike's Information Criterion (AIC) (Milliken and Johnson 2011). Following ANOVA analysis, the least square means for response variables with significant interactions were compared using a Tukey-Kramer adjustment with significance set at $\alpha=0.05$. Least square means for response variables with only significant main effects were compared using an LSD test with a Bonferroni adjustment and significance set at $\alpha=0.017$.

3.3 Results

3.3.1 Weather conditions following planting

Weather conditions for the experimental period were characterized by warm temperatures and high precipitation relative to the previous six years. June had an average temperature of 17 ˚C with 14.6 mm more cumulative precipitation than 2006-2011. July had an average temperature of 20 ˚C, and experienced heavy rainfall events early in the month which resulted in 88 mm cumulative precipitation. Following this period, rainfall events were less frequent, and August received 33.6 mm less cumulative precipitation than 2006-2011 and had an average temperature of 18 °C (Figure 11, 12C, 12D).

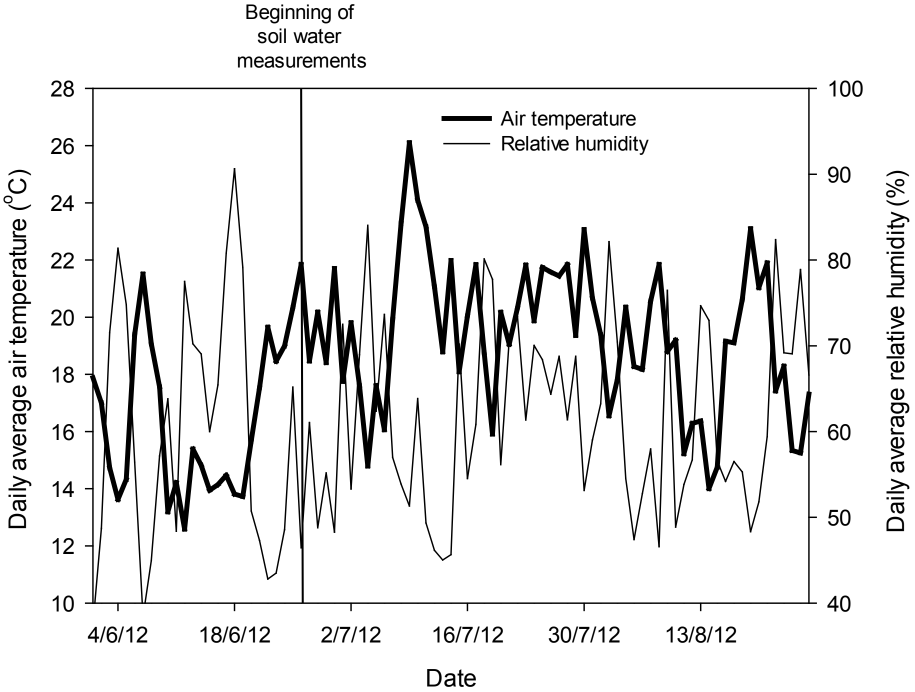

Figure 11. Daily average air temperature and relative humidity from June 1-August 26, 2012. Data was collected from Agroclimate Canada's Mildred Lake meteorological station.

3.3.2 Soil temperature and moisture

Soil temperature at 10 cm depth in *South* plots was on average 1.2 ˚C higher than *North* plots throughout the growing season ($P < 0.001$) (Figure 12A). This temperature difference increased to 2˚C at the end of August. Differences in soil temperature were more subtle between *Control* and *Hydrogel* treatments. *Hydrogel* plots were 0.4 ˚C cooler than *South* plots on average ($P = 0.007$) (Figure 12A). At 20 cm depth, soil temperatures fluctuated similarly to the soil temperatures at 10 cm in all aspect/ soil amendment treatments, however, peaks in temperature typically lagged by a day (all $P < 0.002$) (Figure 12B).

Soil water content was higher at 10 and 20 cm depth on *North* aspects than South aspects during the harvest period (all $P < 0.001$) (Figure 12E). However, soil water content did not differ between *North* and *South* treatments at either depth over the entire course of the growing season ($P = 0.164$ and $P = 0.125$, respectively). This can largely be attributed to heavy precipitation events in June and July, which resulted in consistently high soil moisture in all treatments for the majority of the experiment (Figure 12C, 12D). Soil water potential was higher in the *North* treatment than the *South* treatment overall (all $P < 0.013$) (Figure 12E, 12F).

While daily soil water content in *Hydrogel* plots tended to be higher than in *Control* plots at both 10 cm and 20 cm depth, it did not significantly differ between the *Hydrogel* and *Control* treatments (both $P \geq 0.193$) (Figure 12C, 12D). Soil water potential also did not differ between the treatments at either depth ($P \geq 0.136$) (Figure 12E, 12F), which may be attributed to large early-season rainfall events (Figure 12C, 12D).

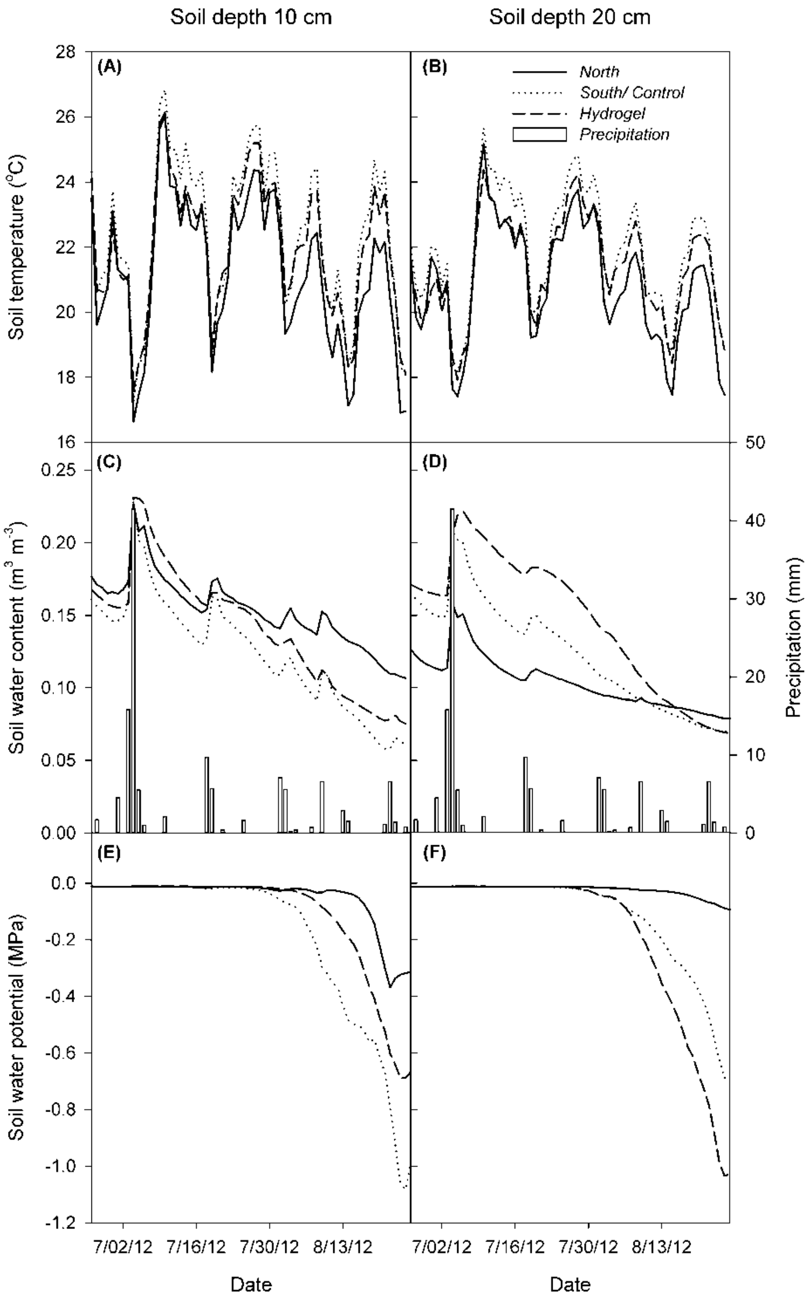

Figure 12. Daily average Soil temperature (A), volumetric soil water content and daily precipitation (C) and soil water potential (E) at 10 cm soil depth, and average soil temperature (B), volumetric soil water content and daily precipitation (D) and soil water potential (F) at 20 cm soil depth of aspect/soil amendment treatments from June 26-August 25, 2012. Precipitation data was collected from Agroclimate Canada's Mildred Lake meteorological station (n=7).

3.3.3 Soil bioavailable nutrients

The nitrogen supply rate (total available N, NO_3^-, and NH_4^+) did not differ between *North* and *South* aspects; however, *South* plots had 1.7 and 2 times greater supply rates of P and K^+, respectively, than the *North* plots (both $P< 0.002$). Supply rates of NH_4^+ did not differ between soil amendment treatments, but *Hydrogel*-amended soils had 4.6 times greater NO_3^- compared to *Control*s ($p< 0.001$), which resulted in higher total nitrogen availability in the *Hydrogel* treatment ($p= 0.002$) (Table 8). Further, soils amended with *Hydrogel*s had 7.8x and 1.8x higher Potassium (K^+) and P supply rates, respectively (both $p< 0.001$).

Table 3. Summary of the average (± SE) vertical nutrient supply of rate of soil bioavailable nutrients measured from the soil surface of the three aspect/ soil amendment treatments (n=10). Different letters indicate differences among means.

	Nutrient supply rate (μg 10 cm^2 32 days^{-1})		
Soil nutrient	*North*	*South/Control*	*Hydrogel*
Total available N	14.4 ± 1.3 **b**	14.6 ± 0.8 **b**	38.6 ± 6.9 **a**
NO_3^-	9.2 ± 1.2 **b**	6.9 ± 1.0 **b**	31.5 ± 6.8 **a**
NH_4^+	5.2 ± 1.1 **a**	7.7 ± 1.0 **a**	7.1 ± 1.1 **a**
P	3.7 ± 0.4 **c**	6.3 ± 0.8 **b**	11.1 ± 1.0 **a**
K^+	125.3 ± 17.8 **c**	251.5 ± 29.4 **b**	1972.5 ± 213.3 **a**

3.3.4 Aspect and stock type selection

3.3.4.1 Early establishment and seedling growth

No seedling mortality was observed in any stock type or aspect/soil amendment treatment. The pattern of height growth and shoot mass growth (data not shown) in response to stock type and aspect were the same for both response variables. All stock types experienced greater stem growth (height and mass) in the *South* than the *North* treatment (both P< 0.001). However, the difference in height and stem mass growth between seedlings growing in the *North* and *South* treatments was greater in the *highRSR* stock type (difference of 13.0 cm and 0.7 g) than in the *highNSC* (4.9 cm and 0.2 g) and *large* (4.0 cm and 0.2 g) stock types, resulting in significant stock type by aspect interaction terms for height growth (P= 0.055) and new stem mass growth (P= 0.005) (Figure 13). Regardless of aspect, the *highRSR* stock type had overall the greatest height growth compared to the other two stock types with the *large* stock type having the lowest height growth (p< 0.001). While height growth was similar between *large* and *highNSC* seedlings on *South*-facing plots, *highNSC* seedlings had greater height growth when grown in *North*-facing plots (Figure 13).

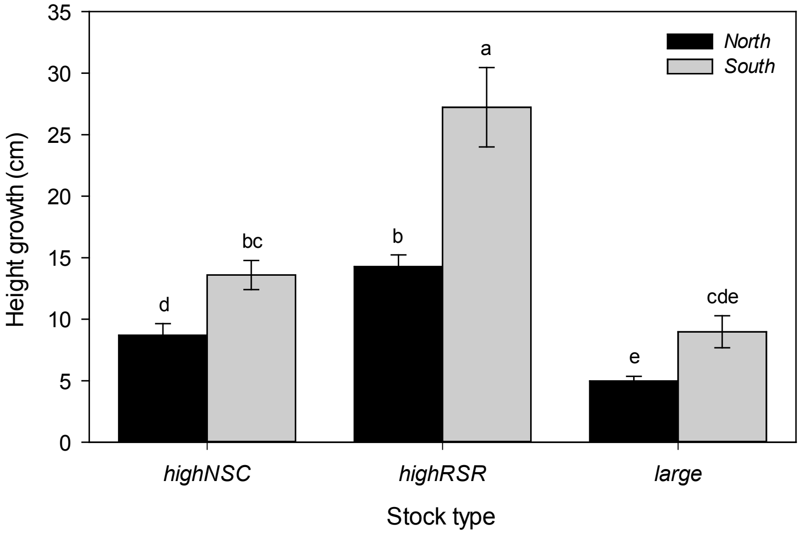

Figure 13. Average height growth after the first growing season of three aspen seedling stock types planted on North and South facing slopes. Error bars represent one standard error of the mean and different letters indicate differences among means (n=10).

Both leaf area and leaf mass during the first growing season after out-planting were 83 % and 73 % greater in seedlings growing on *South* slopes than on *North* slopes (both $P < 0.001$) (data not shown). The *highRSR* stock type produced the highest leaf area (183.18 cm^2) compared to both the the *highNSC* (133.74 cm^2) and *large* (140.62 cm^2) stock types ($P = 0.0153$). *HighNSC* seedlings had less leaf mass (2.1 g) than both the *highRSR* (2.5 g) and *large* seedlings (2.6 g), which did not differ ($P = 0.047$) (data not shown).

There was a slight trend of greater root volume growth of aspen seedlings grown on a *South* aspect ($P = 0.052$) (data not shown). Overall, *highNSC* (10.8 mL^3) and *large* (11.1 mL^3) seedlings had greater root volume compared to the *HighRSR* seedlings (6.76 mL^3) (data not shown). However, all stock types added similar amounts of root volume to their root systems (data not shown).

Overall, seedling root to stem ratio (RSR, without leaves) declined more from initial conditions in the *South* than in the *North* treatment ($P < 0.001$; Figure 14). This decline was driven by high stem growth on *South* plots (Figure 13). Within the *highNSC* and *large* stock types, change in RSR was similar between aspect treatments, while *highRSR* seedlings showed a larger decrease in RSR from initial conditions in the *South* (-3.1 $g\ g^{-1}$) than the *North* (-1.8 $g\ g^{-1}$) treatment; this resulted in a significant interaction effect ($P = 0.029$) (Figure 14). RSR decreased the most in the *highRSR* stock type in both aspect treatments, which can be attributed to their greater stem growth over the first growing season. RSR of *highNSC* seedlings also decreased slightly in both aspect treatments. In contrast, RSR of *large* seedlings increased slightly in both treatments, though this change was minimal (Figure 14).

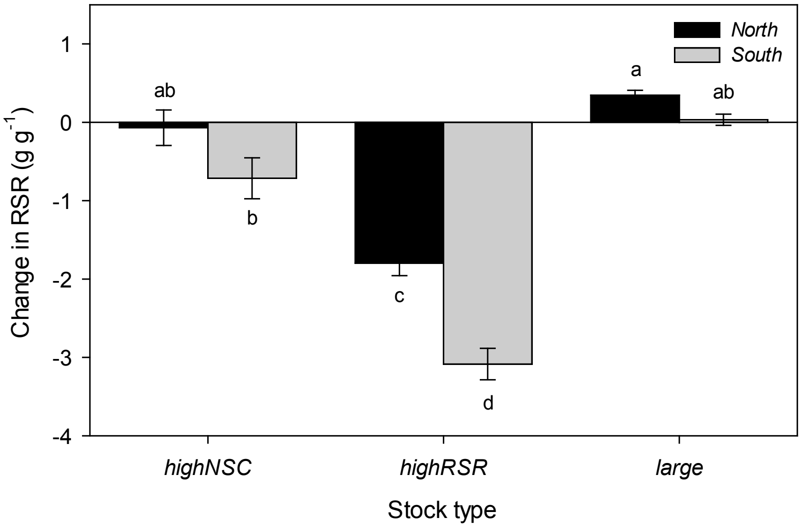

Figure 14. Average change in root to stem ratio (RSR without leaves) from initial conditions after the first growing season for three aspen seedling stock types planted on a North and South facing slope. Error bars represent one standard error of the mean and different letters indicate differences among means (n=10).

3.3.4.2 Physiological responses of aspen seedling stock types

Stomatal conductance (g_s) did not differ among the three stock types and the *North* and *South* aspects (P= 0.746 and P= 0.303, respectively), but seedlings grown on *South* aspects had lower shoot water potentials (-1.79 MPa) than those grown on *North* aspects (-1.55 MPa) (P=0.016). However, shoot water potential did not differ among the three stock types (P= 0.550).

3.3.5 Hydrogel amendment and stock type selection

3.3.5.1 Early establishment and seedling growth

Height growth and stem mass growth were 78 % and 74 % greater in seedlings that grew in soil amended with *Hydrogel* (both P< 0.045) (data not shown). Overall, the *highRSR* stock type had greater stem growth (29.4 cm height growth and 1.3 g stem mass growth) than both the *highNSC* (13.8 cm and 0.7 g) and *large* (12.6 cm and 0.7 g) stock types (both P< 0.001) (data not shown). Total leaf area and leaf mass also did not differ among the three stock types (both P≥ 0.211) (data not shown); however, leaf area and leaf mass in the *Hydrogel* treatment increased by 40 % and 30 %, respectively, (330.5 cm^2 and 2.8 g) relative to the *Control* treatment (197.4 cm^2 and 2.0 g) (both P< 0.023) (Figure 15).

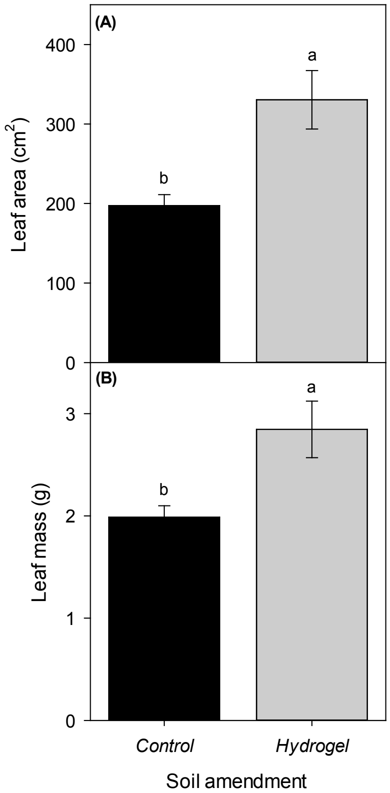

Figure 15. Average leaf area (A), and leaf mass (B) production during the first growing season of aspen seedlings (all three stock types combined) planted in different soil amendments. Error bars represent one standard error of the mean and different letters indicate differences between means (n=30).

Hydrogel application resulted in increased root volume growth (P =0.012); however this was dependent on the stock type. While *Hydrogel* application increased root volume growth by 80 % and 104 % in the *highNSC* and *large* stock types, respectively, it did not affect root volume growth in *highRSR* seedlings. This response resulted in a significant hydrogel by stock type interaction effect (P= 0.015) (Figure 16). *Large* seedlings had the most root volume growth in both *Hydrogel* and *Control* treatments, while *highRSR* seedlings had the least root volume growth in both soil treatments (Figure 16).

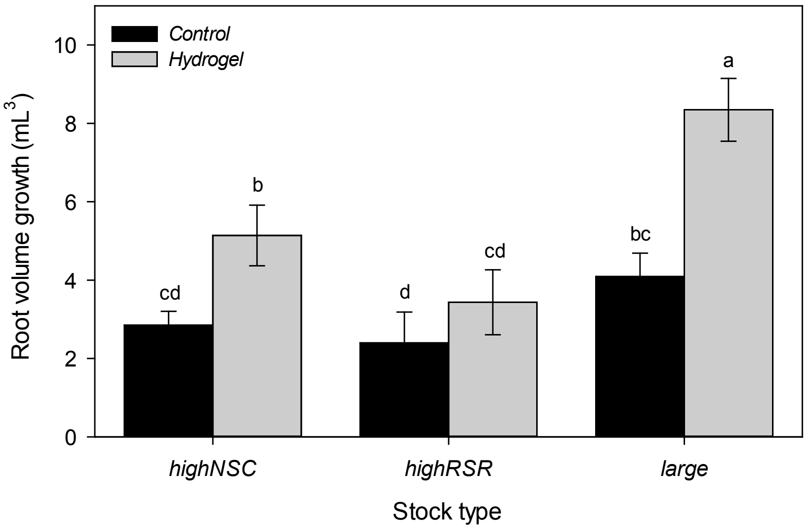

Figure 16. Average root volume growth following one growing season of three aspen seedling stock types planted in different soil amendment treatments. Error bars represent one standard error of the mean (n= 10) and different letters indicate differences among means.

Total seedling size (mass) of the *highNSC* and *highRSR* stock types was unaffected by the *Hydrogel* application, while the *Hydrogel* amendment increased total seedling mass by 4.3 g in the *large* stock type (Hydrogel by stock type interaction effect

P= 0.043) (Figure 17). Similar to initial conditions, the *large* seedlings were still the largest in terms of total mass in both treatments, while *highRSR* seedlings had the lowest total mass (Figure 17).

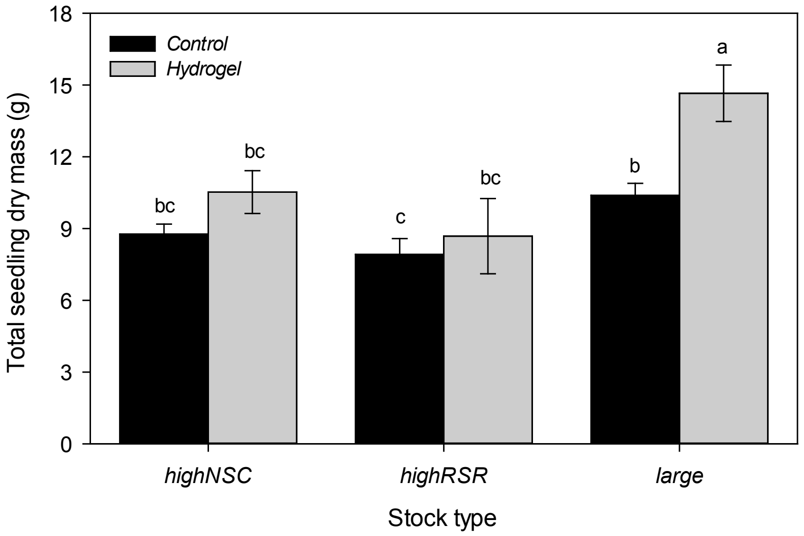

Figure 17. Average total seedling mass following one growing season of three aspen seedling stock types planted in different soil amendment treatments. Error bars represent one standard error of the mean (n= 10) and different letters indicate differences among means.

Overall, *Hydrogel* application had no effect on the RSR of all seedling stock types (P= 0.889) (data not shown). After the first growing season the *highRSR* stock type had the largest decrease from initial conditions, 4.9 g g^{-1} to 2.0 g g^{-1}, followed by the *highNSC* stock type, which decreased from 3.2 g g^{-1} to 2.5 g g^{-1}, and the *large* stock type (from 1.62 g g^{-1} to 1.56 g g^{-1}) (P< 0.001). After one growing season the RSR continued to be higher in the *highNSC* seedlings compared to *large* seedlings (P< 0.001); however, the RSR in *highRSR* seedling was intermediate between the *highNSC* and the *large* seedlings (data not shown).

3.3.5.2 Physiological responses of aspen seedling stock types

Hydrogel application had no effect on g_s or shoot water potential across all three stock types (P= 0.172 and P= 0.188, respectively). Similarly, there were no differences in g_s (P= 0.504) or shoot water potential (P= 0.733) among stock types.

3.4 Discussion

The *highRSR* stock type had the best above-ground growth performance (stem growth and foliar production) of all stock types one year after out-planting in both aspect treatments, despite having the smallest root systems. This stock type's higher initial root allocation may have enabled it to uptake relatively more water and nutrients than the other two stock types (Chapin et al. 1986; Poorter and Nagel 2000; Grossnickle 2005), which allowed for greater water and nutrient allocation to above-ground tissues (Landhäusser et al. 2012a) for foliar development and shoot growth (Hilbert 1990; Van der Werf et al. 1993). However, it is important to note that the *highRSR* stock type had relatively high initial NSC reserves (concentration); NSC reserves provide a readily accessible carbon source for shoot growth in planted aspen seedlings prior to the advent of photosynthesis (Landhäusser and Lieffers 2002), and it is likely that high initial NSC concentration played a role in early shoot growth. As in previous studies assessing aspen seedling quality, we were unable to entirely differentiate the effects of RSR and NSC concentration on planting performance (Martens et al. 2007; Landhäusser et al. 2012b). Despite this, *highRSR* seedlings had greater shoot growth than *highNSC* seedlings, which had similar initial NSC concentrations, suggesting that RSR was the main characteristic driving first-year growth performance.

Conversely, high NSC content and large seedling size did not improve growth performance and height growth and foliar production was actually similar between *highNSC* and *large* stock types overall. This result was surprising, as previous research found that aspen seedlings with high NSC reserves had greater growth rates than large, nursery grown seedlings when out-planted onto reclamation areas (Martens et al. 2007; Landhäusser et al. 2012b). While this suggests that NSC reserves at the time of planting had no influence on seedling growth, the lower growth exhibited in the *highNSC* stock type may have also resulted from growth lags attributed to paclobutrazol application. Jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Ait.) and Eastern larch (*Larix laricina* (Du Roi) K. Koch) seedlings treated with high concentrations of paclobutrazol have experienced stem and foliar growth lags the growing season following application (Rietveld 1988), though no growth lags were observed in out-planted aspen seedlings one season after application (Landhäusser et al. 2012b); however, the active ingredient in the stem growth inhibitor used in this experiment was double that of the one used in the latter study, which may potentially explain the *highNSC* stock type’s low above-ground growth.

Despite clear differences in above-ground growth, root growth did not differ among stock types in either aspect treatment; similar results were observed in Chapter 2, which suggests initial seedling characteristics have little influence on below-ground growth and that root growth is more greatly influenced by environmental factors. As well, the *highNSC* and *highRSR* stock types showed reduced RSR at the conclusion of the experiment and greater growth allocation to stem tissues. While it was initially thought that large seedlings with low RSR would show greater root growth allocation, RSR only slightly increased in the *North* treatment and stayed the same in the *South* treatment despite some root growth on both aspects. The experimental area experienced heavy rainfall early in June and July, and there was ample water available in all treatments for the majority of the experiment. It is likely seedlings were able to disproportionately allocate resources to above-ground growth over root development in the absence of moisture limitation.

All stock types planted in *South* plots experienced greater above-ground growth (stem growth and leaf area development) than stock types planted in *North* plots overall. This result was somewhat surprising, as it was initially predicted that seedlings grown on *South*-facing slopes at Northern latitudes would experience greater transplant shock than seedlings grown on *North*-facing slopes due to low soil moisture availability and high VPD (Hasler et al. 1982; Letts et al. 2009). Soil temperature was higher and soil water potential was lower on *South* slopes than *North* slopes at the time of seedling harvest, which was indicative of moisture stress and consistent with previous research (Leij et al. 2004; Fisher et al. 2005). This resulted in seedling shoot water potential being lower in the *South* (-1.79 MPa) than the *North* treatment (-1.55 MPa). These lower shoot water potentials, however, might not have been translated into stress as aspen do not experience loss of conductivity greater than 50 % until water potentials in the shoot decline below -2.3 MPa (Cai and Tyree 2010). Therefore it is very unlikely that seedlings on South slopes experienced severe drought stress and a significant loss of stem conductivity. Large rainfall events early in the growing season resulted in soil water content and water potential being equivalent to field capacity until late into the growing season (August 9, 2012); additionally, June and July received 42 mm more precipitation in the experimental area than in the previous 6 years, which indicates that there was ample plant available water in both aspect treatments for growth. This suggests that drought conditions did not exist for the majority of the experiment, and that other abiotic site factors may have resulted in the different seedling growth responses observed between aspects.

Greater stem growth and foliar production in the *South* treatment could be credited to warmer soil temperatures. Studies have found that higher soil temperatures decreased hydraulic resistance in the root systems of English oak (*Quercus rubur* L.) (Cochard et al. 2000) and trembling aspen (Way et al. 2013), which resulted in greater hydraulic conductance in leaf tissues and at the whole seedling level in the latter study. High hydraulic conductivity can result in a more balanced seedling water status, which can in turn allow for greater leaf area development and growth. Similarly, warm soil temperatures have been associated with greater above-ground growth in aspen seedlings, including higher leaf area production (Landhäusser and Lieffers 1998; Wan et al. 1999, 2001; Dang and Cheng 2004; Peng and Dang 2003) and root and stem mass growth (Landhäusser and Lieffers 1998; Wan et al. 1999, 2001).

Additionally, high soil temperatures in the *South* treatment may have had a profound effect on soil nutrient availability, which likely also influenced seedling growth. Phosphorus (P) and potassium (K) availability was considerably higher on *South*-facing plots, while total N availability did not differ between aspect treatments. Several studies suggest that N (Matson and Vitousek 1981; Van Cleve et al. 1983; Chapin et al. 1995; Rustad et al. 2001) and P (Mackay and Barber 1984; Van Cleve et al. 1983; Chapin et al. 1995) nutrient availability and uptake increase with warmer soil temperatures. In two field studies conducted in central Alberta, aspen seedlings treated with fertilizer containing high P concentration experienced greater height and volume growth following one growing season (Van den Driessche et al. 2003; Van den Driessche et al. 2006), while other studies found that aspen grown from seed had greater root growth and leaf area when potted in soil containing high P concentration (Chapin et al. 1983; Wolken et al. 2010). Thus, it is likely that high P availability on *South* slopes contributed to the high above-ground growth observed within this treatment.

Unexpectedly, there was no observable difference in N availability between *North* and *South*-facing slopes, despite warmer soil temperatures in the *South* treatment. This has been observed in several studies; Malhi and McGill (1981) observed that nitrification rates of soil samples collected in central Alberta decreased as soil temperatures increased from 20 °C to 30 °C, while Dannenman et al. (2007) found that total available N did not differ between North and South-facing aspects within beech (*Fagus sylvatica* L.)-dominated stands in Southern Germany. While warmer soil temperatures generally result in increased nitrification, decreasing soil water availability can significantly reduce soil microbial activity (Dannenmann et al. 2009) and inhibit gross nitrification (Smith et al. 2003; Gessler et al. 2005). It is possible that increases in soil nitrification at warmer temperatures were offset by the drying soil conditions in August, which could have inhibited N availability in the *South* treatment (Rennenberg et al. 2009).

Hydrogel application did not significantly increase plant available water (bulk soil water content or soil water potential) in amended plots. This is contrary to previous studies where hydrogel treatments applied at similar concentrations doubled soil water content compared to non-amended substrates (Al-Humaid and Moftah 2007; Bhardwaj et al. 2007). In our experiment, high precipitation rates early in the growing season led to high soil water content and less-negative water potential on plots with Southern exposure, which may have reduced the water-holding efficiency and need of hydrogel application during this particular growing season. Subsequently, hydrogel application did not decrease hydraulic stress (shoot water potential) or improve stomatal conductance (g_s) in seedlings.

Contrarily, the bioavailability of N (NO_3^-), P and K^+ were considerably greater in hydrogel-amended soils. Several studies have found that the application of various hydrogels slows the leaching of nutrients including N, P and K^+, though the rate of leaching of NO_3^- was unaffected in these studies (Magalhaes et al. 1987; Bres and Weston 1993; Mikkelsen et al. 1993; Mikkelsen 1994). Alternatively, higher nutrient bioavailability measured in hydrogel-amended plots may have resulted from increased nutrient transport. While soil water content was not higher in amended plots over the entire course of the experiment, it was generally higher on a daily basis; diffusion of soil nutrients generally increases with increasing water content (Klute and Letey 1958; Barber 1962), and it is possible that high water content in the *Hydrogel* treatment increased nutrient transport to the roots of our seedlings. Regardless of the mechanism, it is likely that the higher nutrient availability in the *Hydrogel* treatment led to the greater stem, root and leaf growth observed in this experiment. Shoot growth and leaf development increases significantly in aspen seedlings with increasing P availability (Chapin et al. 1983; Van den Driessche et al. 2003; Van den Driessche et al. 2006; Wolken et al. 2010), while seedlings exposed to high levels of NO_3^- had greater root growth in snow gum (*Eucalyptus pauciflora* Sieber ex Sprengel.) grown in glasshouses (Atwell et al. 2009) and greater leaf area and shoot volume in cultured, one year old aspen (Siemens and Zwiazek 2013). While seedlings with high RSR still experienced the greatest shoot growth overall, large seedlings with low RSR had considerably more root growth and increased the most in total size in the *Hydrogel* treatment. Seedlings with low RSR (*large*) have relatively less nutrient supplies to satiate above-ground hydraulic demands and growth, and it is likely that they were more reactive to the high nutrient availability in *Hydrogel*-amended soils.

3.5 Conclusions

These two field studies suggest that aspen seedlings with high root mass allocation (*highRSR*) perform better in terms of above-ground growth across a variety of field sites, including both *North* and *South*-facing aspects and on amended soils, though high NSC concentration may also be a characteristic beneficial to out-planting success . Contrarily, high NSC reserves (content) do not appear to directly improve growth performance or out-planting success, and large seedling size coupled with low RSR appears to be detrimental to stem and foliar growth, particularly on sites with limited nutrient availability and lower air/soil temperatures. However, out-planting seedlings with large root systems can improve root growth on non-stressed reclamation sites with good growing conditions.

Hydrogel amendments in our study did not increase plant water availability or improve seedling water status, as we had a wet summer. Further study of hydrogel application under moisture-limiting conditions is required to determine the suitability of its use on drought-prone boreal reclamation sites. Despite this, hydrogel application resulted in higher soil nutrient availability, which greatly improved aspen root, shoot and leaf growth. This suggests that hydrogel amendments may still be beneficial to aspen growth on boreal reclamation sites, even under ideal conditions where water is not limiting.

Chapter 4: General discussion and conclusions

4.1 Research summary

In my first experiment, the first-year growth performance and physiological status of seedling stock types with varying size (height and root volume), root to stem ratio (RSR) and non-structural carbohydrate (NSC) reserves (content and concentration) exposed to three varying levels of controlled moisture stress (*Con*, *Ml*, and *Sev*) was assessed in a controlled growth chamber experiment. The results from this experiment showed that drought stress reduced seedling growth and physiological functioning, though responses to drought were dependent on initial seedling characteristics. Under both well-watered conditions and drought stress, seedlings with high RSR (*highRSR*) displayed the greatest stem growth (height and mass) and leaf production, and had higher growth allocation to stem tissues. In contrast, larger seedlings (height and root volume, *large*) and seedlings with high NSC reserves (*highNSC*), both with lower RSR relative to *highRSR*, allocated less growth to above-ground tissues under all watering regimes which resulted in an increase in RSR. While root growth decreased under drought stress, it did not vary among stock types, indicating below-ground growth responses were independent of initial seedling characteristics and were more greatly influenced by drought.

In terms of physiological responses, all seedlings displayed decreased stomatal conductance (g_s) and shoot water potential as drought intensity increased. Seedlings displayed similar shoot water potentials except under *Sev* drought, where *highRSR* seedlings were under greater hydraulic strain. This was attributed to the highRSR seedlings' high leaf area production before the onset of drought, which likely increased transpirational water loss and subsequently led to increased xylem tensions (Struve and Joly 1992; Haase and Rose 1993; Chaves et al. 2003).

While all seedlings reduced g_s under drought stress to limit transpiration, this also restricted photosynthesis and led to reductions in root and total seedling NSC concentrations. In contrast, seedling soluble sugar concentrations increased with increasing drought severity, which was likely to prevent desiccation and to maintain cellular turgor through osmotic adjustment (Chaves 1991; Chaves et al. 2002; Close et al. 2005). NSC concentration in all organs remained higher in seedlings with high initial reserves (*highNSC*) while higher growth in *highRSR* decreased NSC concentration to levels similar to *large*; however, NSC response to drought was similar among stock types despite clear differences in growth. This suggested that initial seedling characteristics had little influence on NSC reserves under drought, and that drought stress had a stronger influence on NSC than growth allocation.

The results from this experiment prompted me to test the first-year growth and physiological performance of the same aspen stock types after planting on a stressful boreal reclamation site. Additionally, I assessed the influence of hill slope and aspect on drought and the performance of planted seedlings.

Growth responses among stock types showed similarities between the controlled drought experiment and the field experiment. In both experiments, seedlings with high initial RSR had the greatest stem growth and foliar production regardless of moisture conditions or aspect treatment, and had the highest growth allocation to above-ground tissues. As well, shoot growth was lower in seedlings with low initial RSR, and the *large* stock type with the lowest RSR showed the poorest above-ground growth performance after out-planting. Much like in the first experiment, environmental factors, such as soil temperature nutrients in this study, had a greater influence on root growth than initial seedling characteristics.

In terms of aspect, shoot water potential was lower in seedlings out-planted on *South* slopes than *North* slopes (-0.24 MPa lower), which indicates that seedlings were under greater hydraulic stress on *South*-facing aspects at the time of sampling. However, unlike the controlled drought experiment, seedlings were not exposed to continuous drought conditions, as large precipitation events early in June and July increased volumetric soil water content and water potential to field capacity conditions for the majority of the experimental period. Rather, growth was influenced by other site factors. Warm soil temperatures due to high solar inputs, coupled with ample water availability, increased phosphorus and potassium availability (MacKay and Barber 1984; Van Cleve et al. 1983; Chapin et al. 1995) on *South* slopes relative to *North* slopes, which resulted in greater stem, leaf and root growth.

In a separate experiment, I tested whether hydrogel soil amendments improved plant water availability and seedling performance under drought stress. In this experiment, all *South* plots were paired with hydrogel plots, as it was initially hypothesized that South-facing hill slopes would be exposed to higher temperatures, vapor pressure deficits and soil water constraints (Hasler 1982; Leij et al. 2004; Fisher et al. 2005). However, wet growing season conditions reduced the water-holding efficiency and need for hydrogel. As a result, volumetric soil water content and soil water potential did not differ between amended and non-amended plots and shoot water status was similar among seedlings. However, NO_3^-, P and K^+ availability was higher on amended plots, which resulted in higher stem, leaf and root growth than on non-amended soils; however, the mechanism behind this increase in nutrient availability is not yet fully understood.

My thesis aimed to evaluate the influence of seedling characteristics on the growth performance and physiological functioning of aspen seedlings exposed to drought stress, and to evaluate how these characteristics influence the growth performance of seedlings planted onto stressful mine reclamation sites. The findings of my experiments suggest that high RSR is the characteristic most beneficial to growth of aspen seedlings under drought stress, and seedlings with this characteristic are better able to overcome transplant shock when planted onto reclamation sites. In contrast, large seedling size (height and root volume) and high NSC content did not improve seedling growth or physiological performance under drought or out-planting success, and low RSR appears to be the characteristic most detrimental for growth on stressful reclamation sites.

4.2 Management implications

Currently, the production of nursery-grown aspen seedlings is focused on generating tall seedlings with large root collar diameters. These characteristics have long been thought to be accurate predictors of seedling quality and establishment success. However, my research indicates that these parameters are poor predictors of out-planting performance on drought-stressed sites. Tall seedlings with low root mass allocation actually had the poorest above-ground growth performance following out-planting and under simulated drought stress. Thus, it is not recommended that these characteristics be used to assess seedling quality in nursery-produced aspen stock.

Recent research has suggested that high RSR and NSC reserves are better predictors of aspen seedling quality and performance following planting on boreal reclamation sites (Martens et al. 2007; Snedden 2010; Landhäusser et al. 2012b). The results of my research reaffirm the findings of these studies, and suggest that seedling stock with these characteristics are suitable for planting under drought stress and thus should be favored for use on stressful reclamation sites. In particular, RSR appears to be the main characteristic driving first-year growth performance under drought stress; high root mass allocation may allow for greater water and nutrient uptake (Chapin et al. 1986; Poorter and Nagel 2000; Grossnickle 2005) and allocation to stem and leaf growth (Landhäusser et al. 2012a; Hilbert 1990; Van der Werf et al. 1993). While it is likely that high tissue NSC concentration aided in early shoot growth (Landhäusser and Lieffers 2002), *highRSR* seedlings had greater above-ground growth than *highNSC* seedlings, despite possessing similar initial NSC concentrations. Selecting aspen stock with high RSR appears to be the best option to reduce transplant shock in out-planted seedlings. By planting high quality seedlings with high RSR on stressful sites, the time required to reach canopy closure could decrease, which could aid in weed suppression and decrease the time required to establish a self-sustaining forest ecosystem.

Therefore, it is recommended that nurseries focus on producing aspen seedling stock with high RSR for use on stressful boreal reclamation sites. While stem growth inhibitors (SGI) such as paclobutrazol have been successfully used to induce bud set and increase RSR and NSC concentrations in treated aspen seedlings (Landhäusser et al.

2012a; Landhäusser et al. 2012b), I found that developing aspen stock by exposing seedlings to outside growing conditions for longer time periods following germination resulted in higher RSR and similar NSC concentrations to stock created using SGI. This method of stock creation is relatively easy to impose and is recommended for nursery production of aspen seedlings, as it resulted in higher quality stock.

4.3 Research Limitations and Future Research

In the controlled drought experiment, seedlings subjected to moisture stress displayed less growth and more negative shoot water potentials than well-watered seedlings. However, we observed very little differentiation in above-ground growth responses and shoot water potential within stock types as drought severity increased from *Ml* to *Sev.* While tight stomatal regulation under drought stress likely prevented shoot water potential from decreasing substantially under *Sev* drought (Tardieu and Simonneau 1998), it is also possible that moisture limitations in the *Sev* treatment were not intense enough to elicit different growth responses from the *Ml* treatment.

The *highRSR* stock type experienced the greatest above-ground growth under drought stress as well as following out-planting. However, *highRSR* was also characterized by its high NSC reserves (concentration), which may have contributed to its superior growth. Much like the research of Martens et al. (2007) and Landhäusser et al. (2012b), we were not able to fully differentiate the influence of NSC concentration and RSR on aspen growth. In order to fully understand which characteristic best improves seedling quality, it may be necessary to develop and contrast stock types that differ entirely in their initial characteristics by altering their growing conditions.

This research provides useful insight into developing and selecting high quality seedlings with characteristics beneficial to planting on dry reclamation sites. While NSC concentration may play a role, our results suggest that high root mass allocation is the characteristic that results in the best out-planting performance in the first growing season. However, most previous research only assesses out-planting performance following one or two growing seasons, and it has yet to be determined if the better initial performance of the *highRSR* stock type would continue in following years. *HighRSR* was able to allocate more growth to shoot tissues following out-planting and exposure to drought, though this subsequently led to large reductions in RSR. It is possible that the high growth performance of this stock type may not be fully replicated in the future due to lower initial RSR at the start of the following growing season.

In the aspect and stock type selection experiment, site conditions were generally wet and we were thus not able to fully determine which seedling stock characteristics best improved performance on droughted reclamation sites. Despite site moisture conditions non-conducive to a drought study, this experiment was successfully able to explore how site factors of different aspects, such as soil temperature and nutrient availability, influenced seedling growth and physiology. However, air temperature data from each aspect treatment was not collected, and the effect of air temperature on seedling growth could therefore not be ascertained. While aspen seedlings exhibit greater shoot, root and leaf growth with warmer air temperatures, hydraulic resistance increases in leaf and shoot tissues at elevated temperatures (Way et al. 2013); as such, high air temperatures increase aspen's vulnerability to cavitation. Soil temperature was consistently higher on South aspects than North aspects, and it is probable that air temperature was as well. Air temperature likely had a profound effect on the growth patterns observed in this study, and in connection with low soil moisture at the time of sampling, may have lowered seedling hydraulic conductivity and resulted in the lower shoot water potentials observed in seedlings grown on South aspects.

Heavy precipitation during the early portion of the growing season reduced the water-holding efficiency and need for a hydrogel amendment in our field experiment. Further study must be conducted to properly evaluate the effectiveness of hydrogel amendment use in boreal reclamation under continuous drought conditions. In contrast, hydrogel amendments increased soil nutrient bioavailability. However, the mechanisms behind hydrogels increasing soil nutrient availability are not yet fully understood. Research assessing how hydrogels improve soil nutrient availability is required, as its application may prove useful in improving growing conditions on nutrient-deficient sites.

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Appendix 1

Table A1-1 PROC MIXED ANOVA results for seedling measurements obtained during the preliminary measurement period. The level of significance used is α = 0.05.

Initial seedling conditions	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
RSR (g g^{-1})	*Stock*	2	63	91.82	**< 0.001**
	Drought	2	63	0.78	0.463
	*Stock*Drought*	4	63	0.55	0.700
Total seedling NSC (g)	*Stock*	2	27	36.62	**< 0.001**
	Drought	-	-	-	-
	*Stock*Drought*	-	-	-	-
Total seedling NSC (%)	*Stock*	2	27	24.83	**< 0.001**
	Drought	-	-	-	-
	*Stock*Drought*	-	-	-	-
Height (cm)	*Stock*	2	63	359.77	**< 0.001**
	Drought	2	63	0.45	0.638
	*Stock*Drought*	4	63	0.29	0.886
Root collar diameter (mm)	*Stock*	2	63	99.81	**< 0.001**
	Drought	2	63	1.44	0.244
	*Stock*Drought*	4	63	0.15	0.962
Shoot mass (g)	*Stock*	2	63	240.96	**< 0.001**
	Drought	2	63	0.24	0.785
	*Stock*Drought*	4	63	0.26	0.902
Root mass (g)	*Stock*	2	63	42.15	**< 0.001**
	Drought	2	63	2.23	0.116
	*Stock*Drought*	4	63	0.87	0.485
Total mass (g)	*Stock*	2	63	102.85	**< 0.001**
	Drought	2	63	1.51	0.229
	*Stock*Drought*	4	63	0.5	0.735
Root volume (mL^3)	*Stock*	2	63	14.15	**< 0.001**
	Drought	2	63	2.6	0.082
	*Stock*Drought*	4	63	2.4	0.059

Appendix 2

Table A2-1 PROC MIXED ANOVA results for seedling morphological responses obtained following the final harvesting period. The level of significance used is α = 0.05.

Morphological response	Effect	Num Df	Den Df	F	P > F
Height growth (cm)	*Stock*	2	81	52.03	**< 0.001**
	Drought	2	81	24.15	**< 0.001**
	*Stock*Drought*	4	81	4.23	**0.004**
New shoot growth (g)	*Stock*	2	81	25.32	**< 0.001**
	Drought	2	81	45.85	**< 0.001**
	*Stock*Drought*	4	81	5.51	**0.001**
Leaf area (cm^2)	*Stock*	2	81	86.88	**< 0.001**
	Drought	2	81	156.4	**< 0.001**
	*Stock*Drought*	4	81	12.01	**< 0.001**
Leaf mass (g)	*Stock*	2	81	34.76	**< 0.001**
	Drought	2	81	150.27	**< 0.001**
	*Stock*Drought*	4	81	9.13	**< 0.001**
Root growth (g)	*Stock*	2	81	1.45	0.241
	Drought	2	81	64.16	**< 0.001**
	*Stock*Drought*	4	81	1.23	0.305
Root volume (mL^3)	*Stock*	2	81	14.87	**< 0.001**
	Drought	2	81	59.25	**< 0.001**
	*Stock*Drought*	4	81	0.69	0.598
RSR ($g\ g^{-1}$)	*Stock*	2	81	62.21	**< 0.001**
	Drought	2	81	1.78	0.175
	*Stock*Drought*	4	81	0.83	0.510
Change in RSR (%)	*Stock*	2	81	112.77	**< 0.001**
	Drought	2	81	1.78	0.175
	*Stock*Drought*	4	81	0.83	0.510

Table A2-2 PROC MIXED ANOVA results for seedling physiological responses obtained following the final harvesting period. The level of significance used is α = 0.05. *denotes a borderline significant interaction term.

Physiological response	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
Stem water potential (MPa)	*Stock*	2	81	9.17	**< 0.001**
	Drought	2	81	63.26	**< 0.001**
	*Stock*Drought*	4	81	2.46	0.052*
Net photosynthesis (μmol m^{-2} s^{-1})	*Stock*	2	81	2.32	0.105
	Drought	2	81	7.76	**0.001**
	*Stock*Drought*	4	81	1.50	0.211
Stomatal conductance (mol m^{-2} s^{-1})	*Stock*	2	81	1.48	0.235
	Drought	2	81	14.34	**< 0.001**
	*Stock*Drought*	4	81	0.67	0.613

Table A2-3 Results for a linear regression analysis between shoot water potential and leaf area in aspen seedlings exposed to different watering regimes. The level of significance used is α = 0.05.

Drought treatment	**df**	**SS**	**MS**	**F**	**P > F**	**R^2**	**Adjusted R^2**
Control	1.000	0.023	0.023	0.457	0.504	0.016	-0.019
Mild	1.000	0.544	0.544	6.286	0.018	0.183	0.154
Severe	1.000	1.469	1.469	23.905	< 0.001	0.461	0.441

Table A2-4 PROC MIXED ANOVA results for total NSC, soluble sugar and starch concentration/content of whole-tree, shoot and root organs in aspen seedlings obtained following the final harvesting period. The level of significance used is α = 0.05.

Seedling NSC fraction	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
Total seedling NSC concentration (%)	*Stock*	2	81	13.51	**< 0.001**
	Drought	2	81	7.29	**0.001**
	*Stock*Drought*	4	81	0.59	0.669
Total seedling NSC content (g)	*Stock*	2	81	21.20	**< 0.001**
	Drought	2	81	56.12	**< 0.001**
	*Stock*Drought*	4	81	0.19	0.942
Total seedling soluble sugar concentration (%)	*Stock*	2	81	26.38	**< 0.001**
	Drought	2	81	28.74	**< 0.001**
	*Stock*Drought*	4	81	1.76	0.145
Total seedling soluble sugar content (g)	*Stock*	2	81	20.06	**< 0.001**
	Drought	2	81	22.98	**< 0.001**
	*Stock*Drought*	4	81	0.39	0.817
Total seedling starch concentration (%)	*Stock*	2	81	5.82	**0.004**
	Drought	2	81	3.83	**0.026**
	*Stock*Drought*	4	81	1.63	0.175
Total seedling starch content (g)	*Stock*	2	81	17.89	**< 0.001**
	Drought	2	81	74.24	**< 0.001**
	*Stock*Drought*	4	81	0.22	0.926
Shoot NSC concentration (%)	*Stock*	2	81	27.09	**< 0.001**
	Drought	2	81	0.61	0.543
	*Stock*Drought*	4	81	1.75	0.148
Shoot NSC content (g)	*Stock*	2	81	68.50	**< 0.001**
	Drought	2	81	22.73	**< 0.001**
	*Stock*Drought*	4	81	0.78	0.541
Shoot soluble sugar concentration (%)	*Stock*	2	81	23.16	**< 0.001**
	Drought	2	81	15.01	**< 0.001**
	*Stock*Drought*	4	81	1.52	0.203
Shoot soluble sugar content (g)	*Stock*	2	81	52.61	**< 0.001**
	Drought	2	81	12.48	**< 0.001**
	*Stock*Drought*	4	81	0.35	0.843
Shoot starch concentration (%)	*Stock*	2	81	33.86	**< 0.001**
	Drought	2	81	24.46	**< 0.001**
	*Stock*Drought*	4	81	3.36	0.014
Shoot starch content (g)	*Stock*	2	81	77.22	**< 0.001**
	Drought	2	81	40.12	**< 0.001**
	*Stock*Drought*	4	81	2.33	0.063
Root NSC concentration (%)	*Stock*	2	81	5.36	**< 0.001**
	Drought	2	81	9.57	**< 0.001**
	*Stock*Drought*	4	81	0.61	0.658
Root NSC content (g)	*Stock*	2	81	17.37	**< 0.001**
	Drought	2	81	56.91	**< 0.001**
	*Stock*Drought*	4	81	0.25	0.910
Root soluble sugar concentration (%)	*Stock*	2	81	2.56	**0.084**
	Drought	2	81	1.13	0.329
	*Stock*Drought*	4	81	0.85	0.501
Root soluble sugar content (g)	*Stock*	2	81	12.29	**< 0.001**
	Drought	2	81	19.24	**< 0.001**
	*Stock*Drought*	4	81	0.40	0.805
Root starch concentration (%)	*Stock*	2	81	4.15	**0.019**
	Drought	2	81	19.81	**< 0.001**
	*Stock*Drought*	4	81	0.46	0.768
Root starch content (g)	*Stock*	2	81	19.23	**< 0.001**
	Drought	2	81	72.95	**< 0.001**
	*Stock*Drought*	4	81	0.28	0.890

Appendix 3

Table A3-1 Physiological responses (average ± SE) of three aspen seedling stock types (n=4) and seedlings of all stock types (n=12) grown on different aspect/soil amendment treatments. * denotes differences in shoot water potential between seedlings of all stock types grown on *North* and *South* aspect treatments.

Physiological response	**Stock type**	**Aspect/ soil amendment**			
		North		*South/control*	*Hydrogel*
Stomatal conductance (mmol m^{-2} s^{-1})	*highNSC*	220.3 ± 72.7		141.7 ± 32.2	206.2 ± 53.6
	highRSR	172.2 ± 44.5		126.8 ± 32.2	182.3 ± 55.2
	large	152.5 ± 36.7		168.3 ± 57.6	223.1 ± 82.9
	All seedlings	181.6 ± 29.3		145.6 ± 22.7	203.9 ± 34.5
Stem water potential (MPa)	*highNSC*	-1.39 ± 0.17		-1.83 ± 0.17	-1.69 ± 0.12
	highRSR	-1.57 ± 0.12		-1.8 ± 0.10	-1.69 ± 0.17
	large	-1.7 ± 0.11		-1.75 ± 0.08	-1.67 ± 0.16
	All seedlings	-1.55 ± 0.08	*	-1.79 ± 0.06	-1.68 ± 0.08

Appendix 4

Table A4-1 PROC MIXED ANOVA results for soil bioavailable nutrients. The level of significance used is α=0.05.

Soil bioavailable nutrient (μg 10cm^2 32 days^{-1})	**Num df**	**Den df**	**F**	**P < F**
Total available N	2	27	6.09	**0.007**
NO_3^-	2	27	11.49	**< 0.001**
NH_4^+	2	27	1.51	0.240
P	2	27	23.15	**< 0.001**
K^+	2	27	42.70	**< 0.001**

Table A4-2 PROC MIXED ANOVA results for soil temperature, volumetric water content and water potential. Comparisons between soil responses for North and South plots in experiment I are denoted in rows labeled aspect, while comparisons between Control and Hydrogel plots in experiment II are denoted in the rows labeled soil amendment. The level of significance used is α= 0.05.

Soil measurement	**Aspect/ soil amendment**	**Effect**	**Num df**	**Den df**	**F**	**P < F**
Soil temperature (°C)- 10 cm depth	Aspect	treatment	1	11	38.00	**< 0.001**
		day	60	660	832.34	**< 0.001**
		treatment*day	60	660	15.73	**< 0.001**
	Soil amendment	treatment	1	11	11.22	**0.007**
		day	60	660	357.50	**< 0.001**
		treatment*day	60	660	3.61	**< 0.001**
Soil temperature (°C)- 20 cm depth	Aspect	treatment	1	12	35.45	**< 0.001**
		day	60	660	614.75	**< 0.001**
		treatment*day	60	660	8.13	**< 0.001**
	Soil amendment	treatment	1	12	14.71	**0.002**
		day	60	660	282.43	**< 0.001**
		treatment*day	60	660	3.18	**< 0.001**
Soil water content (m^3 m^{-3})- 10 cm depth	Aspect	treatment	1	11	2.23	0.164
		day	60	660	45.64	**< 0.001**
		treatment*day	60	660	2.75	**< 0.001**
	Soil amendment	treatment	1	11	1.92	0.193
		day	60	660	56.69	**< 0.001**
		treatment*day	60	660	1.12	0.264
Soil water content (m^3 m^{-3})- 20 cm depth	Aspect	treatment	1	11	2.76	0.125
		day	60	658	33.09	< 0.001
		treatment*day	60	658	4.19	< 0.001
	Soil amendment	treatment	1	11	1.67	0.223
		day	60	658	37.75	**< 0.001**
		treatment*day	60	658	1.33	0.056
Soil water potential (MPa)- 10 cm depth	Aspect	treatment	1	10	9.38	**0.012**
		day	60	660	23.13	**< 0.001**
		treatment*day	60	660	7.33	**< 0.001**
	Soil amendment	treatment	1	9	2.68	0.136
		day	60	540	43.09	**< 0.001**
		treatment*day	60	540	2.45	**< 0.001**
Soil water potential (MPa)- 20 cm depth	Aspect	treatment	1	11	8.75	**0.013**
		day	60	629	16.02	**< 0.001**
		treatment*day	60	629	10.25	**< 0.001**
	Soil amendment	treatment	1	10	2.11	0.177
		day	60	571	62.84	**< 0.001**
		treatment*day	60	571	4.55	**< 0.001**

Appendix 5

Table A5-1 PROC MIXED ANOVA results for seedling morphological responses obtained following one growing season in the aspect and stock type selection study. The level of significance used is α = 0.05. *****denotes a borderline significant interaction term.

Morphological response	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
Height growth (cm)	*Stock*	2	54	31.42	**< 0.001**
	Aspect	1	54	30.93	**< 0.001**
	*Stock*Aspect*	2	54	3.06	**0.055***
New shoot growth (g)	*Stock*	2	54	15.02	**< 0.001**
	Aspect	1	54	51.91	**< 0.001**
	*Stock*Aspect*	2	54	5.79	**< 0.001**
Leaf area (cm^2)	*Stock*	2	54	4.52	**0.015**
	Aspect	1	54	49.12	**< 0.001**
	*Stock*Aspect*	2	54	2.71	0.076
Leaf mass (g)	*Stock*	2	54	3.23	**0.047**
	Aspect	1	54	57.56	**< 0.001**
	*Stock*Aspect*	2	54	2.75	0.073
Root volume (mL^3)	*Stock*	2	54	38.75	**< 0.001**
	Aspect	1	54	3.96	**0.052***
	*Stock*Aspect*	2	54	2.11	0.131
Root volume growth (mL^3)	*Stock*	2	54	2.93	0.062
	Aspect	1	54	3.96	**0.052***
	*Stock*Aspect*	2	54	2.11	0.131
Root mass (g)	*Stock*	2	54	39.52	**< 0.001**
	Aspect	1	54	3.28	0.076
	*Stock*Aspect*	2	54	1.50	0.232
Root mass growth (g)	*Stock*	2	54	1.24	0.297
	Aspect	1	54	3.28	0.076
	*Stock*Aspect*	2	54	1.50	0.232
RSR ($g\ g^{-1}$)	*Stock*	2	54	16.39	**< 0.001**
	Aspect	1	54	26.05	**< 0.001**
	*Stock*Aspect*	2	54	3.80	**0.029**
Change in RSR ($g\ g^{-1}$)	*Stock*	2	54	118.03	**< 0.001**
	Aspect	1	54	26.05	**< 0.001**
	*Stock*Aspect*	2	54	3.80	**0.029**

Table A5-2 PROC MIXED ANOVA results for seedling physiological responses obtained following one growing season in the aspect and stock type selection study. The level of significance used is α = 0.05.

Physiological response	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
Stem water potential (MPa)	*Stock*	2	15	0.62	0.550
	Aspect	1	15	7.35	**0.016**
	*Stock*Aspect*	2	15	1.60	0.234
Stomatal conductance (mmol m^{-2} s^{-1})	*Stock*	2	15	0.30	0.746
	Aspect	1	15	1.14	0.303
	*Stock*Aspect*	2	15	0.67	0.527

Appendix 6

Table A6-1 PROC MIXED ANOVA results for seedling morphological responses obtained following one growing season in the hydrogel amendment and stock type selection study. The level of significance used is α = 0.05.

Morphological response	Effect	Num Df	Den Df	F	P > F
Height growth (cm)	*Stock*	2	36	83.07	**< 0.001**
	Soil Amendment	1	9	3.65	**0.045**
	*Stock*Soil Amendment*	2	36	0.91	0.121
New shoot growth (g)	*Stock*	2	36	3.39	**0.045**
	Soil Amendment	1	9	8.40	**0.018**
	*Stock*Soil Amendment*	2	36	0.22	0.802
Total seedling mass (g)	*Stock*	2	36	19.55	**< 0.001**
	Soil Amendment	1	9	5.45	**0.044**
	*Stock*Soil Amendment*	2	36	3.45	**0.043**
Number of leaves	*Stock*	2	36	29.28	**< 0.001**
	Soil Amendment	1	9	3.77	0.084
	*Stock*Soil Amendment*	2	36	3.47	**0.042**
Leaf area (cm^2)	*Stock*	2	36	1.62	0.211
	Soil Amendment	1	9	7.48	**0.023**
	*Stock*Soil Amendment*	2	36	1.77	0.185
Leaf mass (g)	*Stock*	2	36	1.10	0.344
	Soil Amendment	1	9	10.96	**0.009**
	*Stock*Soil Amendment*	2	36	2.41	0.105
Root volume (mL^3)	*Stock*	2	36	75.87	**< 0.001**
	Soil Amendment	1	9	9.99	**0.012**
	*Stock*Soil Amendment*	2	36	4.72	**0.015**
Root volume growth (mL^3)	*Stock*	2	36	20.38	**< 0.001**
	Soil Amendment	1	9	9.99	**0.012**
	*Stock*Soil Amendment*	2	36	4.72	**0.015**
Root mass growth (g)	*Stock*	2	36	12.31	**< 0.001**
	Soil Amendment	1	9	4.08	0.074
	*Stock*Soil Amendment*	2	36	2.63	0.086
RSR (g g^{-1})	*Stock*	2	36	7.87	**0.002**
	Soil Amendment	1	9	0.02	0.889
	*Stock*Soil Amendment*	2	36	0.54	0.586
Change in RSR (g g^{-1})	*Stock*	2	36	82.34	**< 0.001**
	Soil Amendment	1	9	0.02	0.889
	*Stock*Soil Amendment*	2	36	0.54	0.586

Table A6-2 PROC MIXED ANOVA results for seedling physiological responses obtained following one growing season in the hydrogel amendment and stock type selection study. The level of significance used is α = 0.05.

Physiological response	**Effect**	**Num Df**	**Den Df**	**F**	**P > F**
Stem water potential (MPa)	*Stock*	2	12	0.32	0.733
	Soil Amendment	1	3	2.89	0.188
	*Stock*Soil Amendment*	2	12	0.08	0.926
Stomatal conductance (mmol m^{-2} s^{-1})	*Stock*	2	12	0.73	0.504
	Soil Amendment	1	3	3.19	0.172
	*Stock*Soil Amendment*	2	12	0.01	0.988