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Tolerance mechanisms of black spruce (*Picea mariana*) seedlings exposed to saline oil sands tailings

by

Edmund Bancroft Redfield ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

in

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ABSTRACT

Petroleum extraction from oil sands in Alberta produces tailings with high ion concentrations (Na^+, Cl^-, and SO_4^{2-}). Composite tailings retain water, leaving portions of deposits waterlogged. A potential species for revegetation of tailings is black spruce (*Picea mariana* (Mill.) B.S.P.). It is flood tolerant, so identification of salt tolerance characteristics could allow screening of planting stock. Salt affects plants by inducing water deficit, and ion toxicity. This study used controlled environment and field experiments to examine relationships between drought tolerance and salt tolerance in black spruce. Plant responses to water deficit were examined by measuring water relations parameters derived from Pressure-Volume curves. Parameters indicating drought tolerance were predictors of injury caused by salt stress. These included: lower relative water content at the turgor loss point; higher relative water content of the symplast; lower osmotic potential at the turgor loss point; lower maximum bulk modulus of elasticity; and higher osmotic amplitude for turgor maintenance.

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List of Acronyms and Symbols

CT	Composite Tailings (Syncrude) / Consolidated Tailings (Suncor)
EC	Electrical Conductivity
P-V	Pressure-Volume (curve)
g_s	stomatal conductance
T	transpiration
Ψ_w	water potential
Ψ_p	turgor potential
$\Psi_{\pi tlp}$	osmotic potential at the turgor loss point
$\Psi_{\pi sat}$	osmotic potential at full saturation
$\Psi\Delta\pi$	osmotic amplitude for turgor maintenance
RWC_{tlp}	relative water content at the turgor loss point
RWC_s	relative water content of the symplast
RWC_a	relative water content of the apoplast
ε_{max}	maximum modulus of elasticity

1.0 General Introduction

Open pit mining of oil sands in northeastern Alberta started in the 1920's and the industry has been a major commercial producer of petroleum since 1967. There are an estimated 1.7 trillion barrels of oil in Alberta oil sands deposits, which is one-third of known world reserves (Bennett 1996). Of this, approximately 7-8 % can be accessed with surface mines (OSDC 1998). Oil sands mining provided 20% of Canada's oil needs in 1993. Currently, there are two major production facilities, one operated by Syncrude Canada Ltd., and the other by Suncor Energy Inc., both of which are located near Fort McMurray. The disturbance caused by these operations is a result of more than just excavation. The amount of land required to dispose of oil sands tailings far exceeds that of any other form of mineral processing (Marshall 1982). Although procedures for handling and disposing of tailings are simple, the volume to be dealt with imposes practical constraints on reclamation planning (Moneco 1983).

Until now, tailings have been allowed to naturally segregate into tailings sand, and fine tails, and then disposed of separately. Tailings sand is made up of the granular mineral deposits left after water and sludge removal, and is relatively easy to revegetate. Fine tails make up approximately 5-10% of the tailings. These mostly occur suspended in a liquid state and are thus more problematic to store and reclaim (Moneco 1983).

Production of CT (called "Composite Tails" by Syncrude and "Consolidated Tails" by Suncor) reduces the volume of fine tails by removing most of the water component. The process used presently involves mixing of tailings sand, gypsum and fine tails so that calcium from the gypsum acts as a flocculent, causing the fine clays to settle out with the sand and release the water (Sheeran 1998). However, CT deposits produced by this method are more difficult to reclaim than tailings sand. Composite Tails retain a high water content and high concentrations of sodium, chloride, and sulfate (Renault et al., 1998). Development of anaerobic conditions in the root zone has also been observed in CT, and may exacerbate the effects of salt and other stresses induced by tailings (Croser and Zwiazek 2000).

The need for native flood tolerant and salt tolerant plants complicates the process of revegetation which is vital for both short-term erosion control, and long-term reclamation of tailings affected sites. Additional constraints to revegetation are that:

1) species selection is limited to the use of native species; and 2) the area is part of an industrial Forest Management Agreement, an end land use that calls for use of species which have commercial value (AEP 1999). One potential candidate for reforestation is black spruce (*Picea mariana* (Mill) B.S.P.), which is native, flood tolerant (LeBarron 1948), and commercially valuable. As a consequence, this study has centered on this species, with its objective being to determine if it is possible to identify characteristics of salt tolerant individuals in order to allow the selection of suitable planting stock.

The two main stresses that plants experience as a result of salt exposure are water deficit and ion toxicity (Greenway and Munns, 1980). Renault et al. (1998) have carried out work with many boreal forest species and reported that broadleaf species such as dogwood and poplar had relatively high tolerance of sodium and sulphate. They found that, in general, conifers were more affected by high salt exposure than other tree seedlings. However there was an unusually high individual variability in the response of conifer seedlings to CT water (Renault et al. 1998). Thus, they recommended that a selection process for conifers could utilize these differences to select tolerant seedlings. The mechanisms of genetic control and the major genes controlling salt tolerance in forest tree species have not been identified (Allen et al. 1994). Consequently, genetic variation in salt tolerance can only be demonstrated by measuring the responses of different genotypes, rather than specific genes.

1.1 OBJECTIVES

This study has focused on the use of tissue water relations as a means of identifying suitably tolerant plants. Such a technique may be possible because of the similarities between drought stress and salt stress, as both are forms of water deficit stress. However, the usefulness of this technique could be limited by the sensitivity of black spruce to ion toxicity, particularly chloride.

The ability to cope with osmotic stress has already been examined somewhat in black spruce. Zine El Abidine et al. (1994) identified several water relations parameters derived from Pressure-Volume (P-V) curves, which were indicative of drought tolerance. These included: lower relative water content at the turgor loss point, lower maximum modulus of elasticity, lower osmotic potential at the turgor loss point, and higher osmotic amplitude for turgor maintenance. While the study found these parameters to be significant, there was little ecotypic variation detected. This supports the findings of many other studies which show that genetic variation of the species occurs primarily within stands and among individuals within populations and families rather than between ecotypes such as upland and lowland populations (Fowler and Mullin 1977, Parker et al. 1983, Wang and Macdonald 1993).

Tan et al. (1995) demonstrated that drought tolerance has a significant genetic component, as made evident by the tendency of some families of black spruce seedlings to exhibit greater drought tolerance than others. Significant correlations have been observed between juvenile and mature growth responses in black spruce (Sulzer et al. 1993), suggesting that selection based upon early development is an acceptable practice for this species (Lambeth 1980).

Little work has been done previously to examine ion toxicity in black spruce, though information about other plant species is available. Peanuts, when faced with equivalent water deficit in iso-osmotic solutions of NaCl and Na_2SO_4 suffer greater reductions in chlorophyll content, and carbon assimilation in NaCl than in Na_2SO_4 (Chavan and Karadge 1980). Similar sensitivity has been noted in Serbian spruce (*Picea omorika*), and jack pine (*Pinus banksiana*), (Alt et al. 1982; Croser et al. 1999). Consequently, chloride was expected to be the most likely cause of ion related injury.

The two main objectives of this study have been to:

1) Assess the sensitivity of black spruce to chloride
2) Determine if P-V curves can predict the response of black spruce to salts either in the absence of chloride sensitivity, or at low chloride concentrations

Identification of those tissue water relations parameters which are of benefit to seedlings facing salt induced water deficits, would allow selection of populations, families, or clones of suitable seedlings possessing such characteristics. Allen, et al. (1994) concluded that such screening is the best way of immediately achieving improvement in the salt tolerance of trees. Seedlings selected in this way could then provide sufficient numbers for operational planting of sites to be reclaimed.

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2.0 Literature Review

2.1 MINE SITES

Currently, there are two major oil sands plants operating near Fort McMurray that extract petroleum from ore excavated from open pit mines. One is operated by Syncrude Canada Ltd., and the other by Suncor Energy Inc. There are plans to open a third mine and processing facility, Albian Sands, which is a joint venture between Shell Canada Ltd., Chevron Canada Resources Ltd., and Western Oilsands L.P. (Shell Canada Ltd. 1997). The Syncrude and Suncor mines are both located north of Fort McMurray, near the Athabasca River (Figure 2.1.), at approximately 57° 00' N, 111° 35' W.

Prior to development, the leases supported a mixedwood boreal forest (Rowe 1972). The area is part of the Central Mixedwood section of the Boreal Forest Region (Alberta Environmental Protection 1994). The forests surrounding the mines are dominated by associations of aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*), mixed with white spruce (*Picea glauca*) and black spruce (*Picea mariana*) (Stringer 1976). Stands of jack pine (*Pinus banksiana*) are also common. White spruce is the climax species on relatively well drained sites, however aspen is the forest cover of greatest extent due to its ability to regenerate rapidly following disturbance (Rowe 1972). Jack pine is dominant on sandy areas but mixes with other species on dry glacial till soils. Black spruce occurs mixed with jack pine in the uplands and with tamarack (*Larix laricina*) in poorly drained areas and low lying bogs.

Based upon the present mining plans, it is likely that much of the area to be reclaimed will have higher salinity than predevelopment landscapes (Renault et al. 1998). The long-term plan for affected sites is to establish a mosaic of self-sustaining forests (50%), grasslands (20%), wetlands (10%), and lakes (20%) (Syncrude 1995). Vegetation on these reclaimed end-of-lease landscapes will be exposed to relatively high levels of salt resulting from contact with tailings (Renault et al 1998). Such conditions will impose short, and perhaps, long-term constraints on regeneration, depending upon the topography and natural recharge of the sites. Success of reclamation will depend in part upon the ability of the vegetation to tolerate these salts.

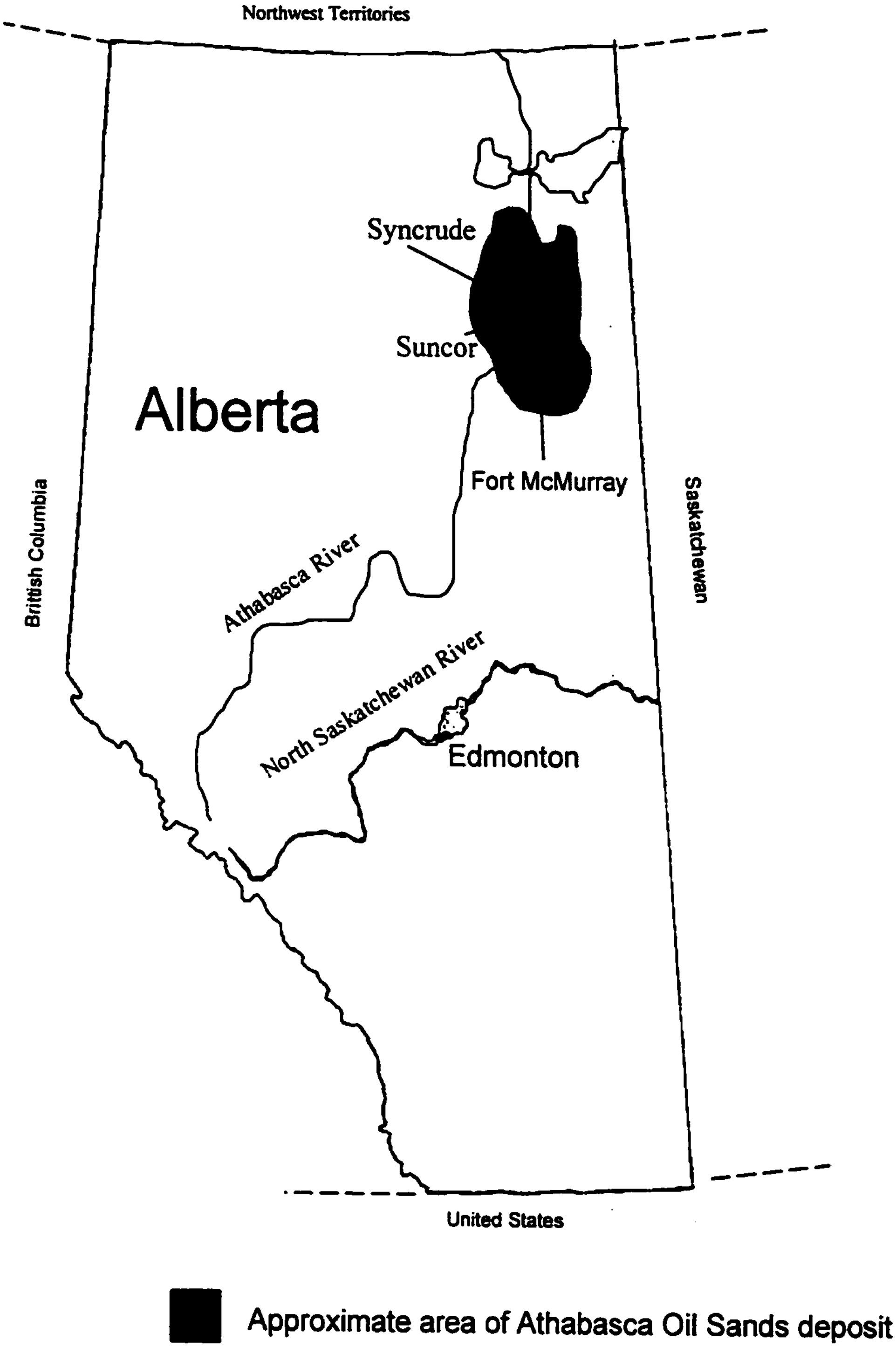


Figure 2.1: Location of Syncrude and Suncor mine sites in northeastern Alberta, with an approximate indication of the subsurface extent of the deposit. (adapted from Stewart 1963)

2.2 TAILINGS

Oil sands tailings consist of the whole ore body plus net additions of water used in processing, less the recovered bitumen product. Reliable technologies for handling and revegetating tailings sand exist (Moneco 1983; Techman 1983). The biggest challenge is disposal of fine tails. These settle slowly and tend to form a highly voluminous sludge (Marshall 1982). One of the main factors preventing settling is the salinity of fine tails, which causes clay particles to repel each other (FTFC 1995), keeping them suspended. Because of the high water content of the sludge (as much as 85%), it retains fluid characteristics and must be stored behind dykes with little possibility of using it as a solid substrate for plant establishment (Marshall 1982; FTFC 1995).

A possible solution to dealing with this material is the production of CT (called Composite Tails by Syncrude, and Consolidated Tails by Suncor). Composite Tails are composed of 30% fine tails, and 70% tailings sand, mixed with gypsum (Sheeran 1998). Through ion exchange, the calcium from the gypsum displaces much of the sodium from the clays, and causes the fines to flocculate and settle out (MacKinnon 1998).

Oil sands ore naturally contains significant amounts of NaCl, and this becomes concentrated in tailings during extraction due to recycling of process waters. The initial amounts of NaCl contained in the ore vary. The deposits that Syncrude is mining are significantly more saline than those of Suncor, and as a consequence Syncrude's tailings materials have higher concentrations of ions than those produced by Suncor (Renault et al. 1998)

The high gypsum dosages required for production of CT (800-1200 g/m^3 of tailings) result in significant addition of ions as sodium displaced from the clays associates with sulphate from the gypsum to form sodium sulphate (Renault et al. 1998). As a consequence, the main ions occurring in CT are Na^+, Cl^-, and SO_4^{2-}. These ions are present in CT in concentrations resulting in electrical conductivities (EC) averaging 4.5 dS/m (Golder Associates 2000), when 4 dS/m is generally considered the threshold at which such materials impose a significant constraint to plant growth (Singer and Munns 1996).

Excess water is slowly released from the CT as it consolidates. Composite tails become trafficable within a year or two, but as the high EC indicates, this material can be quite saline, which continues to create significant problems for revegetation (and thus erosion prevention and reclamation). A comparison of the properties of tailings sand and CT with the target properties for substrate in mixedwood sites is presented in Table 2.2.1. Given that existing technologies for dealing with tailings sand work well, conversion of tailings sand to CT can be seen as a complication and contamination of otherwise benign material. However, some means of disposal of fine tails must be found, and production of CT is one of the most promising options.

2.2.1 The objective of CT production

In order to understand the magnitude of the fine tails problem and the quantities of material involved, it is illustrative to construct a model of tailings production based upon operational numbers, even though ongoing enlargement of the operation has rendered these particular numbers obsolete. When the Syncrude plant opened in 1978, it was designed to produce 125,000 barrels per day of synthetic crude oil (Smith 1979). To meet this level of production 231,000 tons of bituminous ore were required per day (ALCRC 1982), which is approximately 1.85 tons per barrel. The void space created by extraction of one ton of bituminous ore is 0.4 m^3 and the tailings which result from processing the ore occupy 0.6 m^3 (Simpson-Lewis et al. 1979). This means that Syncrude's daily use of 231,000 tons of ore opened up 92,400 m^3 of space but produced tailings which required 138,600 m^3 of storage space - a volumetric discrepancy of 46,200 m^3 per day of operation. This is in spite of the fact that between 7 and 17% of the ore was recovered as bitumen. A large proportion of this discrepancy is due to the process waters locked up in the fine tails.

Tailings are pumped from Syncrude's processing plant to large ponds where the coarse solids settle out to form dykes and beaches of tailings sand while much of the fines and residual bitumen are carried out into the pond as a thin slurry stream (FTFC

Table 2.2.1 Properties of tailings sand and CT as compared to the targets for productive mixedwood sites.

Properties of tailings sand	
Sand	95%
Silt	4%
Clay	1%
pH	8.2
EC	0.6 dS/m
N	<0.5 ppm
P	3.5 ppm
K	40 ppm

(HBT AGRA 1994)

Properties of CT	
Sand	85%
Silt	10%
Clay	5%
pH	7-8.5
EC	2-6 dS/m
N	<0.01 ppm
P	<0.01 ppm
K	10 ppm

(Renault and Zwiazek 1997; Chapter 4, of this thesis: site data)

Target properties for mixedwood sites	
Sand	80%
Silt & clay	20%
pH	4.7-6.5
EC	<2dS/m
Organic matter	3%
N	25 ppm
P	80 ppm
K	265 ppm

(Moneco 1983)

1995). Once there, these fine-grained silts, clays, and residual bitumen sink in the pond to form an accreting layer (the fine tails) which consolidates very slowly. Meanwhile a clarified water layer develops at the surface of the pond and this water is recovered and recycled as extraction process water (concentrating salts naturally present in the ore material). Much of the increase in weight of the tailings (270,000 tons per day) as compared to that of the initial bituminous ore (231,000 tons per day) is accounted for by the process water left behind in which the fines are suspended.

2.2.2 Tailings sand

Tailings sand deposits consist of approximately 95% sand, 4% silt, and 1% clay (HBT AGRA 1994). Most tailings sand is slightly alkaline as a result of sodium hydroxide added during processing to separate oil from the sand (Lesko 1974). Available nitrogen and phosphorus are low, due to the low organic matter content of tailings sand (<1%). All other chemical and physical properties are within acceptable limits for plant growth (Moneco 1983).

2.2.3 Fine tails

Fine tails are 5 - 10% of the tailings (suspended silt and clays). Syncrude's fine tails are about 85% water, 13% clays, and 2% bitumen (FTFC 1995). Fresh fine tails are considered to be acutely toxic for aquatic organisms due to the presence of a number of organic compounds such as naphthenic acids which are derived from the bitumen. However, toxicity declines with time as these compounds degrade (Hunter et al. 1989). It is generally thought that the extremely slow consolidation of this material is related to the dispersed nature of the fine and ultrafine particles as well as the ionic chemistry of the process water (FTFC 1995). The protracted retention of fluid characteristics by this material offers little possibility of establishing vegetation over its surface.

2.2.4 CompositeTailings utilization

The production of CT will reduce the large volume of fine tails which has built up behind dykes over the years, and allow management of future production of fine tails

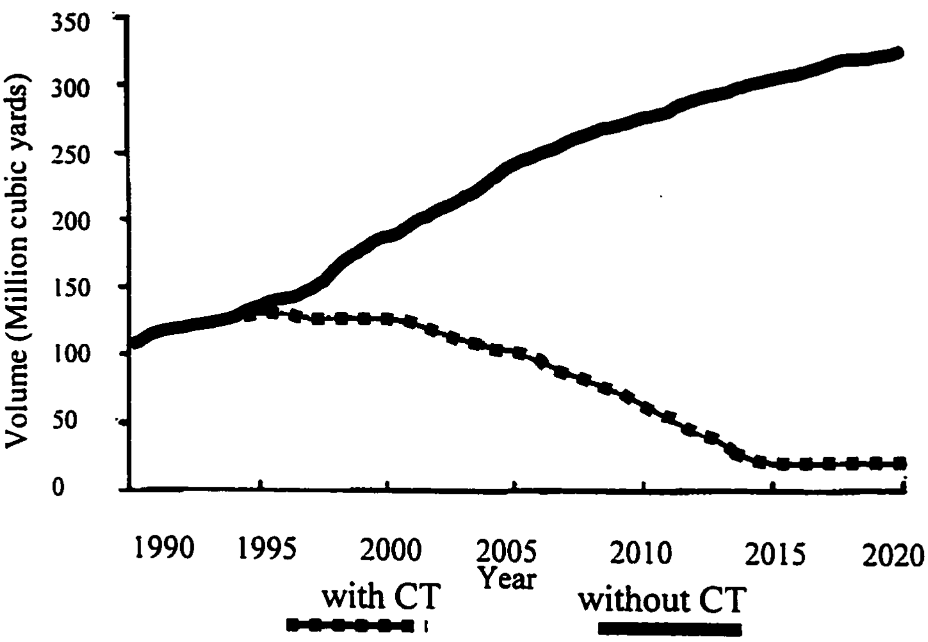


Figure 2.2.1 Impact of CT use on fine tails volume. Data based on Suncor figures. (adapted from Sheeran 1998)

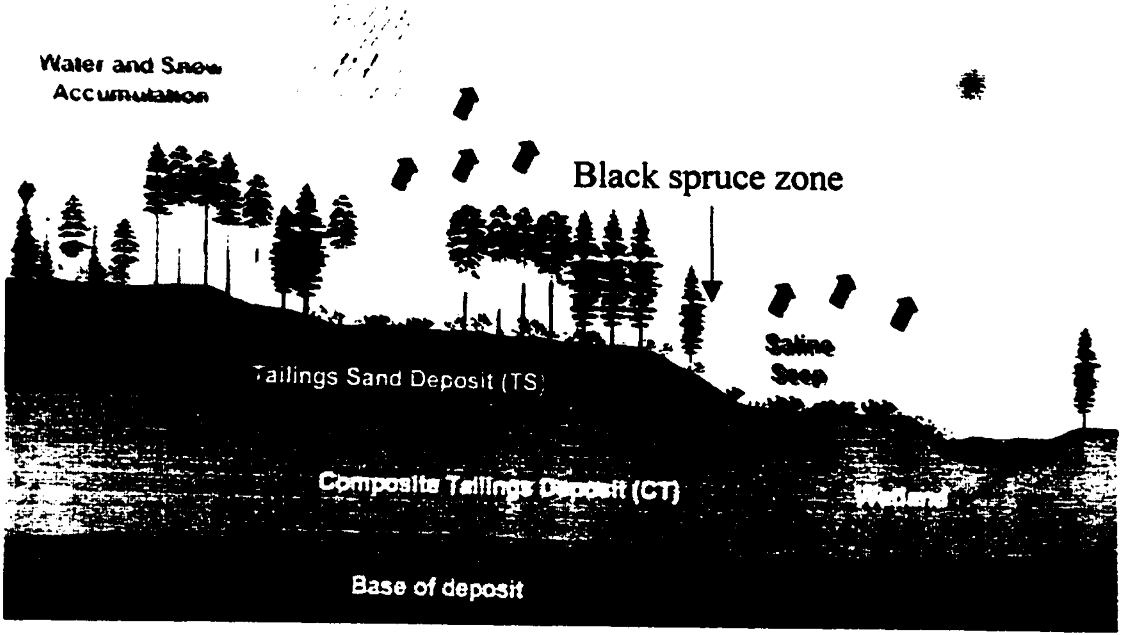


Figure 2.2.2 Hydrology of CT deposit. (Qualizza 1999).

(Figure 2.2.1) to prevent similar accumulation in the future (Sheeran 1998). Release of the water from this material reduces the overall volume of tailings, even though by incorporating tailings sand it increases the proportion of material that will be difficult to revegetate. This difficulty comes from the elevated concentration of ions such as Na^+, Cl^- and SO_4^{2-} found in the CT, as these can be quite damaging to plants (Renault et al. 1998). An additional issue which has been noted is the development of anaerobic conditions in CT which may exacerbate ion toxicities by inhibition of the ability of plants to regulate ion uptake (Croser and Zwiazek 2000).

Reclamation using CT (Figure 2.2.2) would involve a process of dry capping. The CT deposit is covered with tailings sand, and the tailings sand is then capped with reclamation material (salvaged muskeg and non-sodic overburden) following established tailings sand reclamation methods (Moneco 1983). Over much of such an area, conditions would not be expected to be very different than those found on tailings sand deposits which have already been reclaimed. Toe slopes would, however, serve as concentration points subject to the formation of flooded soils and saline seeps. This is the area where salt-tolerant black spruce could become useful.

2.3 SALT TOLERANCE

Plant species that are well adapted to salts are called halophytes. Those not well adapted are called glycophytes. The majority of boreal forest species, including most economically valuable gymnosperms, such as black spruce, are glycophytes. It can be difficult to generalize sensitivity to salt among glycophytes, even for a specific species. Lynch et al. (1982) found that the most sensitive variety of barley at the seedling stage was more tolerant at maturity than other varieties, whereas another variety showed the opposite pattern. Generally, however, woody plants are tolerant of salt while dormant as seeds, very sensitive during emergence and young seedling stages, and become increasingly tolerant as they age (Shannon 1994). In light of this, the focus here will be on the use of nursery reared seedlings for reforestation of sites affected by CT materials. Direct seedling of black spruce under these conditions would be ill advised. Croser et al.

(2000) found that, in black spruce, rates of germination and subsequent root elongation (hence establishment) of seedlings are dramatically reduced by saline conditions.

Concentrations of salts which seeds are exposed to during germination may be significantly higher than those faced by plants at later growth stages (Haywards and Bernstein 1958). This is because the surface layers of soil accumulate salts as evaporation and capillary rise occurs. Germinating seeds are located near the soil surface, which may contain substantially higher salt concentrations than the upper 10-20 cm of the soil. Moreover, the evaporation of moisture at the soil surface increases osmotic pressure and moisture tension in that zone. This exacerbates the water deficit effects of salinity, and reduces the germination rate. Germination can be strongly affected by salinity even in trees considered to be salt-tolerant at older growth stages (Clemens et al. 1983).

Water deficit and ion excess are the two main constraints that salt imposes upon germination. Zekri (1993) notes that the osmotic potential of a soil solution may decrease to the point where it retards or prevents the uptake of water by seeds. It is the intake of water which is needed to activate and mobilize the enzymes in the embryo that break down the food reserves in the endosperm and provide the required energy for it's development (Albrecht 1993). Clemens et al. (1983) observed that the soil solution can become toxic to emerging seedlings, due to excessive ion concentrations. This can result in a significant decrease in the percentage of seeds that successfully germinate and become established. Several *Casuarina* species, which are generally considered to be relatively salt tolerant, showed no relationship between salt tolerance at germination and at later seedling stages (Clemens et al. 1983). As a result, germinant mortality can not be used to screen out sensitive individuals. Consequently, the use of older more tolerant seedlings should improve survival on salt-affected sites.

2.3.1 Constraints imposed by salinity

The stresses limiting germination and establishment of seedlings pose a challenge for plants even after successful establishment. Greenway and Munns (1980) identify water deficit and ion excess as two of the main ways in which salt affects plants.

Water deficit is caused by a decrease in osmotic potential of the soil solution. This can result in adverse effects on the water relations of individual plant cells, without necessarily reducing turgor. The end effects, are similar to drought stress in many ways. Ion excess may take more time to develop. It occurs when ions accumulate to toxic levels in plants and begin to disrupt the plant metabolism directly. Marschner (1995) identifies a third factor affecting plant growth in saline substrates: nutrient imbalance. This can result from depression in uptake and/or shoot transport and impaired internal distribution of mineral nutrients, particularly calcium. It is often not possible to assess the relative contributions of these three major constraints to growth at high substrate salinity, as many factors are involved (Marschner 1995). In the short term, however, the primary stress is likely to be water deficit, as ion excess and nutrient imbalances may take more time to develop. Thus, the ability of the plant to deal with water deficit stress through drought resistance characteristics, can strongly affect its performance when exposed to saline conditions.

2.3.2 Plant response to salts: avoidance and tolerance

When faced with an environmental stress such as salinity, organisms that survive do so by either avoiding the source of the stress, or tolerating it (Levitt 1972). Avoidance occurs when a plant prevents or reduces damage without coming into an equilibrium with the stress. A tolerant plant, meanwhile, attempts to come into equilibrium with the stress, and if successful may not experience serious injury.

Avoidance of salt toxicity can be achieved by preventing harmful accumulations of salt in the plant by exclusion, excretion, or dilution of ions (Levitt 1972). Such sophisticated options are often not available to glycophytes which by definition are not specifically adapted to saline environments. Instead, they frequently depend upon tolerance mechanisms to survive.

Salt tolerance is more subtle than avoidance and thus somewhat more complicated to detect and describe. It varies greatly between species, varieties, and ecotypes, as well as with the growth stage of the plant (Shannon et al. 1994). Common ways in which plants tolerate saline environments are: osmotic adjustment to allow cells access to water in spite of decreasing water potentials; sequestration in roots and stems to protect sensitive photosynthetic apparatus in the shoots; and intracellular compartmentalization, where toxic ions are stored in the cell vacuoles, and the less toxic in the cytoplasm (Waisel 1991).

2.3.2.1 Osmotic adjustment

The water relations of plants are controlled primarily by cell water relations (Kozlowski and Pallardy 1997). When plants are exposed to NaCl, ions reduce the apoplastic water potential and accumulate excessively in the cytosol (Binzel et al. 1988). Plant cells osmotically adjust to the water relations imbalance through synthesizing compatible organic solutes, and accumulating ions from the external environment. In the past, the term osmotic adjustment was used to describe a change in the osmotic pressure of leaves in parallel with that of the soil. The term was used for both drought and salt affected plants to describe an accumulation of solutes in leaves or roots which had the effect of maintaining turgor. However, it has become clear that turgor does not control stomatal conductance or cell expansion, so this term has been redefined. Osmotic adjustment now means: a compensating change in the osmotic pressure of cell sap relative to that outside the cell resulting from an increase in the numbers of solute molecules per cell, rather than from an increase in concentration due to a loss of water and decrease in cell volume (Munns, 1988). The primary purpose of osmotic adjustment seems to be to survive stress, rather than allow growth under stress conditions.

Glycophytes undergoing osmotic adjustment tend to exclude or sequester salts within the roots or stems (Shannon et al. 1994), and then depend upon the synthesis of compatible solutes to increase the solute concentration in cells. Osmotic adjustment must be achieved without the accumulation of potentially toxic ions in the cytosol, from the external environment. The ionic activities of those which do accumulate must be attenuated by osmoprotectants (Binzel et al 1988). Presumably, genotypes which are most adapted to salt tightly regulate ion uptake across the plasma membrane at a rate which is compatible with the capacity for vacuolar compartmentalization.

2.3.2.2 Intracellular compartmentalization

The cytoplasm has a low ionic content with respect to the vacuole, even in halophytes (Jeffrey 1987). Regulation of the ions in the cytoplasm is necessary to protect organelles, particularly the chloroplasts, which are quite sensitive to ion excess (Shannon et al. 1994). It has been suggested that glycophytes are not as capable of maintaining ionic gradients necessary for vacoular compartmentalization of ions (Flowers et al 1977), as are halophytes, and that this may account for their susceptibility to salinity. However, Foster and Sands (1977) note that though *Pinus radiata* is a glycophyte, when exposed to salt stress it stored Cl^- in the vacuoles of living cells leaving the cytoplasmic organelles relatively Cl^- free. Binzel et al. (1988) have also demonstrated that the membrane properties required to achieve and maintain such gradients do exist in glycophytes. The rate at which glycophytes can establish the steep gradients which are necessary is much slower than in halophytes, and this may explain some of the substantial differences in salt tolerance between the two.

Failure to compartmentalize excess ions causes salt poisoning, with protein synthesis, energy metabolism, and lipid metabolism all potentially disrupted by ion activity (Munns 1993; Aspinall 1986). The concentration at which this happens will depend upon the ability of the species to restrict transport to shoots, or failing that, compartmentalize the salts in cell vacuoles. Once the vacuole becomes "full", excess salts will accumulate in either the cell wall, or the cytoplasm (Munns 1993). The cells will then die of either dehydration (by salts in the cell wall), or salt poisoning (if salts accumulate in the cytoplasm).

Many plants experience growth inhibition and foliar injury (marginal chlorosis and necrosis on mature leaves) even at relatively low NaCl levels (Marschner 1995). Under such conditions, water deficit is not even necessarily a constraint (Greenway and Munns 1980). Damage may instead be attributed to ion sensitivity (Maas 1993).

The main ion toxicities occurring in salt-affected soils are in response to Cl^- and Na^+ ions, as in saline substrates these are typically the dominant ions present. Although Cl^- is an essential micronutrient for all higher plants, concentrations in saline substrates can exceed this demand and lead to toxicity in non-salt-tolerant plants (Marschner 1995). Sodium toxicity is less of a concern for most plants as they are generally capable of sequestering this ion in roots or stems. However Na^+ can become more of a problem where low Ca^{2+} concentrations in the substrate or poor soil aeration increase uptake and reduce the ability of plants to exclude or sequester it. Conifers, including spruce, have shown distinct chloride sensitivity. For example much of the damage occurring among *Picea omorika* growing under high in NaCl conditions has been attributed to Cl^- toxicity rather than Na^+ toxicity or water deficit (Alt et al. 1982). Similarly, in *Pinus banksiana*, needle necrosis was significantly greater in NaCl than in iso-molar Na_2SO_4 even though Na_2SO_4 both induced greater water deficit, and contained twice as much Na^+ as the NaCl (Croser et al. 1999).

2.3.3 General effects of salinity

Munns and Termat (1986) lay out a useful model of glycophyte response to salinity. The earliest response is that the leaves of the plant grow more slowly, without a measurable change in leaf water status (Munns and Sharp 1993). The reduction of shoot growth is much greater than the reduction of root growth (Cheeseman 1988). These effects become clear long before ions in the shoot build up to toxic levels (Munns and Sharp 1993), and are elastic (i.e. quickly reversible if the stress is eased).

In the longer term, prolonged transpiration causes accumulation of Na^+ and Cl^- to the shoot, especially the older leaves, eventually killing them (Munns and Termat 1986). If the rate of leaf loss exceeds the rate of new growth, the photosynthetic area of the plant decreases, with a corresponding loss of productivity. Should productivity decline to a point where the plant can no longer maintain itself, it will die. Short of death, these effects are plastic (i.e. they have long term implications, causing damage that will remain with the plant whether or not the level of stress is reduced).

This model nicely accommodates mechanisms of avoidance and tolerance. Chances for survival are improved if: growth is maintained; leaves tolerate higher tissue ion concentrations, ion uptake is decreased; or the photosynthetic efficiency of the leaves increases. In cases where the opposite occurs, chances for survival are further reduced.

2.3.4 Use of the model

The Munns and Termat (1986) model is particularly relevant for examining the sequence of events experienced by glycophyte seedlings planted into saline environments . If seedlings are to be planted in order to take advantage of the increase of salt tolerance which occurs in most tree species with age, then an understanding of the effects of salinity on specific plant processes is necessary.

2.3.4.1 Increase in root to shoot ratio

Reductions in shoot growth, without corresponding reductions in root growth increase the root to shoot ratio. This can be an important adaptation to drought stress (Kozlowski and Pallardy 1997). The same effect occurs with soil salinity, due to the decreased water potential of the rooting solution, rather than the presence of a specific salt in it. This is shown by two lines of evidence: (1) the same early growth reduction in leaf expansion induced by NaCl can be induced by other salts of the same osmotic pressure, and by non-ionic osmotica (2) plants need to be grown for an extended period of time before genotypic differences in salt tolerance show up as differences in growth rate (Munns 1993). Thus, decline in leaf growth in drying soils is not caused by a reduced supply of water to the leaves, and is independent of leaf water status (Munns and Sharp 1993).

Abscisic acid (ABA) is thought to be a major cause of the reduction of growth in salt exposed plants (Munns and Sharp 1993). It was observed that the levels of ABA quickly increase in the leaves of plants growing in soils of declining water potential. The effects of ABA are very similar to the short term effects in the model proposed by Munns and Termat (1986), i.e. inhibition of shoot growth while root growth is maintained. However the speed with which the reaction occurs suggests some sort of as yet unidentified signal from the roots to the shoots, which causes localized ABA production in the leaves. This is because the rate of ABA build up in the shoots (resulting in closure of stomata and stoppage of cell elongation) is thought to be too fast to be purely the result of transport of ABA from the roots (Munns and Sharp 1993).

While reduced shoot growth can be advantageous during water deficits, it is not automatically a benefit under saline stress, as water availability for growth may be unlimited as long as the water potential gradient favors water uptake. The Munns and Termat (1986) model would suggest that unless water is actually limiting, such a reduction is a disadvantage as it reduces the ability of the plant to generate new photosynthetically active tissue. Thus, the less a glycophyte demonstrates this response, the better off it may be. Binzel (1985) concludes that, restricted shoot growth under saline conditions is not a direct constraint of exposure to salt, but instead an inability of the plant to discriminate between desiccation and saline stress.

2.3.4.2 Reduction of photosynthetic area

Salt accumulation in older leaves is the main problem for glycophytes (Munns and Termat 1986). In halophytes older leaves have similar concentrations of salt as are found in younger leaves (Flowers and Yeo 1986). Glycophytes, however, show no signs of regulation of the salt concentration in their leaves, it is just a function of time (Munns and Termat 1986). Thus for glycophytes exposed to high levels of salinity, it is inevitable that ion concentrations will build up in the older leaves, and then the leaves will die.

Kutscha et al. (1977) studied morphological and anatomical damage in *Picea glauca* and *Thuja occidentalis* exposed to NaCl. They found that morphologically, new growth appeared green and vigorous, while that of the preceding season ranged from dark green or reddish brown to yellow and apparently dead. Anatomically, the old growth exhibited significant cell damage, such as fragmented cuticle, distorted stomata, collapsed cell walls, disintegrated chloroplasts, and disrupted phloem. Stewart et al (1973) found similar results examining needle necrosis in *Pinus sylvestris, Pinus strobus, Pinus contorta, Pinus ponderosa,* and *Pseudotsuga menziesii.* They observed collapse of the mesophyll, and hypertrophy in the phloem which disrupted its structure. They concluded that though tip burn was common in older needles of healthy trees, salt was a stress factor which aggravated this necrosis, and accelerated senescence .

At higher NaCl concentrations, Kutscha et al., (1977) found that in spite of the fact that new growth looked healthy, in both *P. glauca* and *T. occidentalis* there were signs of damage to stomata, and the cuticle of *T. occidentalis* was somewhat fragmented. Death or degradation of such tissue or material in living needles further disrupts plant functioning as it generates plastic strains in the plant (Levitt 1972). For example, disturbance of the cuticle could increase vulnerability of the plant to radiation stress, and damage to stomata would compromise the ability of the plant to control transpiration and water balance.

2.3.4.3 Reduced nutrient availability

Salinity often upsets the nutritional balance by one or more mechanisms such as competitive interactions between ions, and effects on membrane selectivity (Kozlowski 1997). Ions of similar charge and dimension such as Na^+ and K^+, or NO_3^- and ClO_3^- exhibit competitive interactions in plants (Greenway and Munns 1980; Dean-Drummond and Glass 1982). Plant membranes may have difficulty discriminating between such ions, and can inadvertently take up one at the expense of the other. When this reduces availability of macronutrients such as N^- or K^+, shortage of these nutrients has serious implications for the plant in addition to the toxicity it is facing.

Symbiotic relationships, which provide the plant with nutrients, may also be inhibited. Reddell et al. (1986) found that saline conditions reduced the N output of some strains of nitrogen fixing bacteria associated with *Casuarina obesa* seedlings. Additionally they found that as the rate of shoot growth fell with increasing salinity, so did the size of nodules housing the bacteria. As a result, even those strains of bacteria showing no reduction in N fixed per gram of nodule, had lower output in saline than in non-saline soils (Reddell et al. 1986; Ng 1987). Although black spruce does not maintain symbiotic relationships with nitrogen fixing bacteria, it does benefit indirectly by receiving nitrogen added to the ecosystem by plants which do. Consequently, impairment of such fixation by saline substrates, particularly in a nitrogen deficient material such as a tailings sand cap over CT, will reduce the nitrogen available to planted black spruce seedlings.

Black spruce maintains mycorrhizal associations with fungi (Quoreshi and Timmer 1998), and these too could be negatively affected by salinity, thus directly affecting black spruce. Foster and Sands (1977) found that Cl^- accumulated in the fungal hyphae of mycorrhizae in *Pinus radiata* , and suggested that Cl^- and Na^+ are in part prevented from entering the root at these sites. Work by Cromer et al. (1982) supports this notion. It is unclear what effect these accumulations have on the mycorrhizae, though diminished endomycorrhizal systems have been found in trees growing in saline soils (Guttay 1976). Such reduced systems resulted in a significant decrease in the quantities of P made available to trees by roots.

Given the importance of nitrogen fixing bacteria and mycorrhizae to plant nutrition, reductions in the nutrient output from these relationships make saline soils less productive habitats. That, coupled with membrane difficulties in differentiating between ions, sets the stage for secondary stresses tied to nutrient deficiency as N, P and K, become less available. Nutrients bound like this are unavailable for other metabolic processes. All this at a time when plants are undergoing osmotic adjustment and have an increased need for nutrients for the synthesis of compatible solutes and osmoprotectants (Munns 1988).

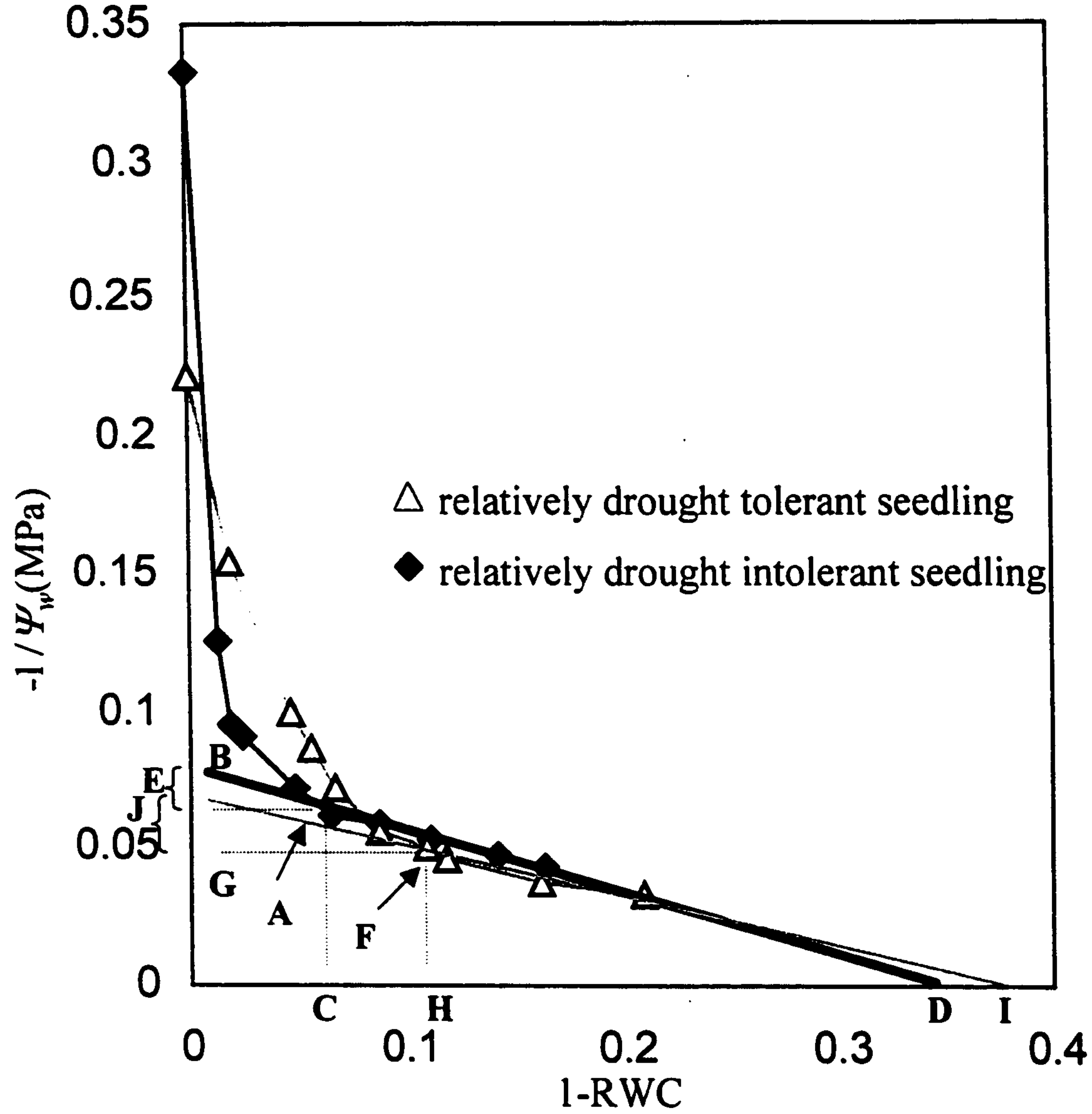
2.4 WATER RELATIONS PARAMETERS

Salt stress and drought stress are forms of water deficit stress. Plant responses to both stresses are governed by many of the same traits. This leads to an examination of water relations parameters useful for predicting seedling response to drought stress in order to assess the utility of these characteristics for predicting seedling response to salt stress. In the short term, the primary stress which seedlings planted in saline substrates are likely to be exposed to is water deficit, as ion excess and nutrient imbalances may take more time to develop (Greenway and Munns 1980). Thus, the ability of the plant to deal with water deficit as indicated by it's drought resistance characteristics, may strongly affect its performance when exposed to saline conditions. This assumes either low sensitivity to ion toxicity or that concentrations of ions in the substrate in which it is growing are below significant toxicity level.

In order to better understand how drought tolerance helps plants deal with water deficit stress, it is useful to examine the Pressure-Volume (P-V) curves of relatively drought tolerant and drought intolerant seedlings (Figure 2.4.1). P-V curves can be constructed for individual seedlings to describe the behavior of their tissue when it is subjected to water deficit stress (Tyree and Hammel 1972; Hinckley et al. 1980; Richter et al. 1980; Turner 1981; Boyer 1995).

The balance pressure, i.e. the pressure necessary to express sap at the cut surface of the branch, provides a measure of water potential, which is recorded on the Y axis. Relative water content, which is determined by weighing the freely transpiring branch, is recorded on the X axis. As plotted in Figure 2.4.1 the points derived from these coordinates, form a curve which traces corresponding changes in water potential and relative water content. Once turgor is lost, the curve becomes linear. Consequently, determination of the point where this occurs, allows derivation of many different plant water relations parameters. Point A indicates the turgor loss point for a seedling that was less drought tolerant, and allows one to read points B and C off the Y and X axis respectively. The first, B, is osmotic potential at the turgor loss point ($\Psi_{\pi_{tlp}}$), which for this seedling is –1.72 MPa. Meanwhile G, -2 MPa, is $\Psi_{\pi_{tlp}}$ for the drought tolerant seedling. . Similarly, point C, 94%, is the relative water content at the turgor loss point (RWC_{tlp}), for the intolerant seedling, and H, 89%, is the RWC_{tlp} for the tolerant seedling. Relative water content of the symplast (RWC_s) is found at point D for the relatively intolerant seedling, and point I for the more drought tolerant one. The former is 35%, and the latter is 38%. These points are determined by extending the regression line of the linear portion of the P-V curve to the point where it crosses the X axis.

Another useful water relations parameter for seedlings is osmotic amplitude for turgor maintenance ($\Delta\Psi\pi$). It is derived by extending the same regression line used for determination of RWC_s to the point where it intersects with the Y axis in order to determine osmotic potential at full saturation ($\Psi_{\pi_{sat}}$). The absolute value of the difference between $\Psi_{\pi_{sat}}$ and $\Psi_{\pi_{tlp}}$ yields $\Delta\Psi\pi$.



A: Turgor loss point for drought intolerant seedling	
B: Osmotic potential at turgor loss point:	-1.72 MPa
C: Relative water content at turgor loss point	94%
D: Relative water content of the symplast	35%
E: Osmotic amplitude for turgor maintenance	0.21 MPa

F: Turgor loss point for drought tolerant seedling	
G: Osmotic potential at turgor loss point	-2.0 MPa
H: Relative water content at turgor loss point	89%
I: Relative water content of the symplast	38%
J: Osmotic amplitude for turgor maintenance	0.55 MPa

Figure 2.4.1: Components of P-V curves used in this study.
(data from black spruce measured for Chapter 4)

These curves were constructed for seedlings used experimentally in the field in Chapter 4. When exposed to saline CT the drought tolerant seedling suffered limited injury, while the intolerant seedling died. Water relations parameters which are associated with such tolerance of water deficit stress are: low RWC_{tlp}; low $\Psi_{\pi tlp}$; low ε_{max}; high $\Delta\Psi_{\pi}$ and high RWC_{s} (low RWC_{a}) (Zine El Abidine et al 1994a; Santakumari and Berkowitz 1989). More detailed discussion of how such traits benefit plants will be found within the discussion sections of Chapters 3 and 4.

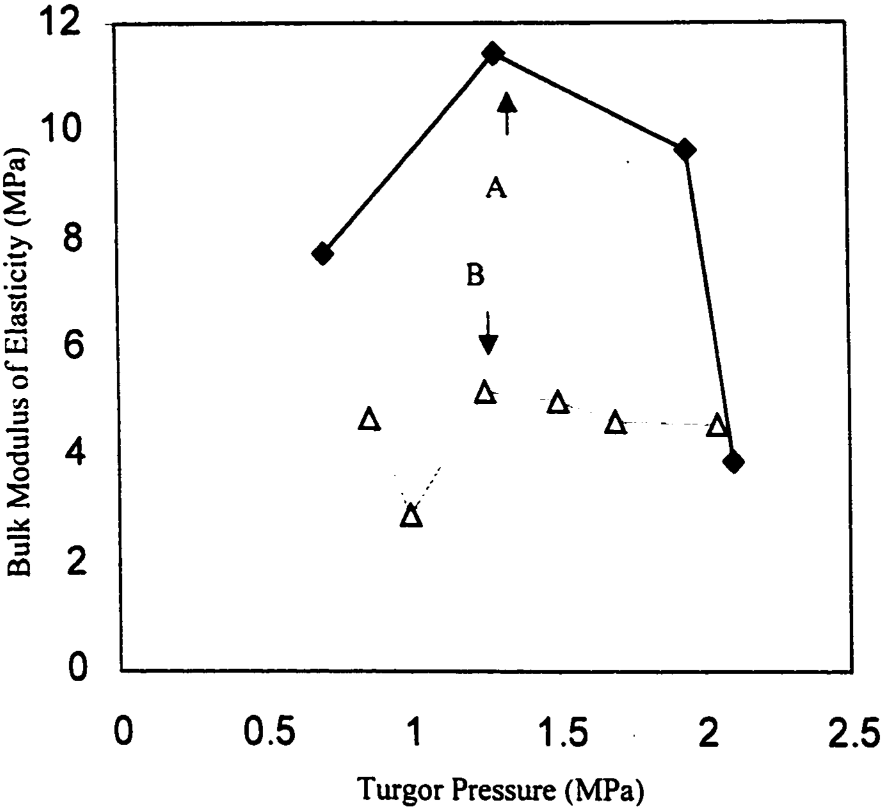
Unlike the other water relations parameters considered here, maximum modulus of elasticity (ε_{max}) is not derived directly from the P-V curve, but is instead plotted at 3% RWC intervals from the same data set using equation 1.0. The modulus of elasticity is derived mathematically and plotted as follows from the data collected to set up the P-V curve (Roberts et al. 1980):

$$\varepsilon = (\Psi_{P1}-\Psi_{P2})/((RWC_{s1}-RWC_{s2})/RWC_{s}) \qquad (1.0)$$

where RWC_{s1} and RWC_{s2} were RWC_{s} at Ψ_{P1} and Ψ_{P2}, respectively. The calculation was from RWC of 100 to 0 at 3% RWC intervals to give ε over the entire range of turgor. The maximum value was then identified.

Example seedlings for ε_{max} are presented in Figure 2.4.2. One of the differences between the two was the lower ε_{max} of the drought tolerant seedling, which indicates greater elasticity. In many plant species ε_{max} occurs at full saturation (Tyree 1976). In black spruce, however, ε_{max} varies considerably and often occurs at less than full saturation (Colombo 1987), as was found in this study.

Construction of P-V curves is a technique that can provide quite a large amount of useful information. Collection of the data necessary to construct a P-V curve was performed here using the free transpiration method (Hinckley et al. 1980). As this process requires a significant investment of time, it may not be practical for screening seedlings on an operational basis. Instead, it provides a means of identifying the characteristics of tolerant seedlings. Operational use could be achieved by planting only



A: maximum modulus of elasticity of relatively less drought tolerant seedling:	11.42 MPa
B: maximum modulus of elasticity of relatively more drought intolerant seedling:	5.17 MPa

Figure 2.4.2: Derivation of maximum modulus of elasticity from plot of modulus of cell wall elasticity versus turgor potential

seedlings from families, populations, or clones known to possess the same characteristics as seedlings which have demonstrated such tolerance of water deficit stress.

2.5 VARIABILTY IN BLACK SPRUCE

Any long-term adaptive potential that a plant may have is useless if the plant cannot survive initial exposure to stress. Thus, while it is difficult to say which seedlings are the best to plant at saline sites, it may be realistic to try to determine which ones to avoid planting, and as a consequence improve the survival rate of planted seedlings. Sands and Clark (1977) demonstrated that pretreatment of seedlings by gradual exposure to salinity can increase survival by triggering adaptive responses while the stress is low. However salt stress in the field is difficult to predict and interpret because soil salinity varies seasonally with soil moisture and rainfall (Reddell et al. 1986). Consequently planting stock should possess an ability to withstand elevated exposure to salt not just at the time of planting (as pretreatment would allow), but a capacity to withstand similar (though perhaps less extreme) fluctuations over the course of it's life. In the case of trees, such as black spruce, this would be expected to be many years.

Though salinity is likely to fluctuate over time it may pose a consistent constraint in many of the places for which black spruce is being considered (Renault et al. 1998). As a result, truly useful salt tolerance must be a lasting physical characteristic rather than a short term response to environment, and will thus be based more on genotype than phenotype. The mechanisms of genetic control and the major genes controlling salt tolerance in forest trees have not been identified (Allen et al. 1994). Thus, relevant genetic variation would be demonstrated indirectly, by measuring the responses of different genotypes. Since both salt stress and drought stress are forms of water deficit stress, plant responses to both are similar and governed by many of the same traits. This suggests that an examination of water relations parameters would be useful for predicting plant response to drought stress in order to assess the utility of these characteristics for predicting plant response to salt stress. Determination of a strategy for selection of trees upon which measurements will be made is an important first step.

One source of variability that has been studied at length in black spruce is edaphic ecotypes. It has been theorized that environmental differences between upland and lowland habitats could create selective pressures that lead to the development of such ecotypes (Wilde 1954; O'Reilly et al. 1985). However, most studies of morphology and isozyme variation have found little difference between the two site types (Fowler and Mullin 1977; Parker et al. 1983). An exception is Yeh et al. (1986), who found some ecotypic isozyme variation in Newfoundland. Work in Alberta by Yeh et al. (1993) also found evidence of differences in growth between upland and lowland populations. However, based upon their genetic data, they concluded that selection pressure on upland and lowland populations was variable and probably not excessively strong. Wang and Macdonald (1993) found that the majority of variability was intrapopulational, and very small between populations or sites

It is understandable why so much work has been done to examine the possibility of soil based ecotypes, considering that *P. mariana* is the most important pulpwood species in Canada (Viereck and Johnston 1990). Consequently it is the most frequently planted tree east of Manitoba (Smyth and Brownright 1984).

Black spruce trees grow much better on upland than on lowland sites; but in upland stands they are usually overtopped by white spruce, aspen, and poplar (Wang 1991). Though upland sites are widely recognized as the most productive, Viereck and Johnston (1990) note that in the southern parts of its range *P. mariana* does well on some heavy peats underlain by considerable amounts of decayed woody material. Jeglum (1974) suggests that when moist forest and swamp are considered, the rate of tree growth is constrained mainly by excess moisture or deficient aeration. However, whenever moist forest and muskeg, or swamp and muskeg are considered, the limiting factors are a combination of reduced nutrients and unfavorable moisture-aeration status.

Wilde (1954), noted a marked difference in the inherent rate of growth of *P. mariana* nursery stock raised from seed collected in muskegs and that of upland soils. Though additional research was necessary, these differences provided an example of the need to consider soil as well as climatic factors in choosing seed sources for reforestation (Wilde 1954). It was also suggested that selective pressure could become significant if forest stands occupy an area of soil large enough to exclude pollination from trees growing on soils of different composition (Wilde 1954). However, the author also recognized that the progeny of phenotypically inferior, or dwarfed trees, located on small areas of infertile soils such as calcareous outcrops, often regain their hereditary good form and high growth potential when planted on reasonably favorable sites. While Wilde advanced the notion of soil ecotypes, he left room for further work to determine their relevance with regards to *P. mariana*.

Morgenstern (1969a, 1969b & 1978) and Morgenstern and Mullin (1990) carried out a series of provenance tests which took in sources spread across the range of *P. mariana* and monitored the progress of these trees over the years since their planting. Little evidence has been found for ecotypic variation, as the performance of populations from dry and wet sites differed less than expected (Morgenstern 1969a). Just as Wilde (1954) observed earlier, rates of germination for the sites varied markedly, with seed from wetter sites performing comparatively poorly. However, Morgenstern (1969a) concluded that this difference in initial vigor was not genotypic, but due instead to the effects that maternal nutrition has on seed composition and seedling survival. Wang (1991) observed similar patterns in Alberta. Germination rate is a juvenile characteristic not indicative of growth potential at later stages. By the time seedlings had survived for two years, seed size became unimportant (Morgenstern 1969a).

Adaptation to soil moisture, and the development of drought resistance were found not to be isolated processes, but instead dependent upon a number of interacting factors in the total environment (Morgenstern 1969a). Additionally, none of the stands tested for drought resistance proved sufficiently isolated to be the distinct breeding populations that Wilde (1954) suggested would be necessary for the development of soil ecotypes. Instead most subpopulations were found in a continuous series from wet through moist and fresh to moderately dry sites (Morgenstern 1969b). This is not surprising given that *P. mariana* has a broad distribution and occurs in multiple forest associations, which allow it to readily grow under many different conditions in mixed stands as well as pure ones (Viereck and Johnston 1990). The primary variation observed was a clinal north to south variation, developed by selection in response to continuous ecological factors such as day length, and regional temperatures (Morgenstern 1969b).

Similarly, Fowler and Mullin (1977) found no evidence of strong edaphic ecotypes of *P. mariana*. Interestingly, their seedlings, unlike those of Wilde, Morgenstern, or Wang, did not show a significant difference in germination rate between seeds from upland and lowland sites. There were, however, some clinal differences, as the northern sources did worse than those from the south. This was attributed to environmental instead of genetic differences. Northern provenances of trees often have low rates of germination due to the inhibitory effects cold temperatures have on successful embryogenesis.

In comparing artificial regeneration on upland versus lowland sites, Wood and Jeglum (1984) found that planted seedlings in uplands were taller and grew faster than seedlings planted in lowland sites. When one considers that much of the seed used in these reforestation programs comes from phenotypically good stands growing in organic lowland soils, even though most of the planting takes place on nonorganic upland soils (Fowler and Mullin, 1977) the difference in performance is interesting. In the very least, it offers support to the notion that pronounced soil ecotypes do not exist.

Work in Alberta by Wang and Macdonald (1992) showed little variation in allozyme and phenotypic traits, though lowland spruce had higher phenotypic plasticity than upland. Fowler and Mullin (1977) concluded that the presently defined regions for seed usage seem sufficient to prevent the use of poorly adapted materials in reforestation programs.

Zine El Abidine et al (1994a) found some significant differences in water relations parameters between upland and lowland populations, however these differences were generally small and, most importantly, were not consistent. Wang (1991) expected upland black spruce to be more drought resistant than lowland, but found no difference between the two. The instances Zine El Abidine et al. (1994a) recorded where P-V curve data suggested a population with superior drought resistance were found among progeny of one lowland population. These traits included: lower relative water content at the turgor loss point, lower maximum modulus of elasticity, lower osmotic potential at the turgor loss point, and higher osmotic amplitude for turgor maintenance. It was concluded that the localized development of a drought tolerant population could have occurred anecdotally because root systems of trees in lowlands are not as well developed as those in uplands and are thus subject to greater stress and selective pressure. Root development and function are severely hampered by high water tables. Denyer and Riley (1964) observed that lowland black spruce can be exposed to significant drought stress because of this restriction of roots systems to surface layers of muck and duff which become extremely dry in drought seasons.

After examining seasonal patterns of water relations parameters in mature black spruce (Zine El Abidine et al. 1994a), the response of seedlings to drought (Zine El Abidine et al. 1994b), and gas exchange and water relations of seedlings (Zine El Abidine et al. 1995), Zine El Abidine et al. (1995) agreed with Morgenstern (1969a) that ecotypic differences in black spruce are neither wide spread, nor well defined. Given the amount of evidence from many other researchers that supports this view, it is reasonable to conclude that meaningful ecotypic variations between upland and lowland black spruce do not occur reliably, if at all. Consequently, most differences observed between the two are probably phenotypic rather than genotypic in origin. Selection of black spruce for drought (and thus salt) tolerance should thus focus on intrapopulational variation, or identification of populations growing in saline environments.

2.6 SYNTHESIS

CT contains NaCl, and Na_2SO_4 at potentially injurious concentrations, which complicates revegetation of CT affected sites. However, the production of CT is preferable to the status quo, which is based upon the accumulation and perpetual storage of fine tails. Of the ions present in CT, Cl^- is likely to be the greatest constraint on plant growth and revegetation. Germination and establishment of seedlings on saline sites is poor, and not an effective means of eliminating salt intolerant individuals. A better strategy would be planting seedlings so as to take advantage of the increase in ability to tolerate salts that occurs as plants age.

Planting is expensive, so it is worth taking steps to improve survival by screening planting stock. While it is difficult to say which seedlings are truly superior and should be planted preferentially, it may be realistic to try to determine which ones to avoid planting, and by doing so, improve the rate of survival. Characteristics of superior seedlings could include improved sequestration of ions in the roots and stems, faster establishment of ionic gradients necessary for vacoular compartmentalization of ions, and a superior capacity to osmotically adjust through production of compatible solutes with minimal use of toxic ions. However, adaptation to and toleration of a stress requires that a plant survive long enough to do so. Plants which die in the short term are dead regardless of any long term adaptive potential they may have had. Unless ion concentrations are high, or seedlings are sensitive to ions, water deficit stress will likely injure seedlings more quickly than ion toxicity does. Thus, if drought tolerance proves helpful, then the superior seedlings will be a subset of those capable of handling the water deficit stress.

Before identification of such a subset is possible, one must determine if the concept of the larger set is actually meaningful. In short, does the ability of black spruce seedlings to deal with water deficit stress actually affect their performance when exposed to saline conditions? This assumes either low sensitivity to ion toxicity or that the concentrations of individual ions are below significant toxicity, as sufficient damage by ions can rapidly kill a plant regardless of it's capacity to tolerate water deficit. Thus, this study has examined the extent to which such assumptions apply to black spruce. It has done so by attempting to answer questions such as: is black spruce sensitive to Cl^- like many other plants (including other species of spruce)? In the absence of toxic concentrations of Cl^- (or if there is little sensitivity to Cl^-), is tolerance of water deficit stress helpful to black spruce? Can that be translated into success in CT in the field, where other environmental stresses are present along with NaCl and Na_2SO_4?

Given that ecotypic variations do not occur reliably within black spruce, the individuals used in each experiment during this study came from single populations in order focus on intrapopulational variation. Water relations parameters were derived using Pressure-Volume curves in order to characterize each seedling's capacity for dealing with water deficit stress. Seedlings were then subjected to salt stress in order to measure any differences in damage suffered by relatively drought intolerant seedlings and relatively drought tolerant seedlings. Rather than trying to identify superior seedlings, the purpose of this study has been to use water relations to determine what separates these from undesirable ones so that by screening planting stock one could avoid planting those least suited to saline conditions, and improve survival. Essentially, it is a search for suitable rather than superior seedlings. Operational use of such water relations based screening techniques could be achieved by planting seedlings from families, populations, or clones known to possess similar characteristics as seedlings which have demonstrated such tolerance of water deficit stress. Future identification of additional mechanisms, such as those identified by the Munns and Termat (1986) model, could allow refinement of screening and more precise identification of the subset of superior seedlings from among those that are suitable.

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3.0 Effects Of Water Deficit Stress And Ion Toxicity On Black Spuce (*Picea mariana* (Mill) B.S.P.) Seedlings

ABSTRACT

Pressure Volume curves were used to determine the drought tolerance characteristics of black spruce (*Picea mariana* (Mill) B.S.P.) seedlings. The seedlings, which had been grown in a growth chamber in solution culture for a month prior to construction of the curves, were then exposed to salt stress induced by NaCl or Na_2SO_4 solutions. Over the next two weeks, measurements of visible injury, water potential, transpiration, stomatal conductance, and electrolyte leakage were made. Visible injury and electrolyte leakage showed that seedlings were significantly more sensitive to NaCl than to Na_2SO_4, although the other measured values did not detect differences. A P-V curve derived drought tolerance characteristic, osmotic potential at the turgor loss point, had predictive value for electrolyte leakage in seedlings exposed to Na_2SO_4 but not in seedlings exposed to NaCl. Black spruce appears to be more sensitive to Cl^- than to Na^+ or osmotic stress. In the absence of injuriously high concentrations of Cl^-, the drought tolerance of an individual contributes to it's ability to cope with salt induced water deficit.

3.1 INTRODUCTION

The amount of land required to dispose of oil sands tailings far exceeds that of any other form of mineral processing (Marshall 1982). Although procedures for handling and disposing of tailings are simple, the volume to be dealt with imposes practical constraints on reclamation planning (Moneco 1983). Production of CT (called "Composite Tails" by Syncrude and "Consolidated Tails" by Suncor) reduces the volume of tailings by removing most of the water component from the fine tails. Nonetheless CT retains a high water content and high concentrations of Na^+, Cl^-, and SO_4^{2-} (Renault et al., 1998). Consequently vegetation on reclaimed end-of-lease landscapes will be exposed to relatively high levels of salt resulting from contact with tailings material.

Water deficit stress and ion excess are the two major constraints to growth of plants exposed to saline conditions (Greenway and Munns 1980). Zine El Abidine et al. (1994) identified several water relations parameters derived from Pressure-Volume (P-V) curves, which were indicative of drought tolerance. However, little work has been done regarding ion toxicity and black spruce, though information about other species is available. Peanuts, when faced with equivalent water deficit in iso-osmotic solutions of NaCl and Na_2SO_4 suffer greater reductions in chlorophyll content, and carbon assimilation in NaCl than in Na_2SO_4 (Chavan and Karadge 1980). Similar sensitivity has been observed in Serbian spruce (*Picea omorika*), a relative of black spruce, and jack pine (*Pinus banksiana*), an associate of black spruce (Alt et al. 1982; Croser et al. 1999). Sodium sensitivity does not pose as much of a challenge for most plants, though Na^+ can also be toxic for plants at sufficiently high concentrations. Sodium becomes a concern where there are low Ca^{2+} concentrations in the substrate, or poor soil aeration (Marschner 1995). Croser and Zwiazek (2000) noted that under hypoxic conditions present in CT, plants have a decreased ability to sequester Na^+ in the roots.

The objective of this experiment was to determine the mechanisms of salt toxicity in black spruce, and to test the hypothesis that drought tolerance factors can help minimize damage to plants exposed to sodium unless excessive chloride is present as well. Pressure-Volume curves were used in this study to examine water relations parameters of individual seedlings, before the seedlings were exposed to NaCl and Na_2SO_4. Iso-osmotic solutions of Na_2SO_4 and NaCl were used to allow a comparison between osmotic and ionic effects. Simultaneously, an iso-ionic solution of NaCl matched the Na_2SO_4 treatment in order to test for sodium sensitivity.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

Seedlings used in this experiment were one-year-old and from a single population (ATSIC# 1758 73-17 W4, 600 m), supplied by the Smoky Lake Forest Nursery (Smoky Lake, Alberta). The seedlings were acquired dormant at the end of the growing season, transplanted into 400 ml peat-filled Spencer-Lemaire containers

(Spencer-Lemaire Industries Ltd., Edmonton Alberta, Canada) and held at 4 °C in a cold room for two months. When buds began to swell, the temperature was increased to 10 °C, and after 1 week, the roots of 96 seedlings were washed and the seedlings were transplanted into solution culture in a growth chamber. Conditions in the growth chamber were set at an 18-hour photoperiod, 20 °C temperature, and 70% relative humidity. The solution culture (Figure 3.2.1) consisted of aerated modified Hoagland's solution (Epstein 1972). Bud flush occurred shortly after transplanting and the seedlings were allowed to recover for four weeks before being exposed to treatment solutions. During this time, they grew an average of 5 to 10 cm in height and produced many lateral branches.

3.2.2 Pressure-Volume curve data

Pressure-volume curves were constructed by the free transpiration method (Hinckley et al. 1980), using a pressure chamber (PMS Instruments, Corvalis Oregon, USA). Tissue sampled for the P-V curves came from the tips of the uppermost lateral branches, approximately 5 cm long. Curves were constructed for each of the 96 seedlings that were subsequently used for experimentation. The parameters derived from these curves were osmotic potential at the turgor loss point ($\Psi_{\pi tlp}$), relative water content at the turgor loss point (RWC_{tlp}), osmotic amplitude for turgor maintenance ($\Delta\Psi\pi$), and maximum modulus of elasticity (ε_{max}).

3.2.3 Treatments

The seedlings were randomly assigned to one of four different treatments (24 seedlings per treatment), each containing aerated modified Hoagland's solution (Epstein 1972). The following treatment solutions were used: control, 60 mM Na_2SO_4, 90 mM NaCl, and 120 mM NaCl. Figure 3.2.2 indicates how the iso-osmotic solutions of 60 mM Na_2SO_4 and 90 mM NaCl were used to allow a comparison between the effects of osmotic stress, and Cl^- sensitivity, while the 120 mM NaCl was iso-ionic with the 60 mM Na_2SO_4 which allowed testing for Na^+ sensitivity. Eight seedlings were placed in each container, with three containers (24 total seedlings) per treatment. Oxgen content of the solutions was monitored during the experiment using a YSI 5000 dissolved oxygen

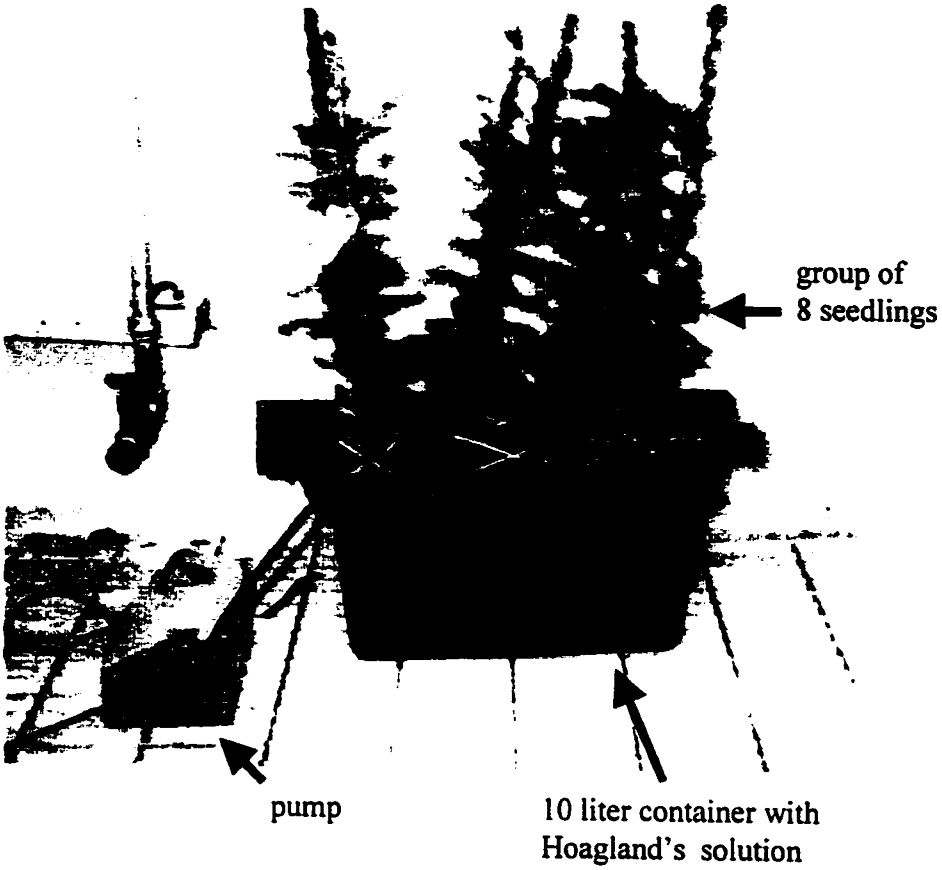
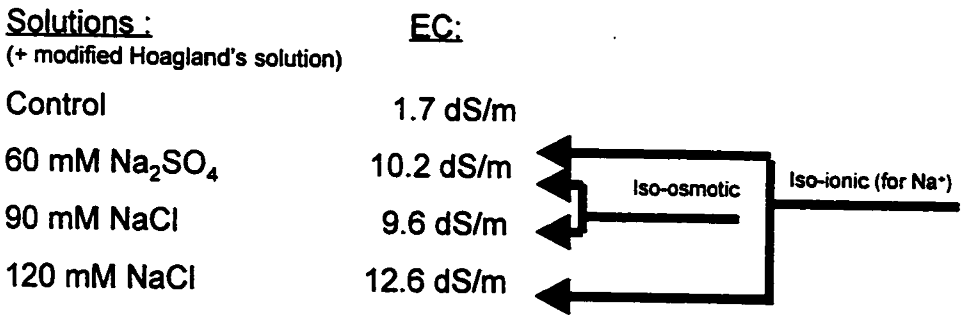


Figure. 3.2.1: Example of solution culture set up used in this experiment, showing pump,seedlings, and container.



Solutions : (+ modified Hoagland's solution)	EC:
Control	1.7 dS/m
60 mM Na_2SO_4	10.2 dS/m
90 mM NaCl	9.6 dS/m
120 mM NaCl	12.6 dS/m

Figure 3.2.2: Experimental design used here to determine sensitivity of black spruce to Cl^- toxicity, water deficit stress and Na^+ toxicity.

meter (YSI Inc, Yellow Springs Ohio, USA), in order to maintain a consistent oxygen content of between 6 and 8 mg O_2 L^{-1} solution.

3.2.4 Visible injury and water relations measurements

Treatment lasted for two weeks with shoot water potential (Ψ_w) measured at the end of week one. Visible injury was recorded just before harvesting at the end of week two. Injury was ranked on a scale of 0-10 (10 being greatest) by examining the leader, two lateral branches at the top of the tree, and two lateral branches at the bottom of the tree. Two locations on each branch were assessed, near the bud and the stem, with one point assigned for discoloration, necrotic tissue, or needle loss at each location. Transpiration (T) and stomatal conductance (g_s), were measured at the end of the second week. Water potential was measured on lateral branches using a pressure chamber (PMS Instruments, Corvalis Oregon, USA), as described by Scholander et al. (1965). Transpiration and stomatal conductance were measured with a LI-1600 steady state porometer (Li-Cor, Lincoln Nebraska, USA).

3.2.5 Electrolyte leakage as a measure of injury & toxicity of solutions

Electrolyte leakage was measured at the end of the second week using live tissue from the lowest branches of each seedling. A conductivity meter (Hanna Instruments Inc., Woonsocket, RI, USA) was used to compare the ratio of electrolytes leaking out after 5 hours to the total electrolytes released from the same tissue after killing it by heating at 121 °C and subsequent freezing at -85°C (Zwiazek and Blake 1991a). This value was expressed as leakage as a percentage of total. Toxicity was defined as a statistically significant finding that a substance caused injury (i.e. elevated electrolyte leakage as compared to controls) following exposure. This study did not attempt to determine the toxic concentration thresholds for Na^+ or Cl^- in black spruce.

3.2.6 Statistical analysis

Statistical analysis was performed using SAS version 6.12 (SAS Institute Inc., Cary, North Carolina, USA). These included linear regression, correlation and analysis of variance (with orthogonal contrasts). For the linear regression, electrolyte leakage was the dependent variable and each of the water relations parameters derived from the P-V curves served as independent variables ($\Psi_{\pi tlp}$, RWC_{tlp}, RWC_s, $\Delta\Psi\pi$, and ε_{max}). The formula was as follows:

$$y_i = \alpha + \beta x_i + \varepsilon_i \tag{3.0}$$

where:

y_i = dependent variable (electrolyte leakage)
α = Y intercept of regression
β = coefficient of regression
x_i = independent variable (each was performed as a separate regression: $\Psi_{\pi tlp}$, RWC_{tlp}, RWC_s, $\Delta\Psi\pi$, and ε_{max})

Correlation matrices were run to test for relationships between variables. An analysis of variance was also conducted for each of the measured dependent variables, with treatment as the factor. The model used in each case was the same:

$$x_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \tag{3.1}$$

where:

x_{ijk} = dependent variable (visible injury, Ψ_w, T, g_s, or electrolyte leakage - - each was performed as a separate ANOVA)
α_i = treatment (control, 60 mM Na_2SO_4, 90 mM NaCl, 120 mM NaCl)
β_j = replicate (1st, 2nd, and 3rd)

A set of 5 orthogonal contrasts were performed for each of these ANOVAs. These were: 1) chloride vs. no chloride; 2) control vs. Na_2SO_4; 3) between chlorides; 4) between iso-osmotic; and 5) between iso-ionic Na. A final ANOVA was performed upon control and Na_2SO_4 treated seedlings with electrolyte leakage as the dependent variable and $\Psi_{\pi tlp}$ and treatment as factors. The model in this case was:

$$x_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \tag{3.2}$$

where:

x = dependant variable (electrolyte leakage)
α_i = treatment (control, 60 mM Na_2SO_4)
β_j = Osmotic potential at turgor loss point group ($\Psi_{\pi tlp} > -2$ and $\Psi_{\pi tlp} < -2$)

3.3 RESULTS

The visible injury index of seedlings indicated a strong treatment effect as shown in Figure 3.3.1a. As opposed to control and Na_2SO_4 treated seedlings, plants in both 90 mM NaCl and 120 mM NaCl showed significant needle necrosis and needle loss. Table 3.3.1 presents the results of an ANOVA performed on the visible injury data. There was a significant treatment effect with orthogonal contrasts indicating that profound differences existed between seedlings that were exposed to toxic concentrations of chloride, and those which were not. There was little difference between control and 60 mM Na_2SO_4 seedlings, although significant differences occurred between the Na_2SO_4 seedlings and the seedlings exposed to iso-osmotic NaCl and iso-ionic (for Na^+) solutions.

Water potential showed significant treatment effects (Figure 3.3.1b and Table 3.3.1). Orthogonal contrasts were performed to determine how the significance was partitioned between treatments and found significant differences in Ψ_w between the seedlings exposed to Cl^- and those which were not, as well as significant differences between control and Na_2SO_4 treated seedlings. No differences were noted in Ψ_w between seedlings exposed to different concentrations of Cl^-, between seedlings in iso-osmotic solutions, or the solutions iso-ionic for Na^+. The same observations were made for transpiration (Figure 3.3.1c and Table 3.3.1) and stomatal conductance (Figure 3.3.1d and Table 3.3.1). Water relations parameters had some effect on stress seedlings suffered in NaCl as a correlation was found between ε_{max} and T for seedlings exposed to 120 mM NaCl, but not the other solutions. Seedlings with low ε_{max} showed a greater reduction in transpiration than those with higher ε_{max} (Table 3.3.1).

Measurement of damage yielded slightly different results. Electrolyte leakage (Figure 3.3.1e and Table 3.3.1) showed significant treatment effects when tested with ANOVA. Orthogonal contrasts were performed to determine how the significance was partitioned between treatments and found significant differences in electrolyte leakage between those seedlings exposed to Cl^- and those which were not, as well as significant differences between control and 60 mM Na_2SO_4 exposed seedlings. Similarly, there was no difference between the levels of Cl^-, however as with the recorded visual injury data, there were significant differences between seedlings in the iso-osmotic solutions, as well

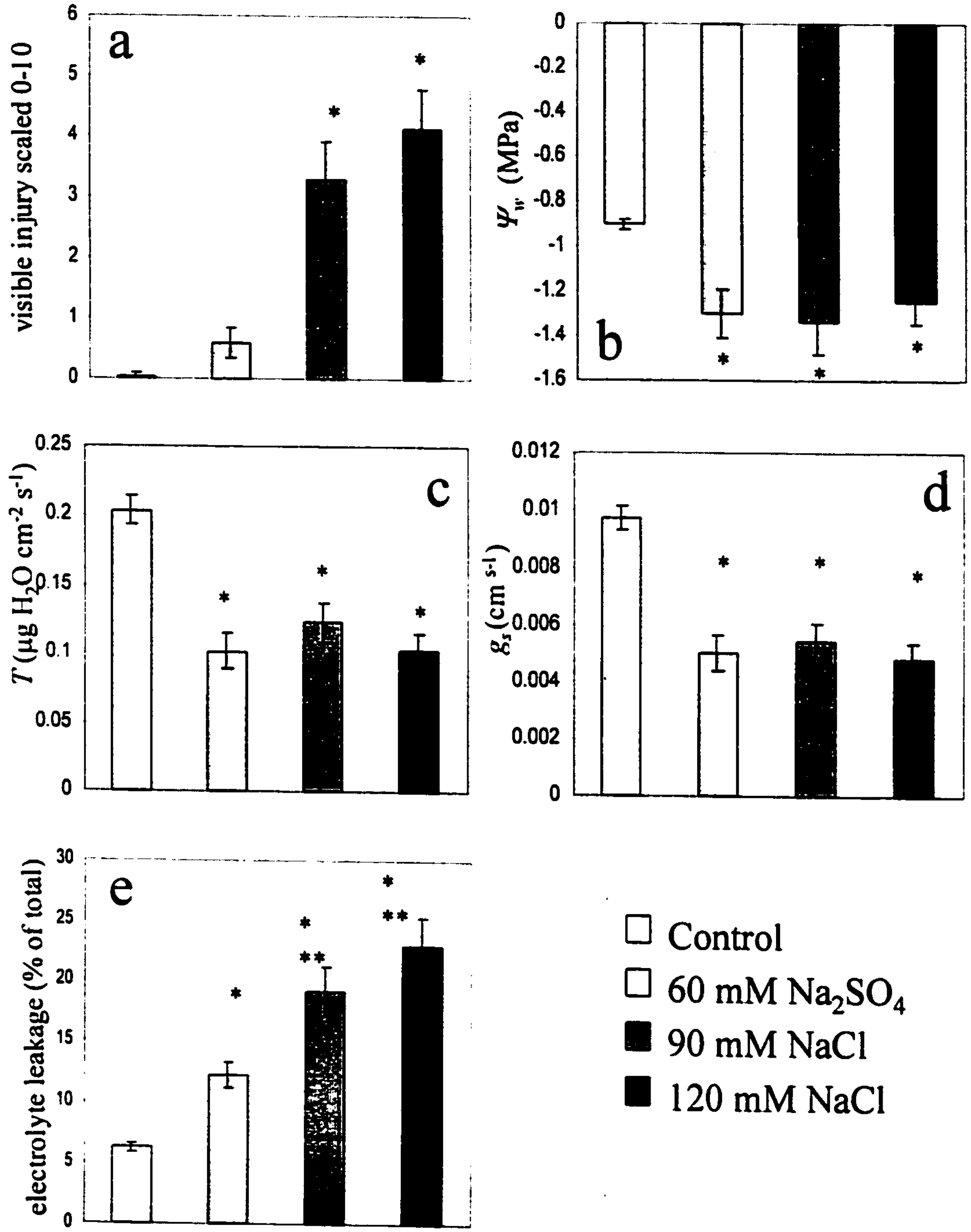


Figure 3.3.1 Dependent variables measured over the course of the experiment: a) Visible injury index; b) water potential after 1 week; c) Transpiration after two weeks; d) stomatal conductance after two weeks; e) electrolyte leakage after two weeks. Error bars represent standard error, n=24 (per treatment).

* indicates significant difference from control at p=0.05

** indicates significant difference from Na_2SO_4 at p=0.05

Table 3.3.1 Statistical analysis of measured variables.

Test	*Parameter* Visible injury Index	Water Potential	Transpiration	Stomatal Conductance	Electrolyte Leakage
ANOVA					
Treatment	p<0.0001	p=0.0170	p<0.0001	p<0.0001	p<0.0001
Orthogonal Contrasts					
chloride vs. w/out	p=0.0007	p=0.0018	p<0.0001	p<0.0001	p<0.0001
control vs. Na_2SO_4	p=0.3878	p=0.0090	p<0.0001	p<0.0001	p=0.0117
between chlorides	p=0.1853	p=0.5193	p=0.2089	p=0.3990	p=0.0989
between iso-osmotic	p<0.0001	p=0.8007	p=0.2004	p=0.5639	p=0.0032
netween iso-ionic Na	p<0.0001	p=0.6947	p=0.9808	p=0.7891	p<0.0001
Correlations	---	---	ε_{max} 120 mM NaCl p=0.0277	---	$\Psi_{\pi tlp}$ 60 mM Na_2SO_4 p=0.0194

Table 3.3.2: ANOVA for osmotic potential at the turgor loss point of seedlings not exposed to injuriously high concentrations of Chloride.

Dependent variable: electrolyte leakage as a measure of injury
Factors: EC and $\Psi_{\pi tlp}$

Two EC groups:		Two $\Psi_{\pi tlp}$ groups:	
Control	60 mM Na_2SO_4	$\Psi_{\pi tlp} > -2$ MPa	$\Psi_{\pi tlp} < -2$ MPa
EC<2	EC>9.9	(-1.47 to -1.96)	(-2 to -2.73)

EC:	p<0.0001
$\Psi_{\pi tlp}$:	p=0.0020
EC X $\Psi_{\pi tlp}$-	p=0.0015
R^2:	0.5824

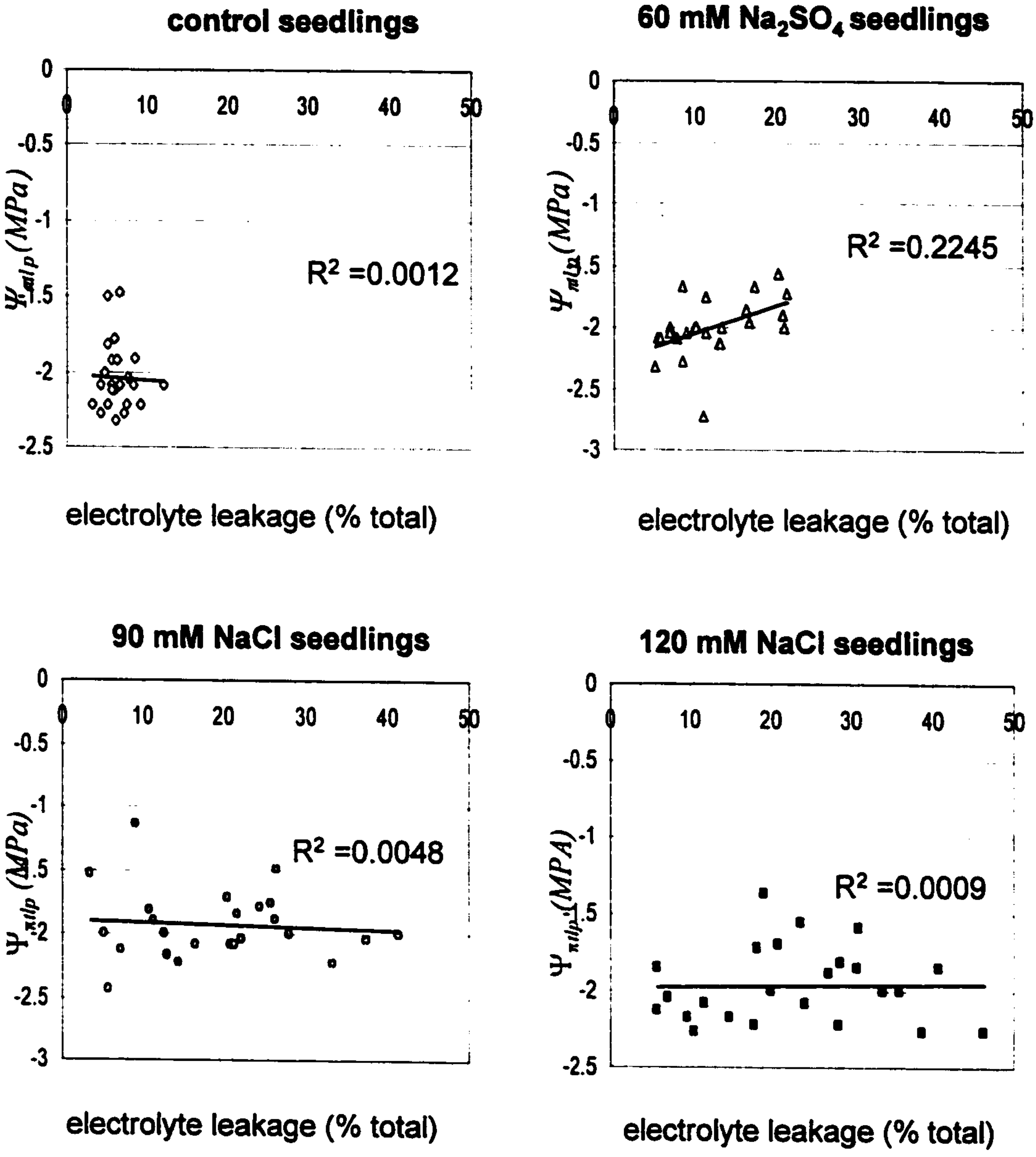


Figure 3.3.2 Electrolyte leakage vs. osmotic potential at turgor loss point for seedlings of each individual treatment.

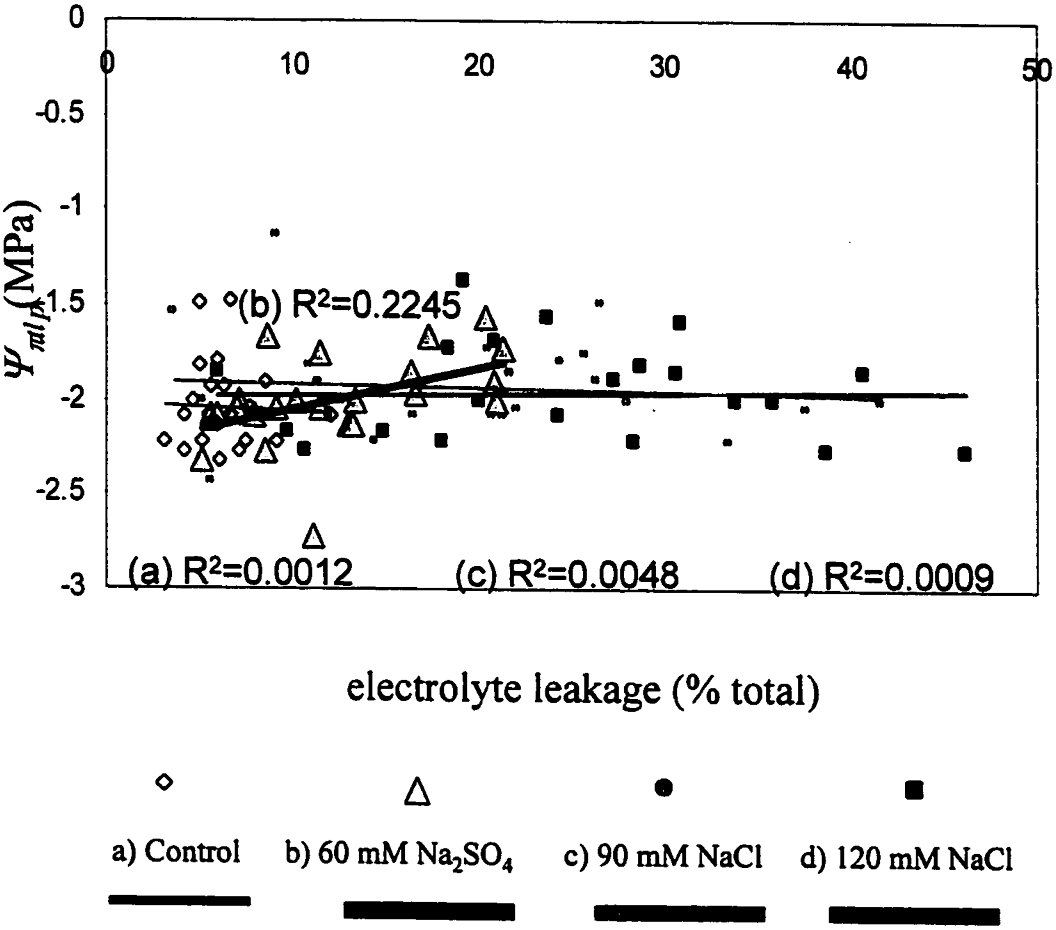


Figure 3.3.3 Electrolyte leakage vs. osmotic potential at the turgor loss point for seedlings of all treatments plotted to allow comparison between treatments. Treatments are plotted individually in Figure 3.3.2.

as in the solutions that were iso-ionic concentrations of Na^+. In 60 mM Na_2SO_4, low $\Psi_{\pi tlp}$ was correlated with less electrolyte leakage (p=0.0194). Osmotic potential at the turgor loss point was plotted against electrolyte leakage and regressions were performed (Figures 3.3.2 and 3.3.3). Other P-V curve derived data did not demonstrate a significant relationship with electrolyte leakage, and are thus not presented here.

An ANOVA on the seedlings exposed to 60 mM Na_2SO_4 further examined the relationship between $\Psi_{\pi tlp}$ and electrolyte leakage, the results of which are presented in Table 3.3.2. Two treatments were considered, the control and the 60 mM Na_2SO_4. The seedlings were divided into two groups: $\Psi_{\pi tlp} > -2$ MPa (these varied from –2.0 to -2.73 MPa), and $\Psi_{\pi tlp} < -2$ MPa (these varied from –1.47 to -1.96 MPa). Where treatment solutions had a high EC, significant injury resulted (p=0.0001). The lower the $\Psi_{\pi tlp}$ the less electrolyte leakage occurred (p=0.0020). Most importantly, there was an interaction between treatment and $\Psi_{\pi tlp}$ (p=0.0015), indicating that low $\Psi_{\pi tlp}$ reduced the injury which was attributable to high EC.

3.4 DISCUSSION

Water relations, Ψ, T, and g_s, were affected by all salt treatments. However, there were few differences detected between the treatments (Figures 3.3.1b, c, and d). Electrolyte leakage, on the other hand showed a distinct progression. These values closely matched the visual injury for the NaCl treatments, but not for the 60 mM Na_2SO_4 treatments. This would suggest that visual observation of injury can provide a good indication of Cl^- toxicity, but is less reliable in assessing damage which results from water deficit or Na^+ toxicity. This is not particularly surprising however, as Zwiazek and Blake (1991b) demonstrated that electrolyte leakage was an effective means of early detection of osmotically induced membrane injury in black spruce.

Changes in membrane function occur in early stages of plant injury, and tend to be proportional to the level of injury (Dlugokecka and Kacperska-Palacz 1978), which would explain why such injury was measurable in Na_2SO_4 treated seedlings before becoming visually evident. When faced with water deficit, drought tolerant plants protect membrane integrity better than those which are less tolerant (Martin et al. 1987).

Comparison of treatments in Figure 3.3.3 where electrolyte leakage is plotted against $\Psi_{\pi tlp}$ illustrates both the significant differences between treatments, and the effect of $\Psi_{\pi tlp}$ on electrolyte leakage. Control seedlings were all clustered between about 2-12% electrolyte leakage (as a percent of total), and so this could be described as the leakage expected of healthy tissue. Seedlings exposed to 60 mM Na_2SO_4 ranged between 5 and 22% electrolyte leakage, with a distinct slope present, indicating a relationship between low $\Psi_{\pi tlp}$ reduced damage. There was little difference between the two NaCl treatments. The 90 mM NaCl ranged from 5 to 37% and had no slope, while the 120 mM NaCl ranged from 7 to 46% and also had no slope. Dramatic differences existed between the R^2 values of the NaCl regression lines and the Na_2SO_4. The regression lines for the NaCl treatments showed no more relationship between $\Psi_{\pi tlp}$ and electrolyte leakage than the regression line for the controls did. Meanwhile, the Na_2SO_4 regression line showed a strong relationship between the two, suggesting that even though $\Psi_{\pi tlp}$ did not account for all the variability in electrolyte leakage among Na_2SO_4 exposed seedlings, it contributed significantly by moderating injury.

Compared with control seedlings, electrolyte leakage in the 60 mM Na_2SO_4 seedlings was significantly higher. In turn, the NaCl treatments resulted in significant increases in electrolyte leakage compared with the Na_2SO_4 treatment. Several of the 120 mM NaCl seedlings did experience more damage than the 90 mM NaCl seedlings, but there was not a significant difference between the two groups of NaCl treated plants. This suggests that most of the membrane sites which were effected by NaCl and resulted in electrolyte leakage, were damaged at the lower concentration.

Chloride causes injury by accumulating in the cytoplasm and disrupting the functioning of organelles, particularly the chloroplasts (Shannon et al. 1994). When exposed to salt stress, glycophytes try to store Cl- in the vacuoles of living cells leaving cytoplasmic organelles relatively Cl- free (Foster and Sands 1977). Sensitivity of an individual to Cl- is partially dependent upon its ability to do this (Binzel 1988). Failure to sequester Cl^- causes disruption of cell metabolism (Munns 1993, Aspinal 1986).

Given the significant differences in injury between seedlings in 60 mM Na_2SO_4 and 90 mM NaCl, even though these are iso-osmotic solutions, it is possible to conclude that Cl^- is more toxic to black spruce than is Na^+ or salt induced osmotic stress (p=0.0032). Similarly, for iso-ionic concentrations of Na^+ as found in 60 mM Na_2SO_4 and 120 mM NaCl, significantly more damage occurred in the solution containing high levels of Cl^- (p=0.0001). This suggests that: when aeration was not an issue, Cl^- toxicity was more of a concern for black spruce than was Na^+ toxicity. Aeration can be a problem in CT. However, since black spruce appears to be more sensitive to Cl^- than Na^+, even if Na^+ uptake increases under poorly aerated conditions (due to reduced capacity to exclude or sequester it), unless Cl^- uptake decreases, Cl^- will remain a significant constraint. Thus even under poorly aerated conditions as are likely to be found in Composite Tailings material (Croser and Zwiazek 2000), the same hierarchy of constraints is likely to exist.

Injury (and thus toxicity) is indicated by the disruption of cell functioning such as damage to membranes indicated by increases in electrolyte leakage (Dlugokecka and Kacperska-Palacz 1978). Figures 3.3.2 and 3.3.3 illustrate this difference between Cl^- toxicity and the combined effects of Na^+ and water deficit on plants. In the absence of toxic concentrations of Cl^-, damage among Na_2SO_4 exposed seedlings is significantly less than other treatments. Injury under such conditions can be regulated and minimized by some plants, as indicated by the slope of the regression line and relatively high R^2 value (as compared to the other treatments). Strong evidence of this is provided by the ANOVA performed on the controls and 60 mM Na_2SO_4 seedlings, reported in Table 3.3.2. The interaction between EC and $\Psi_{\pi tlp}$ indicates that damage that results from osmotic stress caused by Na_2SO_4. is reduced in plants with a higher ability to tolerate water deficit stress. It is logical that P-V curve derived water relations parameters would influence plant response to water deficit stress. Not surprisingly, however, injury due to ion toxicity does not appear to be addressed by such characteristics, unless these affect rates of ion uptake and water use efficiency, neither of which was measured here.

It appears that damage in NaCl treated seedlings is not influenced by the P-V curve derived water relations parameters of seedlings. However there was some evidence of relatively increased stress among those seedlings with a greater capacity to tolerate water deficit. In 120 mM NaCl, low ε_{max} was correlated with decreased transpiration. It is possible that injury among such seedlings might have been due more to ion toxicity than water deficit as these seedlings may have better tolerated the osmotic stress but as a result taken up more Cl^-. Perhaps damage appears similar between more and less drought tolerant seedlings because similar amounts of injury occurred, even thought it was caused by different factors. Drought tolerant trees may have endured the osmotic stress, taken up more ions and thus suffered greater toxicity, while intolerant trees may have succumbed to the osmotic stress directly. Regardless, seedlings exposed to toxic concentrations of Cl^- not only experienced greater damage than those dealing with osmotic stress and little Cl^-, but appeared in general to be less able to regulate or minimize this injury.

It is worth noting that there were seedlings in both the 90 and 120 mM NaCl treatments that showed less electrolyte leakage than many 60 mM Na_2SO_4 seedlings (Figure 3.3.3). Several of these seedlings had comparable leakage to control seedlings, suggesting minimal injury. Due to the focus of this study on water relations parameters, the data available cannot account for the low leakage these seedlings exhibited. The most likely explanation is that such seedlings possess such characteristics as improved sequestration of ions in the roots and stems, faster establishment of ionic gradients necessary for vacuolar compartmentalization of ions, or a superior capacity to osmotically adjust with minimal use of toxic ions from the environment. These, then, would be examples of the subset of best seedlings described in section 2.6, as most of them possessed a relatively low $\Psi_{\pi tlp}$.

Since the results of the ANOVA presented in Table 3.3.2 indicate that low values of $\Psi_{\pi tlp}$ minimize the injury that occurs in black spruce exposed to Na_2SO_4, much of that damage is probably attributable more to water deficit stress than Na^+ toxicity. These data, however, do not allow such a firm conclusion, as the two were not tested separately. Further work comparing Na_2SO_4 with non-ionic osmotica such as polyethylene glycol (PEG) may allow for a clearer determination of a sequence of damage. This technique has proven quite successful with black spruce (Zwiazek and Blake 1991a; Zwiazek and Blake 1991b; Tan et al. 1992). PEG induced osmotic stress provides a more accurate and repeatable water stress than withholding water (Krizek 1985). For seedlings exposed to Cl^-, measurements of ion concentrations in tissue may help identify actual causes of injury. For the purposes of planting seedlings on CT, aeration should probably also be a factor worth considering in future studies.

3.5 CONCLUSIONS

Black spruce is more sensitive to Cl^- than to either water deficit stress or Na^+. If Cl^- is not present at sufficiently high concentrations, low $\Psi_{\pi tlp}$ can help black spruce seedlings resist damage caused by salt induced water deficits. The utility of $\Psi_{\pi tlp}$ for minimizing damage among seedlings exposed to Na_2SO_4 suggests that water deficit stress may be more of a concern for such seedlings than is Na^+, though further work separating the two would be needed to determine if this is actually the case.

Under poorly aerated conditions plant uptake of Na^+ can increase and Na^+ toxicity may become more of an issue (Marschner 1995). Thus, since CT can be poorly aerated, Na^+ toxicity could be more of a concern for plants in the field than it was in this experiment. However, though Na^+ uptake may well increase in CT (Croser and Zwiazek 2000), Cl^- uptake is unlikely to decrease at the same time. Given the sensitivity of black spruce to Cl^-, toxicity of this element is likely to remain the more significant problem of the two. If Cl^- concentrations are high enough, water relations parameters which aid in dealing with water deficit stress, are insufficient to help plants. In CT, where both Na_2SO_4 and NaCl are present, the concern becomes whether Cl^- is at low enough concentrations that drought resistance will be sufficient to help seedlings deal with the water deficit. The variable chemistry of CT suggests that there is not a universally applicable answer, so this must be considered on a site specific basis instead.

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4.0 Impact Of Composite Tailings On Black Spruce (*Picea mariana* (Mill) B.S.P.) Seedlings Under Field Conditions

Abstract

Pressure Volume curves were used to determine drought tolerance characteristics of black spruce (*Picea mariana* (Mill) B.S.P.) seedlings. These seedlings, which had been grown in a growth chamber were then transported to the field and planted in an experimental CT deposit near Fort McMurray, Alberta. The planting occurred along a salinity gradient, and the seedlings remained on the site for three months. Seedling injury was assessed by examining buds for necrosis, and was found to follow a gradient which paralleled the salinity gradient. Using linear regression, several P-V curve derived drought tolerance characteristics were found to be useful for predicting the extent of injury seedlings experienced. These were: lower relative water content at the turgor loss point, higher relative water content of the symplast, lower maximum modulus of elasticity, lower osmotic potential at the turgor loss point, and higher osmotic amplitude for turgor maintenance.

4.1 INTRODUCTION

Composite tailings (CT) technologies used by the oil sands industry in Alberta provide a means for disposal of fine tails that would otherwise continue to accumulate and require perpetual storage. The end result is a trafficable surface that can be capped and revegetated in order to prevent erosion, and eventually return the site to a productive end land use. The chemistry of this tailings material is highly variable. Factors contributing to this variability include differences in the original ore, and leaching due to the hydrology of the deposit. In general, however, CT contains high concentrations of sodium, chloride, and sulphate (Renault et al., 1998). Concentrations of these ions can be expected to be greatest in low spots and toe slopes, or anywhere that ion rich water collects and evaporates (Qualizza 1999).

The primary constraints on growth of vegetation under such saline conditions are water deficits and ion toxicities (Greenway and Munns 1980). Of these, chloride toxicity is often the most significant problem for plants (Alt et al. 1982;

Marschner 1995). Previous experiments also demonstrated that when concentrations of chloride are below acute toxicity, drought tolerance characteristics derived from Pressure-Volume curves can provide a means of determining which plants are better able to tolerate salt induced water deficit stress (Chapter 3 of this thesis).

The purpose of the present study was to examine whether such traits would also be useful for predicting injury among seedlings exposed to CT under field conditions. CT contains both NaCl and Na_2SO_4 in varying amounts. The fact that CT chemistry is not uniform mandates some caution in interpreting these results. In deposits containing greater concentrations of chloride than were present at the site used for this study, screening of planting stock based upon drought tolerance characteristics would be less applicable, as injury due to ion toxicity would become a more significant concern. Alternatively, for CT deposits with lower concentrations of chloride, such as those which result from Suncor Energy's nearby extraction facility (Renault et al. 1998), such a technique could be used with greater confidence.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

Seedlings used in this experiment were one and a half-years-old and from a single population (ATSIC# 1771 13-94-11 W4, 244 m), supplied by the Smoky Lake Forest Nursery (Smoky Lake, Alberta). The seedlings were one-year-old and dormant when acquired. The roots of the seedlings were washed, and the seedlings were then transplanted into 400 ml sand filled Spencer-Lemaire containers (Spencer-Lemaire Industries Ltd., Edmonton Alberta, Canada). These seedlings were grown in a growth chamber for the next 6 months, and fertilized with rapid growth conifer nutrient solution once a week (Wood 1995). Conditions in the growth chamber were held at a 22 hour photoperiod, 20 °C / 18 °C (day/night temperature), with 70% relative humidity. During that time seedling height increased an average of 10 to 20 cm and developed many lateral branches.

4.2.2 Pressure-Volume curves

At the end of May 1999, pressure-volume curves were constructed for the seedlings by the free transpiration method (Hinckley et al. 1980), using a pressure chamber (PMS Instruments, Corvalis Oregon, USA). Tissue sampled for the P-V curves came from the tips of the uppermost lateral branches, approximately 5 cm long. Curves were constructed for 74 seedlings, which were individually tagged to allow tracking of results. These curves were later used to derive the following water relations parameters for the seedlings: osmotic potential at the turgor loss point ($\Psi_{\pi tlp}$), relative water content of the symplast (RWC_s), relative water content at the turgor loss point (RWC_{tlp}), osmotic amplitude for turgor maintenance ($\Delta\Psi\pi$), and maximum modulus of elasticity (ε_{max}).

4.2.3 Site

In early June 1999 the seedlings were transported to the field for planting out in "the U-shaped cell" a CT trial plot located at Syncrude Canada's Mildred Lake facility, near Fort McMurray, Alberta, at 57° 00' N, 111° 35' W. The site was established in 1995, and consists of unamended and uncapped CT. One end of the cell is the high end with an approximately 4% slope running down and curving back down into the low end, much of which is inundated with varying levels of water over the course of the year. The area where the seedlings were planted is in the low end of the cell, pictured in Figure 4.2.1 , and was at the edge of high water line for that year. At this location there were gradients of EC (4.6 dS/m at the low end near the water to 1.4 dS/m up slope) and the ions contributing to that EC (Na^+, Cl^-, SO_4^{2-}) which are listed along with other major aspects of soil (CT) chemistry in Table 4.2.1. It is worth noting that both Ca^{2+} and Sr^{2+} were present in similar gradients.

These values were derived from samples taken at 10 cm depth at each point where a seedling was planted. Samples were measured by extracting leachate from a saturated soil paste. To do this, water content of the CT was first determined by measuring out 5 g of sample, drying it, determining dry weight, and then dividing dry weight by the fresh weight. Then, 100 g of the CT sample was mixed with a quantity of water equal to 5x the water content of the CT. (e.g. if the water content of a 100 g CT sample was 12.11%, then 60.55 g of deionized water were added to it). These materials

Figure 4.2.1: End on view of black spruce seedlings planted in the U-shaped cell. The photo was taken from upslope, looking down along transects 1 and 2 towards the water line.

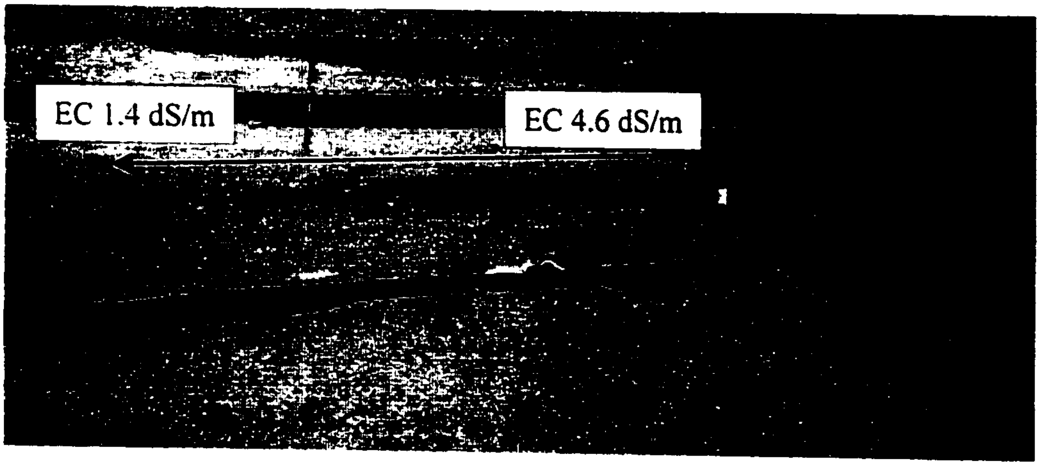

Figure 4.2.2: Side view of black spruce seedlings planted in the U-shaped cell with EC gradient labeled. Those at the low end were exposed to higher salinity than those at the higher end.

Table 4.2.1 Physical and chemical properties of the substrate (Composite Tailings) that the seedlings planted in Transects 1 and 2 were exposed to.

Transect 1

Location	pH	WC%	R-EC	EC	Na	Cl	SO_4	Ca	Sr	B
0 m	8.0	12.2	4.1	3.7	234	190	270	25.9	0.81	0.76
1.5 m	8.4	10.3	3.9	5.4	234	200	210	20.0	0.66	0.70
3 m	8.5	8.9	3.8	5.6	225	180	190	19.1	0.65	0.57
4.5 m	8.1	12.6	3.5	3.5	183	120	210	23.3	0.66	0.63
6 m	8.6	14.2	3.4	2.6	152	100	150	20.4	0.59	0.26
7.5 m	7.5	16.4	3.2	3.8	225	190	220	22.2	0.74	0.77
9 m	8.4	11.8	3.1	3.4	152	120	93	8.9	0.30	0.76
10.5 m	8.6	12.8	2.9	4.1	148	96	100	7.8	0.25	0.70
12 m	8.5	10.8	2.7	3.8	134	79	90	5.0	0.18	0.61
13.5 m	8.1	12.2	2.5	3.2	185	140	93	7.0	0.24	0.84
15 m	7.9	10.2	2.4	1.3	186	140	77	7.4	0.25	0.59
16.5 m	8.9	10.3	2.2	1.8	159	14	7	8.1	0.27	0.97
18 m	7.9	12.3	2.0	2.0	140	100	110	12.7	0.27	0.66

Transect 2

Location	pH	WC%	R-EC	EC	Na	Cl	SO_4	Ca	Sr	B
0 m	8.2	12.5	4.6	4.1	247	120	520	59.4	1.76	0.59
1.5 m	8.1	12.5	4.4	4.0	248	160	400	53.8	1.51	0.32
3 m	7.8	10.7	4.1	3.6	223	120	410	44.2	1.38	0.64
4.5 m	7.5	11.5	3.9	4.2	185	120	290	36.7	1.12	0.67
6 m	8.7	10.6	3.6	5.6	193	150	150	17.9	0.60	0.69
7.5 m	8.2	12.1	3.3	2.7	497	710	440	45.6	1.43	0.94
9 m	8.4	12.2	3.0	3.6	170	13	24	32.4	0.93	0.48
10.5 m	7.9	11.3	2.8	2.4	183	150	220	28.4	0.84	0.61
12 m	8.5	11.5	2.5	2.7	148	88	250	33.8	0.98	0.52
13.5 m	8.2	12.2	2.2	1.9	102	69	190	36.8	0.97	0.41
15 m	7.9	11.6	2.0	1.1	105	58	120	16.7	0.51	0.61
16.5 m	8.3	12.3	1.8	1.8	98	46	60	7.9	0.25	0.66
18 m	8.2	12.5	1.5	1.6	100	52	91	13.3	0.44	0.81

Key:

Location: location along the transect in meters
pH: pH
WC%: % water content
R-EC: EC value as a result of regression
EC: Electrical Conductivity in dS/m
Elements: values in ppm

were mixed and placed on a shaker over night. The following morning it was centrifuged for 15 minutes. The fluid was then poured off from the solid. It was vacuum filtered using #1 Whatman filter paper, and then vacuum filtered again using a 0 .45 micron filter. Electrical conductivity and pH were measured at 22 °C, and the sample was then bottled, and stored in a cold room. The yield averaged about 20-40 ml of leachate per sample. Once the samples were prepared, every third one along each transect was sent to Syncrude Research in Edmonton for ion analysis using Inductively Coupled Plasma Emission Atomic Spectroscopy, and Ion Chromatography.

There was no meaningful variability of other environmental factors detected. No gradients were found of pH, moisture content, or nutrients. The small area involved suggests that climatic conditions which seedlings were exposed to were similar in terms of precipitation, temperatures, light and wind.

4.2.4 Experimental design and statistical analysis

The 74 seedlings were randomly assigned to a location, and then planted at 50 cm spacing in two parallel 18-m-long transects (37 per transect), running along the slope at 90° to the contour, parallel to the EC gradient (Figure 4.2.2). The actual EC value measured where each seedling was planted is presented as the EC in Table 4.2.1. Since ions are mobile and the experiment lasted for 3 months, the EC derived from the regression line (R-EC) was used in the statistical analysis to classify the EC that seedlings were exposed to. After planting, the seedlings remained on site for three months, were harvested in September 1999, and brought back to the lab for further examination.

Injury to seedlings was measured by assessing the buds for signs of necrosis. This system was used instead examination of foliage and measurement of electrolyte leakage (as was done in the growth chamber (Chapter 3)) because electrolyte leakage was impractical in the field, and many seedlings had lost large numbers of needles, so needle based data would have been too coarse to be useful. This injury index was ranked on a scale of 0-10 (10 being the greatest) by examining the leader, two lateral branches at the top of the tree, and two lateral branches at the bottom of the tree. Two points were assigned for dead buds or those showing signs of necrosis, at each location.

Regression analysis was performed for various water relations parameters (derived from P-V curves) to test the ability of these characteristics to predict the injury suffered by seedlings. For the linear regression, injury index was the dependent variable and each of the water relations parameters derived from the P-V curves served as independent variables ($\Psi_{\pi tlp}$, RWC_{tlp}, RWC_s, $\Delta\Psi\pi$, and ε_{max}). The formula was as follows:

$$y_i = \alpha + \beta x_i + \varepsilon_i \qquad (4.0)$$

where:

y_i = dependent variable (injury index)
α = Y intercept of regression
β = coefficient of regression
x_i = independent variable (each was performed as a separate regression: $\Psi_{\pi tlp}$, RWC_{tlp}, RWC_s, $\Delta\Psi\pi$, and ε_{max})

Correlation matrices were run to test for relationships between variables. Statistical analysis using regression and correlation, was performed using SAS version 6.12 (SAS Institute Inc., Cary, North Carolina, USA).

4.3 RESULTS

The extent and distribution of damage seedlings experienced is presented in Figure 4.3.1. In both transects, the injury index gradient closely paralleled the EC, and this correlation was statistically significant for both Transect 1 ($p=0.0148$), and Transect 2 ($p=0.0126$). There was no significant difference in injury between the two transects ($p=0.2468$), and a strong correlation between EC and injury ($p=0.004$) was observed. There was also a strong correlation between location along the transect and injury ($p=0.0157$), though no differences in site environment were detected aside from the EC gradient and the ions contributing to it.

Using linear regression, relative water content at the turgor loss point (RWC_{tlp}) was found to be a predictor of injury index values for seedlings exposed to high salinity (>4 dS/m), but not at lower salinity. As illustrated in Figure 4.3.2a and Table 4.3.1, low RWC_{tlp} was associated with lower injury index values in salt stressed seedlings ($p=0.0039$).

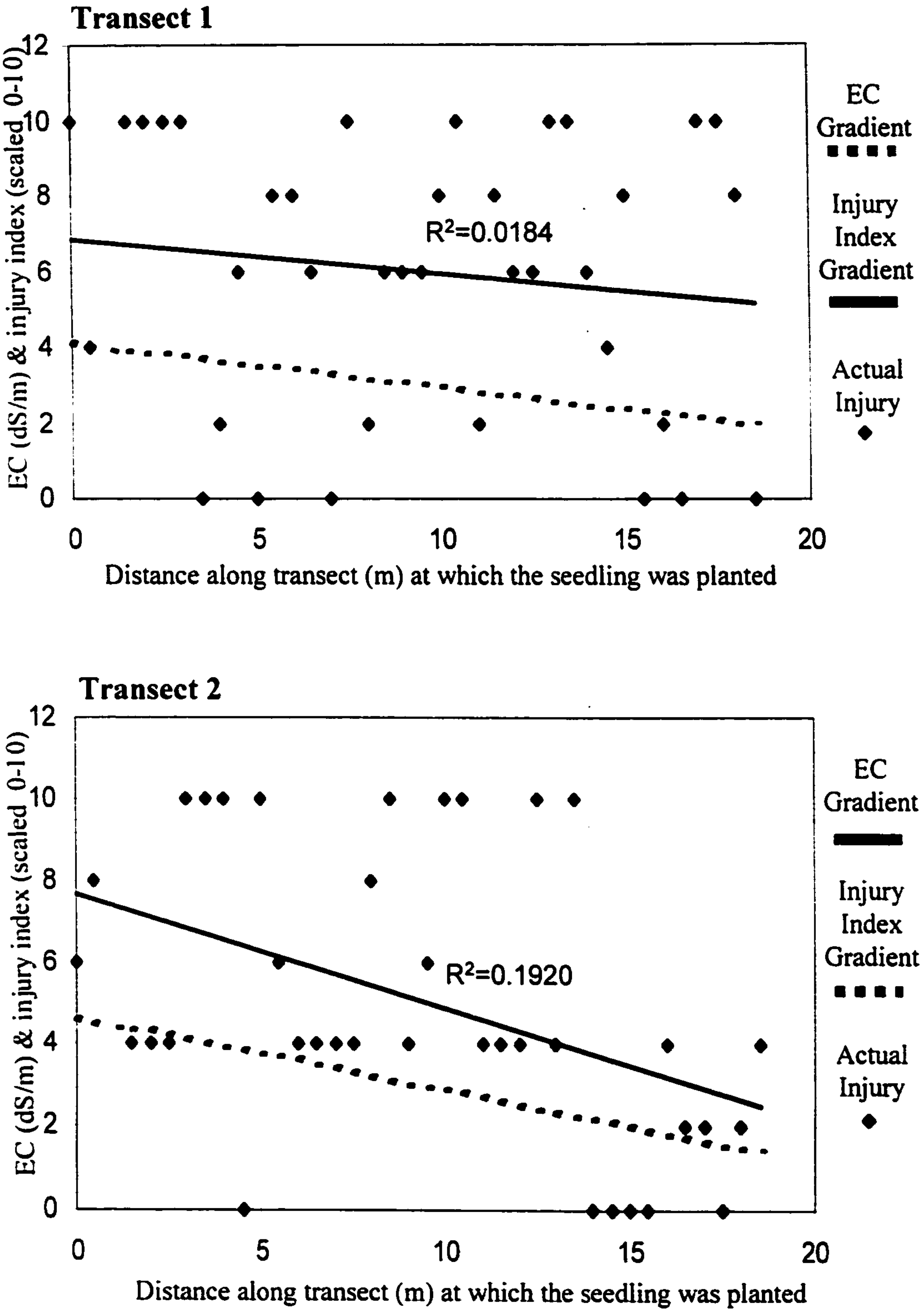

Figure 4.3.1 Parallel damage and electrical conductivity gradients in transects 1 and 2.

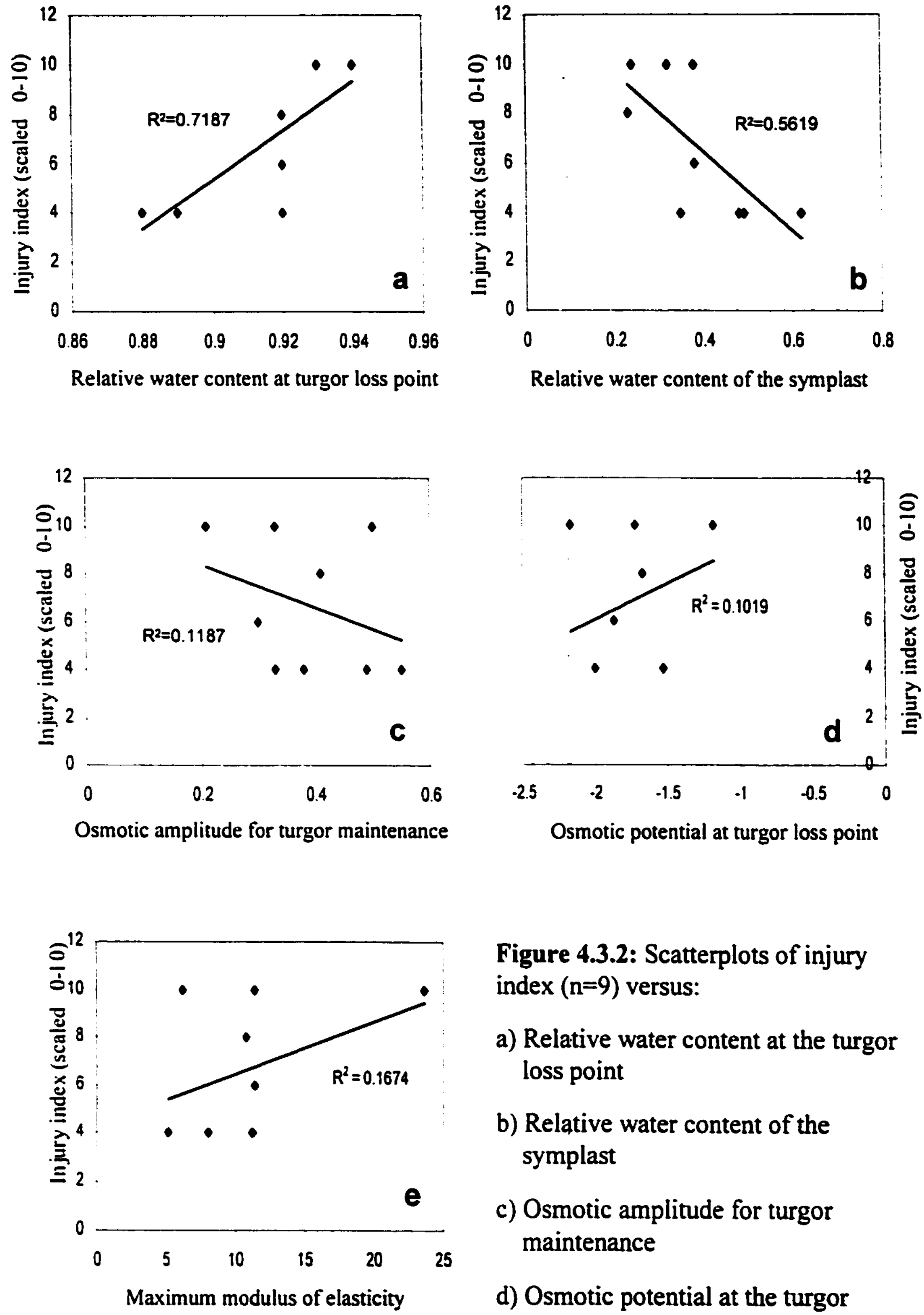

Figure 4.3.2: Scatterplots of injury index (n=9) versus:

a) Relative water content at the turgor loss point

b) Relative water content of the symplast

c) Osmotic amplitude for turgor maintenance

d) Osmotic potential at the turgor loss point

e) Maximum modulus of elasticity

Table 4.3.1: Relationship between relative water content at the turgor loss point and injury

Seedling	RWC_{tlp}	Damage
7	0.88	4
24	0.88	4
83	0.89	4
35	0.92	8
50	0.92	4
65	0.92	6
70	0.93	10
14	0.94	10
20	0.94	10

Regression

Predictive ability at EC above 4dS/m ($p=0.0039$)
$R^2 = 0.7187$

Predictive ability at EC below 4dS/m ($p=0.664$)

Table 4.3.2: Relationship between relative water content of the symplast and injury

Seedling	RWC_s	Damage
24	0.62	4
7	0.38	4
50	0.48	4
65	0.49	6
83	0.38	4
14	0.35	10
20	0.23	10
35	0.32	8
70	0.24	10

Regression

Predictive ability at EC above 4dS/m ($p=0.0200$)
$R^2 = 0.5619$

Predictive ability at EC below 4dS/m ($p=0.151$)

Table 4.3.3: Regression of relative water content of the symplast and osmotic amplitude for turgor adjustment at EC above 4 dS/m

	Seedling	RWC_s	$\Delta\Psi\pi$	Damage
High RWC_s & Midrange $\Delta\Psi\pi$: did reasonably well	24	0.62	0.38	4
	Seedling	RWC_s	$\Delta\Psi\pi$	Damage
Midrange RWC_s & Low $\Delta\Psi\pi$: mixed performance	50	0.48	0.33	4
	65	0.49	0.30	6
	Seedling	RWC_s	$\Delta\Psi\pi$	Damage
Midrange RWC_s & High $\Delta\Psi\pi$: did reasonably well	7	0.38	0.49	4
	83	0.35	0.55	4
	Seedling	RWC_s	$\Delta\Psi\pi$	Damage
Low RWC_s & low $\Delta\Psi\pi$: died	14	0.32	0.21	10
	70	0.38	0.33	10
	Seedling	RWC_s	$\Delta\Psi\pi$	Damage
Low RWC_s & High$\Delta\Psi\pi$: poor performance	20	0.23	0.50	10
	35	0.24	0.41	8

RWC_s and $\Delta\Psi\pi$ combined have a good ability to predict injury at EC above 4dS/m ($p=0.0060$)
Contribution by $\Delta\Psi\pi$ ($p=0.0272$) Contribution by RWC_s($p=0.0030$) ($R^2=0.8178$)

The same ability does not exist at EC below 4 dS/m ($p=0.237$)

Table 4.3.4: Values, performance, and regression for maximum modulus of elasticity and osmotic potential at turgor loss point

	Tree	ε_{max}	$\Psi_{\pi tlp}$	Damage
Low or Midrange ε_{max} & low $\Psi_{\pi tlp}$: low injury	24	7.98	-2	4
	50	11.20	-2	4
	83	11.20	-2	4
Low ε_{max} & High $\Psi_{\pi tlp}$: mixed performance	7	5.17	-1.53	4
	70	6.2	-1.18	10
Midrange to high in both: poor performance	35	10.78	-1.67	8
	14	11.34	-1.72	10
	65	11.42	-1.87	6
High ε_{max} & low $\Psi_{\pi tlp}$: died	20	23.66	-2.17	10

ε_{max}-and $\Psi_{\pi tlp}$ combined have a good ability to predict injury at EC above 4dS/m ($p=0.0063$)
Contribution by ε_{max} ($p=0.0030$) Contribution by $\Psi_{\pi tlp}$ ($p=0.0037$) ($R^2=0.8154$)

The same ability does not exist at EC below 4 dS/m ($p=0.323$)

Table 4.3.5: Mean values for significant water relations parameters of seedlings exposed to EC > 4dS/m grouped by injury index class

Injury	RWC_{tlp}	RWC_s	DYp	Y_{ptlp}	ε_{max}	n
0	-	-	-	-	-	-
2	-	-	-	-	-	-
4	0.89	0.47	0.44	-1.88	8.88	4
6	0.92	0.49	0.30	-1.87	11.42	1
8	0.92	0.32	0.41	-1.67	10.48	1
10	0.94	0.27	0.35	-1.69	13.73	3

Units:

Injury Index:	scaled 0-10
RWC_{tlp}:	% water
RWC_s	% water
DYp	MPa
Y_{ptlp}	MPa
ε_{max}	MPa

Similarly, regression analysis also indicated that the relative water content of the symplast (RWC_s) was a predictor of injury index values among seedlings exposed to high salinity (>4 dS/m), but not at salinity below that. High RWC_s was associated with reduced damage in seedlings (p=0.0200) (Figure 4.3.2b and Table 4.3.2). Multivariate regression using both RWC_s and osmotic amplitude for turgor adjustment ($\Delta\Psi\pi$) accounts for more variability than does RWC_s alone. The larger the $\Delta\Psi\pi$, the lower the seedling injury index values (p=0.0060). Figure 4.3.2c plots injury vs $\Delta\Psi\pi$. Results for this multivariate regression are presented in Table 4.3.3.

Multivariate regression analysis using osmotic potential at the turgor loss point ($\Psi_{\pi tlp}$) and the maximum modulus of elasticity (ε_{max}) also allow prediction of injury index values at high salinity (>4 dS/m), but not below that. Low $\Psi_{\pi tlp}$ and ε_{max} were both associated with reduced damage (p=0.0063). Figures 4.3.2d and 4.3.2e plot injury vs $\Psi_{\pi tlp}$, and injury vs ε_{max} respectively. Results for this regression are presented in Table 4.3.4. A summary of means is presented in Table 4.3.5.

4.4 DISCUSSION

Seedlings were grown in sand and planted in unamended and uncapped CT. The sand plugs, in which seedlings were grown, forced the roots into contact with CT. These conditions were harsher than would be the case for seedlings planted for reclamation operationally. The goal was to produce extreme conditions, as spruce planted in the future will be expected to survive within a saline environment (with seasonal fluctuations) for many years, and will likely face harsh conditions at some point during their lives (Renault et al. 1998).

Seedlings were exposed to nearly uniform conditions in terms of soil (CT) texture, moisture, pH, nutrients, rainfall, wind, sunlight, and temperatures. The only significant difference that was measured was the EC gradient. After three months of exposure to the CT, the seedlings exhibited a bud injury gradient that roughly paralleled the salinity gradient. Given that there were no other differences detected in growing conditions, much of the injury that occurred among the seedlings was likely related to this salinity gradient. Table 4.2.1 presents the chemistry of the CT. Even though both NaCl and Na_2SO_4 were present in the substrate, the extent to which SO_4^{2-} exceeded Cl^-, suggests that across much of the site, Na_2SO_4 was the dominant salt, although there were pockets where Cl^- exceeded SO_4^{2-}.

Response of the seedlings agrees with this assessment, since water relations parameters indicative of tolerance of water deficit stress were useful in the field, much as they had been for seedlings exposed to Na_2SO_4 in the growth chamber experiment discussed in Chapter 3. There, black spruce demonstrated sensitivity to Cl^- that exceeded its sensitivity to either Na^+ or water deficit stress. Although Cl^- was present in the field, the concentration was lower than the NaCl concentrations used in the growth chamber experiment. As a consequence, while some of the necrosis that seedlings exhibited in the field was likely due to Cl^-, the dominant stress appears to have been due to salt-induced water deficit. Had Cl^- been a major factor, it is unlikely that drought tolerance characteristics would have had as strong a relationship with the injury index values.

4.4.1 The turgor loss point: relative water content and osmotic potential.

Both low $\Psi_{\pi tlp}$ and low RWC_{tlp} have been identified as being indicative of drought tolerance (Zine El Abidine et al. 1994). The differences in values of $\Psi_{\pi tlp}$ and RWC_{tlp} for relatively drought tolerant and intolerant seedlings indicate how the former can maintain turgor under more severe osmotic stress than can the latter As discussed in Chapter 2, this explains one reason why relatively drought tolerant seedlings had lower injury index values than the comparatively intolerant ones, as these seedlings could maintain turgor beyond the point of water loss at which the intolerant seedlings could not.

In the growth chamber experiment, $\Psi_{\pi tlp}$ was individually significant as a predictor of injury due to water deficit stress, and RWC_{tlp} was not. In the field, RWC_{tlp} was capable of doing so individually, while $\Psi_{\pi tlp}$ was significant only in conjunction with ε_{max}. Significance of $\Psi_{\pi tlp}$ on its own in one case and not in the other is likely a result of variability of conditions and seedlings. The same is true of RWC_{tlp}. In both the growth chamber and the field the same trends were evident, with relatively drought tolerant seedlings being better suited to such conditions than less drought tolerant seedlings. Consequently these slight differences in results do not constitute an inconsistency. Instead, since both characteristics tend to be found together, and as the relative importance of any one mechanism at any given time is hard to measure, it can be said that greater drought tolerance in general suggests a heightened capacity for dealing with salt induced water deficit stress.

4.4.2 Relative water content of the symplast

The effect of RWC_s on the extent of injury among salt stressed seedlings is a little more difficult to explain than $\Psi_{\pi tlp}$ and RWC_{tlp}. To understand it, one must first recognize what this value represents. Partitioning of water between the symplast and apoplast is a zero-sum division, in that water is within one of the two compartments. Thus, relative water content of the apoplast (RWC_a) is just the reciprocal of $\mathrm{RWC_s}$, and consequently when one is high the other is low. Statistically the information carried by the two values is identical, as both indicate the ratio between symplastic and apoplastic water. The significance of high RWC_s might equally be described as being due to low RWC_a. What is needed in order to understand how high RWC_s may be advantageous to plants, is to examine what is occurring biologically.

Santakumari and Berkowitz (1989) found that the volume of the symplast declines dramatically (as much as 50%) once leaf Ψ_w falls below −1.0 MPa. Loss of photosynthetic capacity of chloroplasts may be more closely related to reduction in symplast or chloroplast volume than low Ψ_w in dehydrated cells (Berkowitz and Kroll 1988). The Munns and Termat (1986) model of plant response to salinity indicates that any effect on photosynthetic efficiency (in the face of declining photosynthetically active leaf area) can have significant implications for survival of salt affected seedlings. Seedlings possessing a greater RWC_s may have a improved capacity to withstand such effects as the initial symplastic volume will have been greater.

Apoplastic volume tends to increase as a result of water deficits (Tyree and Jarvis 1982; Gunnasekera and Berkowitz 1992). Rascio et al. (1990) suggest that this is due to increases in hemicellulose within the cell wall which has important properties such as ion binding and water-holding capacity. It is possible that plants having a greater apoplastic fraction may be better protected against dehydration (Rascio et al. 1997). The same may not be true when salt is involved. Oertli (1968) hypothesized that much of the damage from salt toxicity could be attributed to apoplastic salt accumulation leading to cell dehydration, which could also explain increases in bound (apoplastic) water.

There is some question about the extent to which changes in apoplastic water occur, as membrane leakage from stressed and dying cells can alter osmotic potentials, potentially exaggerating measurements of the apoplastic fraction (Vos and Oyarzun 1988). However, Flowers et al. (1991) found evidence supporting the Oertli hypothesis using X-ray microanalysis. They detected extracellular salt accumulation, and concluded that it could be the factor initiating salinity damage. In any event, changes occurring in RWC_a were not found to confer any significant advantages to seedlings in by maintaining Ψ_p or photosynthesis at low Ψ_w (Gunnasekera and Berkowitz 1992).

Regardless of cause, if even a limited real increase in RWC_a occurred, it would happen at the expense of RWC_s. If this occurred during a water deficit, then symplastic water would be decreased. Such a loss could interfere with chloroplast functioning (Gupta and Berkowitz 1987) and then reduce the efficiency of the remaining photosynthetically active leaf surface, with negative implications for salt affected plants. This effect would be consistent with the observations of Gunnasekera and Berkowitz (1992), and could explain the importance of high RWC_s in this experiment.

Additionally, even when concentrations of Cl^- are below the threshold for necrosis, the photosynthetic capacity of tissue may be diminished by Cl^- ion activity which reduces the levels of biochemical components of CO_2 assimilation, such as ribulose 1,5-biphosphate carboxylase activity, triosephosphate, and phosphoglycerate. (Ziska et al. 1990). Plants with higher initial RWC_s (lower RWC_a) could be less susceptible to this due to a dilution effect provided by the relatively higher symplastic water content.

Binzel (1985) indicates that one of the typical glycophyte responses to water deficits, decrease in shoot growth, occurs during salt induced water deficits because plants cannot distinguish between the two. The Munns and Termat (1986) model indicates the potentially lethal consequences of failure to do so. Similarly, though the increased water holding capacity of the cell wall associated with increasing RWC_a could offer some benefits while causing limited harm during drought stress, the same may not be true during salt stress, particularly if it interferes with photosynthesis. Loss of older leaves to necrosis induced by ion toxicity, makes any reduction in photosynthetic efficiency of the remaining leafs much more expensive. Given the absence of photosynthesis measurements in this study it is impossible to assign this explanation to the importance of RWC_s in this instance with any certainty, but it merits consideration as a possible explanation.

4.4.3 Osmotic amplitude for turgor maintenance

This parameter describes the range of osmotic potentials between full saturation and the turgor loss point, across which the plant cells are capable of maintaining turgor. The wider this range, the more drought tolerant the seedling is (Zine El Abidine et al. 1994). In this experiment high $\Delta\Psi\pi$ was significant in conjunction with high RWC_s. Though RWC_s was significant independently of $\Delta\Psi\pi$, its ability to predict damage was improved with multivariate regression which included $\Delta\Psi\pi$. Thus, while in this instance the effect of high $\Delta\Psi\pi$ was insufficient to influence seedling injury alone, seedlings possessing this trait in addition to high RWC_s, had lower injury index values than those seedlings which lacked it.

4.4.4 Maximum modulus of elasticity

Low values for ε_{max} indicate greater elasticity of the cell walls. Therefore, the cells are able to contract as a result of water deficits, decreasing volume and maintaining turgor. The greater an ability of a plant to do this, the more capable it is of tolerating water deficit and minimizing injury. While ε_{max} can be indicative of drought tolerance, being a finite value and subject to some seasonal variation, it alone does not fully reflect the drought resistance of the plant (Colombo 1987). It is possible for two plants to have almost the same ε_{max} but to differ markedly in their ability to maintain turgor. Consequently, the findings here, i.e. that, as a direct physical mechanism, this characteristic contributes to tolerance of salt induced water deficit stress, without being sufficient to make much difference for plant in and of itself, are consistent with observations made by Colombo (1987). Differences in the physical properties of the cell wall result in part from differences in its chemical composition. Consequently, it is also possible that differences in the chemical make up of the cell wall may influence accumulation of ions outlined by the Oertli hypothesis (1968), and thus measurement of ε_{max} may actually be detecting these indirect effects as well.

4.4.5 Drought tolerance as a multi-trait concept

Although both RWC_{tlp} and RWC_s had sufficient influence to be capable of indicating the likelihood of injury when considered independently, and other parameters, $\Delta\Psi\pi$, $\Psi_{\pi tlp}$ and ε_{max} were useful in combination with each other, the seedlings which suffered the least damage typically possessed several of these characteristics. Table 4.4.1 shows how seedlings #7, #24, #50, and #83 all suffered relatively less damage than the other seedlings exposed to high salinity, and all of these possessed 4 or more drought tolerance characteristics. Seedlings possessing few of these traits fared worse, and all of those which died possessed few, if any, drought tolerance characteristics. Thus, the more drought tolerant a seedling was, the better it seemed to tolerate the stress. Section 2.6 laid out the concept that it may be possible to use water relations to identify those seedlings to avoid planting in CT or other saline conditions, i.e. the less drought tolerant ones. This would leave one with a group of those seedlings which are better suited to such conditions, although it does not identify which of those seedlings would be expected to suffer the least injury.

Some water relations parameters measured in this experiment appeared to be more helpful to salt affected seedlings than others were. For example, the seedlings that died had a relatively high RWC_{tlp}, suggesting that their tissue was more vulnerable to turgor loss at less of a water deficit than the more drought tolerant seedlings. Seedlings which survived but were significantly injured, i.e. #35 and #65, both had a low RWC_{tlp}, but few other drought tolerance traits. Thus there was an important effect of having the trait as opposed to not having it. Some traits were less important. For example high $\Delta\Psi\pi$, appeared to be just as common as low RWC_{tlp} (both occurred in approximately half of the seedlings). Otherwise drought tolerant seedlings lacking this trait did no worse than those drought tolerant seedlings with it. But having a high $\Delta\Psi\pi$ alone appeared to have been insufficient to help otherwise intolerant seedlings cope with the water deficit they experienced. The same was true of ε_{max}. The data available is

Table 4.4.1: Summary of significant water relations parameters at EC > 4dS/m*
*All parameters were insignificant at EC below 4 dS/m

Tree	7	14	20	24	35	50	65	70	83
Parameter									
L/M RWCtlp	X			X	X	X	X		X
H/M RWCs	X			X		X	X		X
L ε *max*	X			X		X		X	X
L $\Psi\pi$*tlp*			X	X		X			X
H $\Delta\Psi\pi$	X		X		X				X
Damage	4	10	10	4	8	4	6	10	4
Mortality		A	A					S	

H=High, M=Midrange, L=Low
Bold indicates parameter was significant individually
Italics indicates parameter was significant in conjunction with other parameters
A= Died by August
S= Died by September

insufficient to assign a hierarchy of importance to these traits, however the important point to take from this is that in general, possession of more water relations parameters indicative of drought tolerance was a sign that seedlings were better suited for dealing with salts.

4.4.6 Potential for strontium toxicity

Strontium, Sr^{2+} was present in gradient that paralleled the EC and damage gradients along with Na+, Cl^- and SO_4^{2-}, and along with these ions contributed to the measured EC gradient. Consequently it is possible that Sr^{2+} somehow contributed to the damage gradient as well. It is chemically similar to Ca^{2+}, and both are thought to play similar roles in many cellular metabolic processes (Iserman 1981), with uptake of the two being somewhat competitive. Kim and Heinrich (1997) found that chlorophyll content in leaves was reduced because of increased peroxidase activity stimulated by uptake of Sr^{2+}, but only when Ca^{2+} was limiting. They also concluded that Sr^{2+} was more mobile as it tended to be found to the exclusion of calcium in new leaves, and was gradually replaced in older leaves. Consequently Sr^{2+} toxicity could interfere with the productivity of new growth, which, considering the Munns and Termat (1986) model may pose some problems for salt exposed plants. Overall however, since CT contains abundant Ca^{2+}, which had a gradient that paralleled that of Sr^{2+}, it is unlikely this was a major constraint, though it is worth noting, and may warrant further observation.

4.5 CONCLUSIONS

Seedlings were exposed to uniform conditions in terms of soil (CT) texture, moisture, pH, nutrients, rainfall, wind, sunlight, and temperatures. The only significant difference in seedling environment that was detected was the EC gradient, and those ions which contributed to it. These were primarily NaCl and Na_2SO_4, and of these two the Na_2SO_4 was the more significant. The seedlings exhibited a gradient of injury index values that paralleled the EC gradient, and in the absence of other differences in growing conditions, salts are the most logical explanation of this damage gradient.

At high salinity (defined as R-EC above 4 dS/m) those seedlings which were relatively more drought tolerant suffered less damage than those which were less tolerant. Drought tolerance was assessed by constructing P-V curves for seedlings before stressing them. The following characteristics were found to be useful for seedlings: lower relative water content at turgor loss point; higher relative water content of the symplast; lower osmotic potential at turgor loss point; lower maximum bulk modulus of elasticity; and higher osmotic amplitude for turgor maintenance

The chloride content of the CT most seedlings were exposed to was less than the toxic concentrations used in the growth chamber in Chapter 3. Consequently the fact that drought tolerance characteristics helped these seedlings cope with salt induced water deficit stress is consistent with results from the growth chamber study. This indicates that use of water relations parameters indicative of drought tolerance to screen black spruce planting stock for use on saline sites should be possible where Cl^- content is low.

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5.0 Synthesis

5.1 INTRODUCTION

Reclamation of sites used for disposal of consolidated tailings presents many challenges. Revegetation of this material can be hampered by high concentrations of NaCl, and Na_2SO_4, flooding, and anaerobic conditions (Renault et al 1998; Croser and Zwiazek 2000). Nonetheless, production of CT is preferable to the alternative of continued accumulation and perpetual storage of fine tails (Sheeran 1998). Over much of a CT deposit, if a sufficiently thick tailings sand cap and organic amendments are used, then conditions would not be expected to be very different than those found on tailings sand deposits which have already been reclaimed. Tailings sand has been successfully revegetated with upland species such as white spruce and aspen (Techman 1983; Moneco 1983). It is anticipated that reclamation of some locations will be more difficult. Toe slopes and low spots would be expected to serve as concentration points subject to the formation of flooded soils and saline seeps, inhibiting the establishment of vegetation. There are also societal constraints, as it is required that species used for revegetation be native, and one of the end land uses (commercially productive forest) calls for species which have commercial value (AEP 1999).

A potential species for the revegetation of those low sites is *Picea mariana.* Black spruce is native, flood tolerant, and commercially valuable. Consequently, the objective of this study has been to examine if it is possible to identify characteristics of salt tolerance in order to allow screening of black spruce planting stock for use on such sites. Renault et al. (1998) observed unusually high variability in the response of conifer seedlings to CT water, suggesting that such selection could be useful. Allen et al. (1994) indicate that this sort of screening is probably the best way of immediately improving the salt tolerance of forest trees. Germination and establishment of black spruce on saline sites is poor (Croser et al. 2000), and not an effective means of eliminating salt intolerant individuals, while maintaining salt tolerant ones (Hayward and Bernstein 1958, Clemens et. al 1983). A better strategy is planting seedlings to take advantage of the increase in salt tolerance that occurs in most plants as they age (Shannon et al. 1994).

Doing so with seedlings which have been identified as being relatively salt tolerant is an improved version of this strategy.

5.2 EXPERIMENTATION

The two main stresses that plants experience as a result of salt exposure are water deficit and ion toxicity (Greenway and Munns, 1980). Given that ecotypic variations do not occur reliably within black spruce, the individuals used in each experiment during this study came from single populations in order to focus on intrapopulational variation. Drought tolerance parameters were derived using Pressure-Volume curves in order to characterize each seedling's capacity for dealing with water deficit stress. Seedlings were then subjected to salt stress in order to measure any differences in damage suffered by relatively drought intolerant seedlings and relatively drought tolerant seedlings.

The growth chamber portion of this study found that black spruce is more sensitive to Cl^- toxicity than it is to either water deficit stress or Na^+ toxicity. Toxicity was defined here as a statistically significant finding that a substance caused injury following exposure. This study did not attempt to determine the toxic concentration thresholds for Na^+ or Cl^- in black spruce. Chloride sensitivity is consistent with the findings of Alt et al. (1982) for serbian spruce (*Picea omorika*), and Croser et al. (1999) for jack pine (*Pinus banksiana*). When Cl^- was not present at toxic concentrations, then water relations parameters indicative of drought tolerance helped black spruce seedlings resist damage caused by salt-induced water deficits.

In the field trial, seedlings were exposed to CT which contained substantial concentrations of both NaCl and Na_2SO_4, instead of one or the other as had been the case in the growth chamber. However, even at the most saline portions of the study site, concentrations of Cl^- were lower than in the NaCl treatments in the growth chamber. As a consequence damage due to Cl^- toxicity was sufficiently limited so that drought tolerance was a useful characteristic for these seedlings as well. The least injured seedlings at high salinity exhibited characteristics identified as consistent with drought tolerance (Zine El Abidine et al. 1994; Santakumari and Berkowitz 1989). These included: lower relative water content at the turgor loss point; higher relative water content of the symplast; lower osmotic potential at the turgor loss point; lower maximum bulk modulus of elasticity; and higher osmotic amplitude for turgor maintenance.

5.3 GENERAL DISCUSSION

In both the growth chamber and in the field the same trends were evident. Relatively drought-tolerant seedlings were more tolerant of saline conditions than less drought-tolerant seedlings. The relative importance of any one water relations parameter at any given time is hard to measure, and many occur in conjunction with one another. Consequently it can be said for black spruce that in general, greater drought tolerance confers a heightened capacity for dealing with salt-induced water deficit stress. Though it is difficult to say which seedlings are superior and should be planted preferentially, it is possible to identify characteristics of those which are inferior, and should not be planted at all. Preventing use of such seedlings would, in and of itself, improve the rate of seedling survival .

Rather than trying to identify superior seedlings, the purpose of this study was to use water relations to determine what separates these from undesirable ones so that by screening planting stock one could avoid planting those least suited to saline conditions, and improve survival. It did so, by demonstrating that drought tolerant seedlings cope better with salt induced water deficit than intolerant ones. Essentially, this has been a search for suitable rather than superior seedlings.

Interestingly, in the growth chamber there were several seedlings amongst the NaCl treatments which showed little injury (both visually and as measured by electrolyte leakage) in spite of the significant water deficit stress and toxic concentrations of ions to which they were exposed. In a few cases their condition was even comparable to that of the control seedlings as can be seen in Figure 3.3.3. Most of these were relatively drought-tolerant seedlings, however there were similarly drought-tolerant seedlings which were significantly damaged by the same treatments. Consequently their condition could be attributed to a mechanism other than tolerance of water deficit stress. The focus on water relations parameters in this study means that the data available is insufficient to explain the relatively low damage the former drought-tolerant seedlings exhibited as compared to the latter similarly tolerant seedlings.

One possible explanation is that such seedlings possess other characteristics such as: improved sequestration of ions in the roots and stems; faster establishment of ionic gradients necessary for vacuolar compartmentalization of ions; or a superior capacity to osmotically adjust with minimal use of toxic ions from the environment (Waisel 1991; Shannon et al. 1994). If so, these seedlings lend credence to the existence of a subset of superior seedlings as discussed at the end of Chapter 2, though this study does not provide a means of identifying the additional characteristics which define them.

Regardless, these observations support the notion that relatively drought-tolerant seedlings are better suited to deal with salt stress than relatively intolerant seedlings. An outline of the response of planted black spruce seedlings to substrate salinity is presented in Figure 5.1. Drought tolerance makes seedlings more attractive candidates for planting than those lacking such characteristics. Sensitivity of black spruce to Cl^- toxicity is a significant concern however. If Cl^- concentrations are high enough, water relations parameters which aid in dealing with water deficit stress, appear insufficient to help a plant without the aid of additional mechanisms, and may in fact allow additional injury. In CT, where both Na_2SO_4 and NaCl are present, the concern becomes whether Cl^- concentrations are low enough that drought resistance alone is sufficient minimize salt stress in seedlings. The variable chemistry of CT suggests that no one answer exists to this question, though the field experiment indicated that even at a location where Cl^- ions had been hydrologically concentrated, toxicity was low enough that drought tolerance remained beneficial. Engineering CT with low initial concentrations of Cl^- would obviously be preferable, but is probably not practical, as aside from expense, that still leaves the issue of safe disposal of excess Cl^-. If, in practice, greater concentrations of Cl^- are frequently found in the field than those found at the CT deposit used for this study, then identification and propagation of "superior" seedlings with additional characteristics for dealing ions may become desirable. Otherwise, drought tolerance should be sufficient on its own.

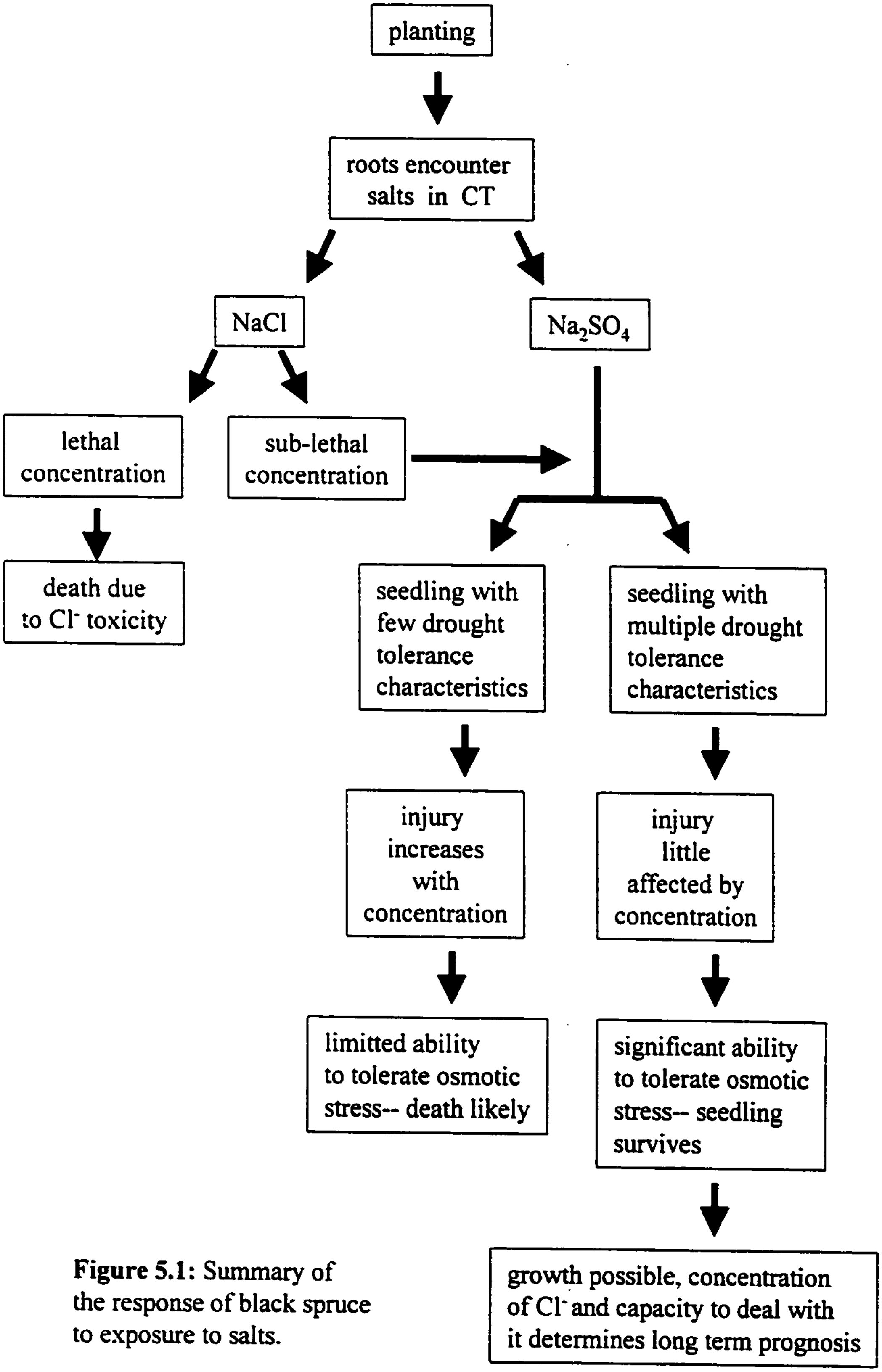

Figure 5.1: Summary of the response of black spruce to exposure to salts.

5.4 CONCLUSIONS

There are three main points regarding the response of black spruce seedlings exposed to salinity that can be taken from this research:

1) Although salinity poses both ionic and osmotic challenges for black spruce, the seedlings are more sensitive to chloride toxicity than to sodium toxicity or osmotic stress.
2) Osmotic stresses caused by salinity can be very similar to stresses caused by drought, and consequently relatively drought tolerant plants can handle these stresses better than less drought tolerant plants.
3) In the absence of toxic concentrations of chloride, drought tolerance characteristics can be used to predict the survival of seedlings in the short term, though accumulation of toxic ions may eventually present long-term challenges for seedlings.

Any adaptive potential that a plant may have is useless if it cannot survive initial exposure to stress. Identification of superior seedlings (i.e. those capable of both withstanding osmotic stress long enough to adapt, and of managing ion accumulation well enough to survive) is difficult. The results of this study suggest that such seedlings do exist as a subset of the relatively drought tolerant seedlings, and may be possible to identify through additional work. Based upon these current results, recognition and exclusion of those seedlings which cannot tolerate osmotic stress is possible. Doing so offers one means of focusing planting efforts on more suitable seedlings, even if it does not identify superior individuals from among these. Screening planting stock in this manner should improve the over all survival rate of seedlings, and can be achieved by using seedlings from populations, families or clones possessing drought tolerance characteristics. The utility of such a technique, however, is dependent upon the chloride content of the substrate involved, as black spruce is sensitive to chloride toxicity.

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