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Low temperature effects on physiological characteristics of dormant white spruce (*Picea glauca*) bareroot seedlings

by

Yingfang Wang ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirement for the degree of **Doctor of Philosophy**

in

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ABSTRACT

The effects of low temperature storage on spring-lifted and fall-lifted white spruce (*Picea glauca* (Moench) Voss) bareroot seedlings were studied. Seedlings grown in the nursery beds photosynthesized in early spring. This photosynthesis resulted in a drastic increase in carbohydrate levels in both needles and roots before spring lifting. The carbohydrate levels were positively correlated with new root growth of seedlings planted in the forest and with terminal shoot growth in the second year following planting. The recovery of photosynthesis was accompanied by changes in the composition of soluble carbohydrates. These changes included an increase in sucrose and a decrease in raffinose and monosaccharides. Spring changes in seedling physiology also included decreases in cell wall elasticity, osmotic potential and relative water content at turgor loss point followed by an increase in osmotic potential and relative water content at turgor loss point shortly before bud break.

Spring-lifted seedlings could be stored at –2°C and +4°C for a limited time without negative effects on their physiology. The duration of safe storage depended on seedling physiological status at lifting time. The maximum recommended storage duration was 9 weeks. Seedlings stored for longer than 9 weeks showed bud flushing or terminal bud injury in storage and poor survival and reduced shoot growth after planting. Storage temperature did not significantly affect seedling total non-structural carbohydrate levels. However, –2°C storage resulted in an increase in sugar levels in needles and roots while 4°C storage maintained similar sugar levels to those before storage. Root growth potential declined with the duration of –2°C storage, but in 4°C storage, high root growth

potential was maintained for 6, 9 and 3 weeks in seedlings lifted on April 20, April 27 and May 4, respectively.

Fall-lifted seedlings could be safely stored at temperatures lower than -2°C. However, lowering storage temperature did not significantly affect tissue carbohydrate levels. Storage temperature affected seedling water relations, gas exchange, bud dormancy release and root growth potentials. Lower storage temperatures resulted in higher osmotic adjustment, decreased cell wall elasticity and delayed bud flushing following planting. At -6°C storage, higher electrolyte leakage was measured in shoots and roots, and seedlings had lower root growth potential compared with those at -2°C and -4°C. Seedlings stored at -4°C had superior stress resistance characteristics including relatively low osmotic potentials at turgor loss point and at full saturation, rigid cell walls, high carbohydrate content and high root growth potential.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

General Introduction

Numerous container and bareroot conifer seedlings are planted every year in North America. Container seedlings are seeded in containers and grown in greenhouses until they achieve the required sizes. Bareroot seedlings are seeded directly in nursery beds or they are seeded in containers that are placed in greenhouses for about six months and then transplanted into nursery beds. Bareroot seedlings usually grow in nursery beds for one to three years before they are lifted. Lifting can take place in fall or spring. Fall-lifted seedlings are stored below 0°C (frozen) through winter, and spring-lifted seedlings are usually stored above 0°C (cold) for a short time before planting.

Container seedlings are grown in greenhouses and their roots are not separated from the soil medium at planting, minimizing damage due to handling. However, bareroot seedlings are larger and better adapted to the outside environment compared with the container stock. Larger seedlings are often better equipped to compete with other plants and tend to survive and grow better than smaller seedlings when planted in the forest (South 1993, McGrath and Duryea 1994). Therefore, in some forest sites, reforestation can be more successful with bareroot seedlings.

In Alberta, white spruce (*Picea glauca* (Moench) Voss) bareroot seedlings are lifted either in fall or in spring. Fall-lifted seedlings are usually stored at -2°C for seven months or longer until spring planting, and spring-lifted seedlings are planted immediately or stored for a short time at 1 - 4°C. Spring-lifted seedlings were found to have higher starch content, earlier bud break and root growth, higher photosynthetic rates, and were more resistant to drought stress compared to fall-lifted seedlings (Jiang et al. 1994, 1995). Present planting schedules favor earlier planting of spring-lifted seedlings than the fall-lifted stock. However, Jiang et al. (1994, 1995) recommended fall-lifted seedlings for early spring planting because of their delayed bud flushing following planting. If fall-lifted seedlings receive planting priority, spring-lifted seedlings may require longer storage at low temperature to prevent mold growth and bud flushing. At present, there are no guidelines for safe low temperature storage of spring-lifted seedlings since the effects of low temperature storage on spring-lifted seedlings have not been thoroughly studied. Similarly to fall-lifted seedlings, the cold or frozen storage tolerance of spring-lifted seedlings will depend on their physiological status at the time of lifting. In the spring, seedlings undergo a relatively slow transition from winter dormancy to growth, but the exact time when seedlings lose their tolerance to prolonged low temperature storage has not been determined. Similarly, the effects of lifting time, storage temperature and storage duration on the post-planting performance of spring-lifted seedlings have not been investigated.

Outplanted seedlings depend on carbohydrate reserves until photosynthesis can meet the demands for growth and respiration. The high carbohydrate content of spring-lifted seedlings may be due to early spring photosynthesis before lifting. However, it is not clear whether and to what extent early spring photosynthesis increases seedling carbohydrate levels and how the increase in carbohydrate content affects seedling growth, survival and stress resistance after planting.

The low carbohydrate content of fall-lifted seedlings may be partly due to respiration during long-term storage. Lower storage temperature may reduce carbohydrate consumption (Ritchie 1987). However, it is not known whether fall-lifted white spruce seedlings can be safely stored at less than -2°C, and how these lower temperatures affect seedling carbohydrate levels and their physiological characteristics.

The principal objectives of the present study were to:

(1) Examine the effects of early spring photosynthesis on carbohydrate levels and field performance of white spruce seedlings.

(2) Help develop guidelines for safe frozen storage (<0°C) and cold storage (>0°C) of spring-lifted white spruce bareroot seedlings.

(3) Determine the effects of frozen storage temperatures on physiological characteristics of fall-lifted white spruce bareroot seedlings.

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Chapter II

Literature Review

Seedling planting is widely used in forest regeneration programs throughout North America. The post-planting success of seedlings depends on their quality and on environmental characteristics of the planting sites (Ritchie 1984a, Duryea 1985). The quality of seedlings is determined by their morphological and physiological characteristics. Although morphological characteristics have long been employed to evaluate stock quality (Johnson and Cline 1991), physiological characteristics determine how seedlings perform in the field. Morphological characteristics change little between lifting and planting, while physiological characteristics during that time may undergo dramatic changes (McKay 1997). Cold and frozen storages have been shown to affect seedling physiological characteristics (Hellmers 1962). Understanding how these physiological characteristics affect reforestation success and how they are altered by lifting and storage would help optimize lifting and storage procedures and improve reforestation success.

2.1 Seedling physiological characteristics and reforestation success

The main seedling physiological characteristics that affect reforestation success are water stress resistance, root growth potential, carbohydrate levels, gas exchange characteristics, and frost hardiness (Duryea 1985).

2.1.1 Water stress resistance

Poor survival and growth after planting may be due to water stress (Burdett 1990, Margolis and Brand 1990), which is caused by root confinement, poor root-soil contact, and low absorption of water into roots (Kozlowski and Davies 1975, Sands 1984, Grossnickle and Heikurinen 1989, Burdett 1990, Carlson and Miller 1990). Since seedlings have limited ability to take up water but relatively high transpiration rates, water stress can appear even at high soil water potential (Sands 1984, Caldwell et al. 1986, Sutton 1995). Due to the water deficit, shoot growth in white spruce can be reduced for several years following planting (Mullin 1963, Vyse 1981, Burdett et al. 1984).

Burdett (1990) pointed out that high plant water potential after planting allows the onset of a positive cycle: initiation of root growth supported by photosynthesis, and maintenance of photosynthesis supported by root growth. Relative water content (RWC), water potential and osmotic potential are commonly measured to determine plant water status (Joly 1985). These parameters can be used to assess water deficit at a particular time. However, plant water status changes with changing environmental conditions. A complete description of plant water stress resistance can be obtained by determining the relationship between relative water content and water potential. From this relationship, osmotic potentials at turgor loss point and at full turgor, cell wall elasticity, turgor potential and osmotic volume are obtained. These parameters can be related to plant water stress resistance (Cheung et al. 1975).

To achieve high water potential after planting, seedlings must absorb water from soil and control water loss from transpiration. New root growth after planting is effective in reducing water stress (Grossnickle and Reid 1984, Nambiar 1984, Sands 1984, Grossnickle and Blake 1987, Grossnickle 1988, Grossnickle and Heikurinen 1989, Guehl et al. 1989). The extension of new roots improves root-soil contact and facilities more efficient water uptake (Chung and Kramer 1975). However, in boreal forest, root growth following spring planting is usually restricted due to low soil temperature (Baker and Swan 1966, Larsen 1980, Ritchie 1985, Vapaavuori et al. 1992, Folk et al. 1995), and (or) lack of photosynthesis (Ritchie and Dunlap 1980, van den Driessche 1987, Vapaavuori et al. 1991). Lack of root growth at low temperature has been found in conifer seedlings grown in hydroponics (Vapaavuori et al. 1992) and soil (Nambiar et al. 1979, Lopushinsky and Kaufmann 1984, Anderson 1986, Lopushinsky and Max 1990). In some conifers, root growth depends primarily on current photosynthesis (Carlson 1976, 1977, Ritchie and Dunlap 1980, van den Driessche 1987, 1991, Ritchie and Tanaka 1990,Thompson and Puttonen 1992, Noland et al. 1997). However the recovery of photosynthesis usually does not occur immediately after planting and it may take several weeks for seedlings to achieve a positive carbon balance (Marshall 1985, Vapaavuori et al. 1992). Therefore, carbohydrate reserves may be necessary for root growth (Carlson 1976, 1977, Philipson 1988, Binder et al. 1990, Omi et al. 1991).

Due to restricted uptake of water following planting, seedlings must have the ability to conserve water and tolerate water deficit stress. Maintaining low stomatal conductance after planting was effective in avoiding drought stress in white spruce and jack pine (Blake 1983, Grossnickle and Heihurinen 1989). However, since low water potentials commonly develop in newly-planted seedlings, the ability to function under low water potential conditions is necessary for seedlings to survive transplanting stress.

Adaptation of plants to water stress depends mostly on their turgor maintenance capacity (Jones and Turner 1978, Ritchie and Shula 1984) which, in turn, is affected by osmotic potential and cell wall elasticity (Jones and Turner 1978, Kikuta and Richter 1986). High turgor maintenance capacity is associated with a large difference between osmotic potentials at the full turgor (π_{100}) and at the turgor loss point (π_0) (Jones and Turner 1978) and with low osmotic potentials and relative water contents at the turgor loss point (Jane and Green 1983). The relationship between turgor maintenance capacity and cell wall elasticity is complex. Plants with elastic cell walls can lose large volumes of water with a small decline in turgor (Ritchie and Shula 1984; Grossnickle 1988). In contrast, plants with rigid cell walls undergo large changes in turgor and water potentials for small changes in water content (Colombo 1987). This produces a large increase in water potential gradient that enables plants to take up water from dry soil without undergoing a large tissue water deficit. Thus, an increase or decrease in elasticity of cell walls can both contribute to dehydration tolerance.

2.1.2 Root growth potential

Root growth potential (RGP) is a measure of the ability of seedlings to grow new roots under optimum conditions. It is an important estimate of seedling quality because it integrates numerous morphological and physiological factors into one measurable characteristic. RGP is closely related to seedling dormancy and cold hardiness (Ritchie and Shula 1984, Ritchie 1986, Tinus et al. 1986). It increases after deep dormancy (rest) has been attained, peaks with fulfillment of the chilling requirement and with the development of maximum cold hardiness, and falls abruptly approximately at the time of bud break and concurrent loss of cold hardiness (Stone et al. 1963, Ritchie and Dunlap 1980, Ritchie 1986, Tinus et al. 1986).

RGP and field survival have been positively correlated in various conifers, including *Pinus* (Burdett 1979, Sutton 1980, Burdett et al. 1983, Feret and Kreh 1985, Hallgren et al. 1993, Brissette and Burdett 1993, Simpson and Vyse 1995), *Pseudotsuga* (Jenkinson and Nelson 1978, 1983, McKay 1992, Sharpe and Mason 1992), and *Picea* (McMinn 1980, Sutton 1980, Burdett et al. 1983, McKay 1992, Sharpe and Mason 1992, Simpson et al. 1994, Simpson and Vyse 1995, Binder and Fielder 1995). However, it has also been reported that RGP was not related to field survival and growth (Pendl and D'anjon 1992, Simpson and Vyse 1995). RGP may not always predict field performance since it is assessed under conditions that may not reflect those in forest planting sites (Simpson and Ritchie 1997). Planted seedlings are usually unable to grow new roots because of low soil temperature and water deficit stress, even though RGP may be high. The poor correlation between RGP and field survival could also be due to the higher importance of classes of the root system than the new root growth measured in RGP test (Johnson-Flanagan and Owen 1985a, b). Johnson-Flanagan and Owen (1985a, b) found that lateral root elongation in early spring enlarged the root system and growth of hair-covered roots in late spring increased the absorptive surface area of the root system. The hair-covered roots, i.e. absorbing roots, are responsible for water and nutrient uptake (Lyr and Hoffman 1972). Stone et al. (1962) reported high seedling survival during the springtime when there was a decrease in RGP. On the other hand, Ritchie (1985) proposed that RGP may be a good indicator of field performance because it correlates with other quality attributes which directly impact field performance. The measure of RGP obtains an estimate of relative cold and stress resistance in seedlings and these properties not the ability to grow roots decide how the seedlings will perform in the field (Ritchie 1985).

2.1.3 Carbohydrate reserves

Seedlings depend on carbohydrate reserves from the time they are lifted until photosynthesis is sufficient to meet the demands for growth and respiration. To survive the planting stress, seedlings must grow new roots, which increase the water uptake and stimulate the positive cycle of photosynthesis and root growth (Burdett 1990). The initial root growth was reported to depend on carbohydrate reserves (Carlson 1976, 1977, Philipson 1988, Binder et al. 1990, Omi et al. 1991, Noland et al. 1997) since the photosynthesis of newly-planted seedlings is usually inhibited (McNabb 1985, van den Driessche 1987). It was suggested that seedlings may not survive planting if they do not have enough carbohydrate reserves at planting (Marshall 1985). Engelmann spruce seedlings had very poor survival when their carbohydrate levels were lower than 300 mg/g dry weight (Ronco 1973), and Scots pine seedlings had increased mortality when the total glucose content fell below 2% of needle dry weight (Puttonen 1986). Poor seedling survival due to depletion of carbohydrate reserves during cold storage was also reported in Jeffrey and ponderosa pines (Hellmers 1962), Scots pine (Puttonen 1986), white spruce (Hocking and Ward 1972), and radiata and mugo pines (McCracken 1979). Shoot growth in spring was reported to depend largely on carbohydrate reserves (Gordon and Larson 1970, Kozlowski and Winget 1964, Krueger 1967). The depletion of carbohydrate reserves inhibited shoot growth of Scot pine (Ericsson et al. 1983). Decreased carbohydrate reserves decreased needle growth, reduced the number of terminal buds, disturbed leader shoot formation, and consequently limited shoot elongation of planted Scots pine seedlings (Puttonen 1986). Seedlings with high carbohydrate content also produced more new roots (Rose 1992, Jiang et al. 1994). In addition to providing energy for growth, adequate carbohydrate reserves increase stress resistance of seedlings (Puttonen 1986, Ritchie 1982). Soluble sugars are the main osmoregulators (Jones et al. 1980, Ackerson and Herbert 1981, Borowizka 1981, Zwiazek and Blake 1990) and are related to drought resistance and frost hardiness (Levitt 1972, Ritchie 1982, Egger et al. 1996).

2.1.4 Photosynthesis

Although carbohydrate reserves can, for limited time, supply energy for planted seedlings, quick establishment, i.e. shoot and root growth, will mainly depend on the recovery of photosynthesis (Ritchie and Dunlap 1980, van den Driessche 1987, Ritchie and Tanaka 1990, Vapaavuori et al. 1992, Noland et al. 1997). Photosynthetic rates are usually low in newly-planted seedlings and it may take several weeks before the seedlings achieve positive carbon balance (Marshall 1985, Vapaavuori et al. 1992). Since photosynthesis is less sensitive to water stress than shoot elongation (Larcher 1995), there have been relatively few studies that correlated photosynthetic rates with field performance. However, photosynthesis depends on the opening of stomata, which results in the movement of water through the soil-plant-atmosphere continuum (Grossnickle and Blake 1985, 1986, 1987). Therefore, a positive carbon balance may indicate seedling acclimation to the specific planting site.

2.1.5 Frost hardiness

Frost hardiness is defined as the lowest temperature to which a plant can be exposed without being damaged (Levitt 1980). It is a desirable physiological characteristic of seedlings for low temperature storage and planting in the temperate and boreal forests. Frost hardening occurs in early fall with growth cessation and bud set. It develops gradually in response to shortening daylength or drought, and more abruptly in response to cold temperature (Levitt 1980). With the accumulation of chilling hours, cold hardiness intensifies, and dormancy is released. When plants are frost-injured, the sites of injury are cell membranes, which lose their selective permeability. Thus, the electrolytes move more freely and diffuse out of cells. By measuring the conductivity of tissue diffusate, an estimate of injury can be made. Electrolyte leakage has been used successfully for estimating frost hardiness of shoots (van den Driessche 1976, Green and Warrington 1978, Colombo and Hickie 1987, Murray et al. 1989) and needles (Burr et al. 1986). This method was also used to test the vitality of seedlings after cold storage. The electrolyte leakage from fine-roots following cold storage was closely correlated with survival and shoot growth of Sitka spruce seedlings (McKay and Mason 1991, McKay 1992).

2.2 Cold storage and seedling physiology

Cold storage of conifer seedlings is widely practiced in the temperate and boreal regions of North America because it facilitates nursery and planting schedules. The success of cold storage will depend on the seedling physiological status at the time of lifting and on storage environment (Camm et al. 1994). Cold storage has been found to affect seedling carbohydrate levels (Ritchie 1982, Cannell et al. 1990, Omi et al. 1994, Jiang et al. 1994), dormancy status (DeWald and Feret 1985, Ritchie et al. 1985, Rietveld 1989), root growth potential (Duryea and McClain 1984, Omi et al. 1994), gas exchange (Omi et al. 1991), and stress resistance (Ritchie 1982, Ritchie 1986, Faulconer 1988).

2.2.1 Lifting time

Seedlings develop the maximum stress tolerance between rest (deep dormancy, i.e. when the buds have set and chilling is required before the buds will burst) and quiescence (post dormant, i.e. when chilling requirement has been satisfied but growth has not started) (Ritchie 1986). This period falls in the mid-winter, and in the boreal region of North America, the ground often freezes before December and remains frozen until March or later. Since lifting seedlings is impossible when the soil is frozen, tree nurseries must lift seedlings either in fall and store them until spring planting, or in spring for short-term storage or immediate planting. In either case, the seedlings are not at the stage of maximum stress tolerance and may show poor field performance after storage. Numerous studies (Lavender and Wareing 1972, Garber and Mexal 1980, Mullin 1980, Sutton 1984, Ritchie et al 1985, Ritchie 1986, Racey 1987, Lavender and Silim 1992) confirmed Hocking and Nyland's (1971) conclusion, "that for overwinter storage, lifting should be delayed as long as possible." Ponderosa pine seedlings lifted in early fall had poor root growth and lower stomatal conductance at the end of storage compared to those lifted in late fall (Stone and Schubert 1959, Omi et al. 1991). Poor survival of seedlings lifted in early-fall has also been reported in Douglas-fir (Lavender 1964, Lavender and

Wareing 1972), western hemlock (Nelson and Lavender 1979), black spruce and jack pine (Lavender and Silim 1992). Seedlings lifted shortly before bud flush in the spring had lower survival rates after planting compared with those lifted and stored in winter (Winjum 1963, Aldhous 1964, Lavender 1964). However, Jiang et al. (1994, 1995) found that spring-lifted white spruce seedlings had higher carbohydrate content and photosynthetic rates and were more drought resistant than seedlings lifted in fall and stored over winter. Spring-lifted ponderosa pine seedlings also had higher RGP, starch content and post-planting survival rates than winter-lifted seedlings (Omi et al. 1994). The high carbohydrate content of spring-lifted seedlings may be due to winter and (or) spring photosynthesis because many conifer species have the ability to photosynthesize in winter and early spring. This photosynthesis resulted in accumulation of carbohydrates (Hagem 1962, Little 1970, Pomeroy et al. 1970, Ericsson 1980, Ericsson and Persson 1980, Glerm 1980, Mattsson 1982, Snyder 1990, Fischer and Hill 1991). However, there is little information on the ability of spring-lifted seedlings to tolerate cold storage.

2.2.2 Carbohydrates

In dark cold or frozen storage, seedlings do not photosynthesize, but respiration continues with the intensity dependent on storage temperature. A decline in carbohydrate levels during cold and frozen storage has been reported in Engelmann spruce (Ronco 1973), mugo and radiata pines (McCracken 1979), Douglas-fir (Ritchie 1982, Cannell et al. 1990), Sitka spruce (Cannell et al. 1990), and white spruce (Jiang et al. 1994) and was attributed to maintenance respiration. In some studies (Forry and Zaerr 1988, Omi et al 1994), the root starch reserves were completely depleted during cold storage. Most of the decline in carbohydrate reserves occurred during the first several months of storage (McCracken 1979, Ritchie 1982, Cannell et al. 1990, Jiang et al. 1994). Starch and sugar conversions may also occur during storage (Halmer and Berley 1982, Ritchie 1982). Lower storage temperature limits the decline of carbohydrate reserve (Ritchie 1987). On the other hand, Winjum (1963) found that starch and reducing sugars did not change in Douglas-fir and Noble fir seedlings during cold storage. Chomba et al. (1993) reported that in Engelman spruce seedlings, needle sugars were not strongly affected by two and four months of frozen storage but starch was reduced by more than 50%.

2.2.3 Dormancy release

In the annual life cycle of conifer seedlings in temperate and boreal regions, dormancy is the period during which there is no visible shoot elongation but active lateral cambial growth as well as differentiation may occur. It starts at the time of bud set in mid-summer and ends at bud break the following spring. When dormancy is imposed by the environment, e.g. decreasing daylength, drought stress, or low temperature, the dormant bud is said to be quiescent (Lavender 1985). The period of quiescence is usually from mid-summer to early fall and from late winter to early spring. When dormancy is maintained by agents within the bud itself, the dormant bud is said to rest (Romberger 1963). A resting bud cannot resume growth even under a favorable environment. It must be vernalized to satisfy the transition from rest to quiescence. Fall-lifted white spruce seedlings are usually at the stage of rest, and spring-lifted ones are at the stage of quiescence. Cold or frozen storage could fulfill the chilling requirement of fall-lifted seedlings (Garber 1978, Carlson 1985, DeWald and Feret 1988). Subfreezing temperatures are less effective in fulfilling the chilling requirement than temperatures between 4 to 6°C in many forest tree species (Hinesley 1982, Ritchie 1984b). The dormancy release of cold-stored seedlings is slower than that in the nursery (Hermann 1967, Ritchie and Stevens 1979, Ritchie 1984b, Ritchie et al. 1985). This slow release may be due to constant low temperature or lack of temperature fluctuation and photoperiod (van den Driessche 1975, Campbell 1978, Lavender 1985). The delay of dormancy release of cold-stored seedling may be advantageous for planting in an area susceptible to spring frost. Prolonged cold storage (above 0°C) of spring-lifted seedlings may result in bud flushing in storage because the buds are already quiescent. However, the possibility of frozen storage of spring-lifted seedlings to prevent bud flushing and extend storage duration has not received an adequate attention.

2.2.4 Root growth potential

Root growth potential is determined by seedling physiological condition (Stone 1970) and the extend of root system development (DeWald and Feret 1988, Deans et al. 1990). The RGP of seedlings lifted in mid-winter was not reduced by cold storage (Winjum 1963, DeWald and Feret 1988). However, RGP in conifer seedlings lifted in fall or in spring declined during storage (Winjum 1963, Ritchie 1984b, Ritchie et al. 1985, Burr and Tinus 1988, DeWald and Feret 1988, Tabbush 1988, McKay and Mason 1991). The detrimental effect of cold storage on seedlings lifted in early fall has been attributed to the lack of physiological readiness for chilling in non-dormant stock (Stone and Schubert 1959, Ritchie and Dunlap 1980, Kramer and Rose 1985). Cold storage decreases RGP of spring-lifted seedlings presumably because of increased metabolism in the buds once the chilling requirement has been met (Ritchie et al 1985). The roots of woody plants are more sensitive to low temperature than shoots (Parker 1959, Pellett and White 1969, Smit-Spinks et al. 1985); therefore, the RGP reduction after frozen storage (Lindstrom and Stattin 1994) could also be due to larger sections of root tissue becoming injured as the temperature drops, with the younger roots dying off first (Studer et al. 1978, Smit-Spinks et al. 1985, Lindstrom and Mattsson 1989). Roots assessed in the RGP test are produced mainly by the elongation of pre-existing fine roots (DeWald and Feret 1988, Deans et al. 1990). However, the reasons for sudden decline in RGP after 6 months (Ritchie 1982) and 22 weeks (Camm and Harper 1991) of frozen storage are not clear.

2.2.5 Gas exchange

Photosynthetic decline after cold and frozen storage has been shown in several conifers (Pharis et al. 1970, McCracken 1978, Harper and Camm 1993). Photosynthesis was progressively reduced as storage duration increased (McCracken 1978). The decreased photosynthetic rates resulted from the disruption of the photosynthetic system due to persistent darkness and low temperature (McCracken 1978, Camm et al. 1993). Seedlings stored at subfreezing temperature showed a slower recovery in photosynthesis than those stored at above zero temperature (Mattsson and Treong 1986). The slow recovery of photosynthesis was due to low stomatal conductance (Mattsson and Treong

1986) and (or) disruption of CO_2 fixation (McCracken 1978, Camm et al. 1993). The low stomatal conductance of frozen-stored white spruce seedling enables them to better adapt to dry conditions (McCracken 1978, Blake 1983). However, Harper and Camm (1993) reported increased stomatal conductance of white spruce seedlings after 22 weeks of frozen-storage.

In general, cold storage as an important part of seedling production has many effects on seedling physiological characteristics. Seedling physiological status is crucial for the successful establishment of planted seedlings. A better understanding of the effects of cold storage on seedling physiology would help forest tree nurseries produce good quality seedlings and would improve reforestation success.

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Chapter III

Effects of Early Spring Photosynthesis on Carbohydrate Content, Bud Flushing and Growth of White Spruce (*Picea glauca*) Bareroot Seedlings[1]

3.1 INTRODUCTION

Rapid growth of shoots and roots is important for successful establishment of planted tree seedlings. Rapid shoot growth allows seedlings to compete with surrounding vegetation and rapid root growth is crucial for efficient water uptake by newly planted seedlings (Burdett 1990). Due to the short growing season in central and northern Alberta, delayed root growth and bud flush of fall-lifted white spruce bareroot seedlings following spring planting have caused a great deal of concern.

Delayed bud flush in fall-lifted white spruce seedlings stored at -2°C may be related to low carbohydrate levels (Jiang et al. 1994) and (or) slow photosynthetic recovery after planting (Jiang et al. 1995). The low levels of carbohydrates in fall-lifted seedlings compared with those lifted in spring could be due to respiration during cold storage (Cannell et al. 1990) and lack of late fall and early spring photosynthesis. Evergreen trees have the potential to photosynthesize in late winter and early spring (Helms 1965, Ludlow & Jarvis 1971, Ericsson & Persson 1980, Schaberg et al. 1995). Significant increases in carbohydrate levels in spring has been observed in several conifer species including red pine (Pomeroy et al. 1970), balsam fir (Little 1970a,b), and Scots pine (Ericsson & Persson 1980, Fischer & Höll 1991). Net photosynthesis is typically low in all seedlings during the first several weeks following planting. This is likely due to poor root establishment and resulting water stress (Grossnickle & Heikurinen 1989, Burdett 1990, Jiang et al. 1994) and may result in a depletion of energy reserves and an overall negative carbon balance (Marshall 1985, Vapaavuori et al. 1992, Jiang et al.

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[1] A version of this chapter has been accepted for publication. Wang, Y. and Zwiazek, J. J. 1998. Scan. J. For. Res.

1994). Therefore, seedling carbohydrate levels may be crucial for the establishment of newly planted seedlings.

The objective of the present study was to examine the effects of early spring photosynthesis on carbohydrate content, bud flushing and growth of white spruce bareroot seedlings. We tested the hypothesis that early spring photosynthesis before spring lifting of white spruce bareroot seedlings results in a significant increase in seedling carbohydrate content which, in turn, improves root and shoot growth after planting.

3.2 MATERIAL AND METHODS

3.2.1 Plant material and experimental design

White spruce [*Picea glauca* (Moench) Voss] bareroot seedlings were grown in the Pine Ridge Forest Nursery (54° 07' N, 112° 29' W), Smoky Lake, Alberta, Canada, from the Whitecourt seed source (seed lot no. DS-88-SW). The seeds were germinated and plants grown for six months in containers in the greenhouse and then planted outside in nursery beds for another 1.5 years (P +1.5). The average seedling height at start of the experiment was approximately 30 cm.

The experiments were conducted in 1994. Air temperature in April, 1994, fluctuated with the maximum reaching above 20°C and the minimum of about -15°C. The soil temperature in the first half of April was relatively stable and remained at about 0°C (Fig. 3.1).

To examine how the absence of early spring photosynthesis affects seedlings, one group of white spruce seedlings for spring lifting was covered (SC) with perforated white cardboard boxes between March 25 and April 18, 1994. The boxes were 60 cm x 35 cm x 55 cm. The light and temperatures inside and outside boxes were monitored using a datalog. The temperature difference between the inside and outside of boxes was less than 2°C and the photosynthetically active radiation (PAR) inside the boxes was 5 to 10 μmol $m^{-2}s^{-1}$ during day time. On March 25, 1994, all seedlings were still covered with snow. Approximately 240 seedlings were covered with six boxes (40 seedlings per box) and the boxes were arranged in two-meter intervals in the nursery bed. The seedlings between the boxes served as uncovered control (SU). Also, one group of 240 fall-lifted seedlings stored at -2°C between October, 1993 and March 25, 1994, was removed from frozen storage and placed with the spring-lifted seedlings in the nursery beds (FO). The roots of the seedlings were covered with moist peat moss and the seedlings were watered every 5 days. The second group consisting of 240 fall-lifted (FF) seedlings remained in frozen storage until measurements were taken. Needle and root samples for carbohydrate analysis were collected from each treatment (SU, SC, FO and FF) on April 7 and April 18, 1994. FO and FF seedlings were used for carbohydrate analyses and not for root growth potential tests and outplanting due to extensive new root growth of FO seedlings after two weeks in the nursery beds. In the early morning of April 18, 1994, seedlings were uncovered and shoot water potentials, relative water contents and gas exchange were measured at mid-day. One seedling from each of the six boxes and six uncovered seedlings were randomly selected for the measurements. After the measurements, all covered and uncovered seedlings were lifted and packed in plastic bags inside waxed cardboard boxes and placed in cold storage at +4°C.

On May 10, 1994, SU and SC seedlings were planted in two forest sites near Devon (53° 22' N, 113° 45' W), Alberta, Canada. The planting design was a randomized complete block design. There were two blocks and two treatments (SU and SC) in each of the blocks. The two blocks were 20-year-old open sites of aspen and white spruce. There were 40 seedlings per treatment planted in each of the two sites. The major shrub species in the planting sites, *Rosa acicalaris* L., was about 1-m tall and sparsely scattered on both sites. The dominant grasses were *Bromus inermis ssp pumpellianus* and *Agropyron trachycaulum* (Link) Mailte. The annual precipitation averaged from 1964 to 1994 by a nearby weather station was 468 mm, with June and July being the wettest months. The average slope on both sides was less than 2% (south facing).

3.2.2 Gas exchange measurements and water relations

Gas exchange parameters were measured at midday in the field with a portable infrared gas analyzer (IRGA) LCA-2 (Analytical Development Company Ltd., Hoddesdon, Hertfordshire, UK). The measurements were taken on a clear sunny day with

PAR higher than 1000 µmol m^{-2} s^{-1}. The uppermost branches were selected for the measurements, with six seedlings per treatment. Immediately following gas exchange measurements, shoot water potentials were measured using a Scholander pressure chamber (PMS Instrument Co. Corvallis, OR). The leaf area of each sample was determined with a LI 3000 Leaf Area Meter (Lambda Instruments Corp., Lincoln, NB). Water use efficiency (WUE, µmol CO_2/ mmol H_2O) was calculated as the ratio of net assimilation (NA) to transpiration (E) rates.

3.2.3 Carbohydrate analysis

SU, SC, FO and FF seedlings were thoroughly washed and roots and needles separated. The tissue was freeze-dried for 48 h and soluble carbohydrates (sugars) were extracted from 0.2 g of needles and 0.4 g of roots with 85% boiling ethanol for 10 min (Jiang et al. 1994). The extracts were filtered through Whatman No.1 filter paper, and the residue extracted two more times with 85% ethanol. The filtered extracts were combined, reduced to water phase in a vacuum evaporator at 40°C and made up to 20 ml with distilled water. Soluble sugars were measured using the anthrone method (Ashwell 1957) as previously reported (Jiang et al. 1994). The tissue residues were freeze-dried and used for starch analysis. Starch was extracted with perchloric acid (Rose et al. 1991) and quantified as glucose equivalents using the anthrone method (Jiang et al. 1994).

3.2.4 Root growth potential

At the time of forest planting, the root growth potential of SU and SC seedlings was tested in the greenhouse at 25/18°C (day/night) temperature and 18-h photoperiod. Twenty seedlings from each treatment were planted in 4-L plastic pots (one seedling per pot) with a mixture of peat moss and sand (3:1) and watered every 3 days. After 4 weeks, the seedlings were lifted and new roots longer than 10 mm were counted.

3.2.5 Field growth and bud flushing

In 1994, the flushing of terminal buds in SC and SU seedlings was recorded once a week following planting in the forest. In 1995, bud flushing was recorded once a week beginning on May 10. The annual height growth was measured at the end of September, 1994 and 1995. New root growth was measured in the field-planted seedlings 2 and 4 weeks following planting by lifting 20 seedlings from each treatment and counting new roots longer than 10 mm.

3.2.6 Data analysis

Effects of treatments on water contents (WC), water potentials (WP), net assimilation (NA), stomatal conductance (g_s), water use efficiency (WUE) and root growth potential were assessed by analysis of variance (one-way ANOVA). Data of root growth and sugar and starch contents were analyzed by two-way ANOVA, with treatment and time as main effects and with interaction. Shoot elongation was analyzed using the GLM procedure. The planting sites served as randomized blocks and the main effects are treatments and year and with interaction. Bud flushing was tested using repeated measures analysis of variance. The t-test was used to separate the differences of means. All calculations were done on SAS software package (version 6.10).

3.3 RESULTS

Covering the seedlings in early spring from March 25 to April 18, 1994, did not appear to affect their photosynthetic capacity (Table 3.1). However, stomatal conductance was lower in SC seedlings compared with SU seedlings, resulting in lower transpiration rates (data not shown) and higher water use efficiency values (Table 3.1). SC seedlings also had significantly higher (less negative) water potentials and water contents than those in the uncovered seedlings (Table 3.1).

Covering seedlings resulted in significant difference in carbohydrate levels (Table 3.2). On April 7, SU and SC seedlings had similar needle sugar levels but about 20% higher starch content compared with SC seedlings. In roots, both sugar and starch contents were similar in SU and SC seedlings (Fig. 3.2). However, on April 18, needle sugar and starch contents of SU seedlings were about 16% and 120%, respectively, higher compared with those in SC seedlings (Fig. 3.2). At the same time, root starch content of SU seedlings was about 20% higher than that in SC seedlings (Fig. 3.2). When sugars and starch were added to calculate the total non-structural carbohydrates (TNC), the total TNC needle content increased from 275 mg g^{-1} DW on April 7 to 316 mg g^{-1} DW on April 18 in SU seedlings and decreased from 240 mg g^{-1} DW to 195 mg g^{-1} DW in SC seedlings. During the same time, root TNC content increased from 160 mg g^{-1} DW to 226 mg g^{-1}DW in SU seedlings and from 151 mg g^{-1}DW to 194 mg g^{-1} DW in SC plants. An significant increase in starch content was also measured in FO seedlings when placed outside in early spring (Table 3.3, Fig. 3.3). On April 18, both needle and root starch levels were about 70% higher in FO seedlings compared with FF plants (Fig. 3.3). Although root sugar levels were significantly lower in FO seedlings than those in FF plants (Fig. 3.3), on April 18, the total TNC content of FO seedlings was about 25% higher than that in FF seedlings.

Covering treatment significantly affected terminal bud flushing in the first year but not second year following planting (Table 3.4). In the first year, three weeks after planting, terminal buds flushed in about 30% of SC seedlings and less than 5% of SU seedlings (Fig. 3.4). Four weeks after planting, approximately 90% of SC seedlings and 80% of SU seedlings had opened buds (Fig. 3.4). Within five weeks of planting all seedlings had flushed terminal buds. In 1995, the second year following planting, SC and SU seedlings planted in 1994 had similar pattern of timing of bud flushing (Fig. 3.4).

After 4 weeks of growing in the greenhouse, SC seedlings had 70.3 ± 8.3 (n=20 seedlings ± SE) new roots ≥10 mm compared with 78.2 ± 10.3 in SU seedlings and the difference was not statistically significant. However, 2 and 4 weeks after planting in the field, SC seedlings produced significantly fewer roots than SU seedlings (Table 3.5, Fig. 3.5).

At the end of the first growing season, the length of terminal shoots was similar in both experimental groups of seedlings (Fig. 3.6). The terminal shoots of SC seedlings were shorter in the second growing season compared with those in SU plants, and the second year shoot length was much shorter in both groups of seedlings than that of the first year (Table 3.6, Fig. 3.6).

3.4 DISCUSSION

In winter, low temperature and snow cover prevent photosynthesis in seedlings that are grown in tree nursery beds in the boreal regions. However, with the snow-melt in early spring, photosynthesis may commence with increasing day temperatures. Due to low night temperatures (Fig. 3.1) and the lack of shoot and root growth, early spring photosynthesis may result in an accumulation of carbohydrates. A pre-budbreak accumulation of starch has been reported in the needles of *Abies balsamea* (Little 1970a, b), *Pinus resinosa* (Pomeroy et al. 1970) and *Pinus sylvestris* (Ericsson & Persson 1980, Fischer & Höll 1995). Our findings also demonstrated that early spring photosynthesis can result in a large increase of carbohydrate content in white spruce seedlings before spring lifting (Figs. 3.2 & 3.3). The increase in root carbohydrate levels in both covered and uncovered spring-lifted seedlings (SC and SU) (Fig. 3.2) and in the fall-lifted seedlings placed outside (FO) in early spring (Fig. 3.3) suggests the translocation of carbohydrates from shoots to roots in early spring. Similar findings were reported for *Pinus sylvestris* and *Picea abies* (Ericsson et al. 1983, Ericsson 1984). Due to the lack of photosynthesis, needle carbohydrate contents of SC seedlings decreased from April 7 to April 18 (Fig. 3.2). This decrease could be due to consumption by respiration and (or) due to the translocation to roots. However, day time respiratory demands for carbon were likely met in SC seedlings by the low PAR intensity inside the boxes which was at about the photosynthetic light compensation point for white spruce seedlings (Man & Lieffers 1997a).

In the present study, the seedlings with higher carbohydrate levels produced more roots but starch levels did not have positive correlation the timing of bud flushing following planting (Fig. 3.4). Carbohydrates are important in newly-planted seedlings for osmoregulation and energy storage. Several studies demonstrated that newly-planted seedlings suffered from water-deficit stress (Grossnickle & Heikurinen 1989, Burdett 1990) and had negative carbon balance for several weeks following planting (Marshall 1985, Vapaavuori et al. 1992). Rapid root growth can help seedlings overcome water stress (Grossnickle & Blake 1985, Burdett 1990). Current photosynthates from the shoots were shown to be necessary for root elongation (Andersen et al. 1986, van den Driessche

1987). However, variations among species have also been reported (Philipson 1988, Vapaavuori et al. 1992). Philipson (1988) and Vapaavuori et al. (1992) showed that in Norway spruce and Sitka spruce seedlings, root growth began about one week before seedlings achieved positive CO_2 balance, suggesting that root growth was initially facilitated by carbohydrate reserves. In our study, white spruce seedlings with the higher carbohydrate content had higher rates of root growth following planting in the forest (Fig. 3.5). A similar correlation between carbohydrate content and early root growth was reported in planted white spruce seedlings (Jiang et al. 1994). In root growth potential (RGP) tests, root growth usually does not correlate with carbohydrate levels (Ritchie 1982). This may be due to favorable environmental conditions used for testing RGP which result in fast photosynthetic recovery following planting. In the field, growth of new roots may depend more on the stored energy reserves. This is consistent with our observations that 2 and 4 weeks following field planting, SU seedlings produced more roots than SC seedlings but their root growth potentials were not significantly different.

Upon fulfillment of chilling requirement, the time of bud flush largely depends on environmental conditions in the spring (Nienstaedt 1966, Perry 1971). Since bud flush is caused by the hydration and expansion of cells, shoot water relations were shown to affect the timing of bud flush (Marsden et al. 1996). Our results showed that about 30% of SC seedlings flushed their terminal buds 3 weeks after planting compared with less than 5% of SU seedlings. In the absence of light, transpiration demands of SC seedlings were lower, resulting in improved shoot water relations which likely promoted bud flush. It is also possible that the slightly different temperatures and relative humidities experienced by SU and SC seedlings resulted in the different timing of bud flush. Man & Lieffers (1997b) reported that buds in white spruce seedlings grown under the forest canopy and in open sites flushed at different time due to temperature differences. Marsden et al. (1996) noted that white spruce seedlings grown in higher humidity break buds earlier than seedlings grown in the lower humidity. In the present study, the timing of bud flush in the second growing season was similar in both treatments because all seedlings experienced similar spring conditions.

Shoots of white spruce are largely preformed in the overwintering buds. This type of growth, referred to as fixed or preformed growth, is affected by the environmental conditions of both the previous and current seasons (Zahner 1968). In red pine, water stress in the year of bud formation and in the year of shoot elongation affected shoot growth (Garrett & Zahner 1973). In the present study, the lengths of terminal shoots in SU and SC seedlings were similar (Fig. 3.6). This was probably due to favorable environmental conditions that both groups of seedlings received when the buds were set in the nursery. However, the decrease of shoot growth observed in the second year was probably due to water stress during bud formation and less nutrients in the second year because of the limited absorption area of roots (Burdett 1990). SC seedlings had low carbohydrate content that likely contributed to reduced root growth following planting in the forest and resulted in reduced shoot growth the following year. High carbohydrate content before planting also correlated with high shoot growth rates in *Pinus sylvestris* (Ericsson et al. 1983). The planting check, commonly taking place in planted white spruce seedlings (Mullin 1963, Burdett et al. 1984, Sutton 1992, 1995), is considered to be caused by water and mineral nutrient stresses (Baldwin & Barney 1976, Nambiar & Zed 1980).

In conclusion, we found that early spring photosynthesis resulted in an accumulation of starch in needles and roots of white spruce bareroot seedlings in the nursery. The high carbohydrate content did not significantly affect seedling root growth potential, the timing of bud flush and terminal shoot elongation during the first growing season. However, seedlings with high carbohydrate levels produced more roots after planting in the forest and had higher rates of shoot growth in the second year following planting.

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Table 3.1 Shoot water content (WC), water potential (WP), net assimilation (NA), stomatal conductance (g_s), and water use efficiency (WUE) of white spruce seedlings. Measurements were taken in seedlings grown in nursery beds on April 18, 1994, before spring-lifting. Means (n=6) ± SE are shown. Means followed by different letters indicate statistically significant differences at 0.05 level determined by t-test. SC, spring-lifted seedlings covered from March 25 to April 18, 1994; SU, spring-lifted uncovered seedlings.

Treatment	WC (%DW)	WP (MPa)	NA (μmol m^{-2}s^{-1})	g_s (mmol m^{-2} s^{-1})	WUE (μmol CO_2 mmol H_2O^{-1})
SC	112 ± 3 (a)	-0.97 ± 0.02 (a)	8.74 ± 0.37 (a)	106 ± 5 (a)	7.76 ± 0.18 (a)
SU	104 ± 2 (b)	-1.04 ± 0.02 (b)	8.16 ± 0.44 (a)	143 ± 8 (b)	6.16 ± 0.29 (b)

Table 3.2 ANOVA – probability values for sugars and starch in needles and roots of spring-lifted white spruce bareroot seedlings.

	Needles		Roots	
Sources	Sugars	Starch	Sugars	Starch
Treatment (T)	0.0001	0.0001	0.5752	0.0003
Date (D)	0.0001	0.0001	0.0001	0.0001
T*D	0.1947	0.0001	0.0007	0.0020

Table 3.3 ANOVA – probability values for sugars and starch in needles and roots of fall-lifted white spruce bareroot seedlings.

	Needles		Roots	
Sources	Sugars	Starch	Sugars	Starch
Treatment (T)	0.6637	0.0001	0.0001	0.0001
Date (D)	0.1081	0.0001	0.1960	0.0013
T*D	0.0595	0.0001	0.0280	0.0035

Table 3.4 ANOVA – probability values for terminal bud flushing of spring-lifted white spruce bareroot seedlings following planting in the forest.

Sources	Terminal bud flushing	
	1994	1995
Treatment	0.0086	0.7121

Table 3.5 ANOVA – probability values for new root growth of spring-lifted white spruce bareroot seedlings following planting in the forest.

Sources	New roots
Treatment (T)	0.0001
Date (D)	0.0001
T*D	0.5736

Table 3.6 ANOVA – probability values for terminal shoot elongation of spring-lifted white spruce bareroot seedlings following planting in the forest.

Sources	Terminal shoot length
Site	0.0827
Treatment (T)	0.0020
Year (Y)	0.0001
T*Y	0.0111

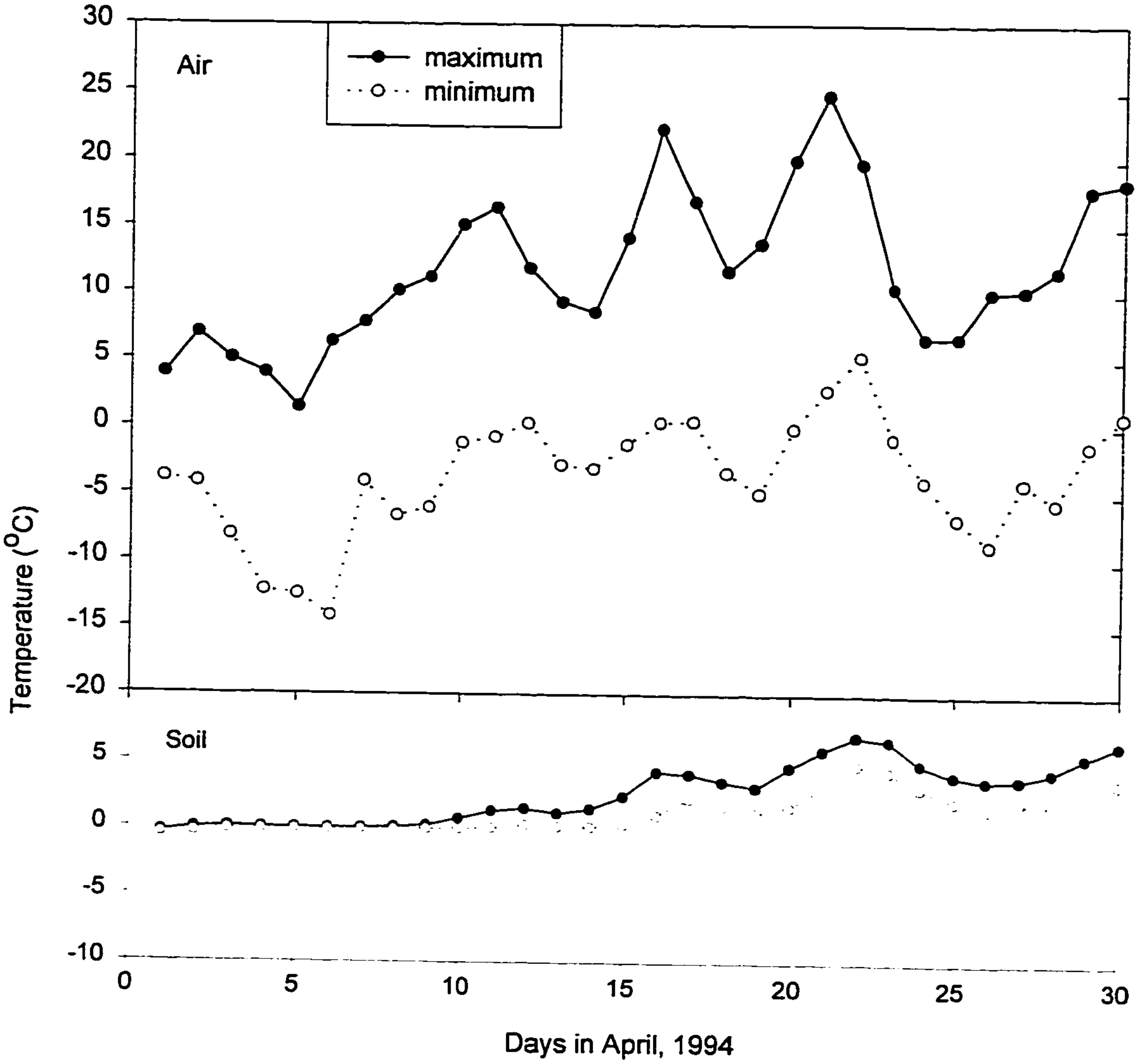

Figure 3.1 The maximum and minimum air and soil temperatures in April, 1994, in the Pine Ridge Forest Nursery, Smoky Lake, Alberta, Canada. Data were obtained from the Pine Ridge Forest Nursery Weather Station.

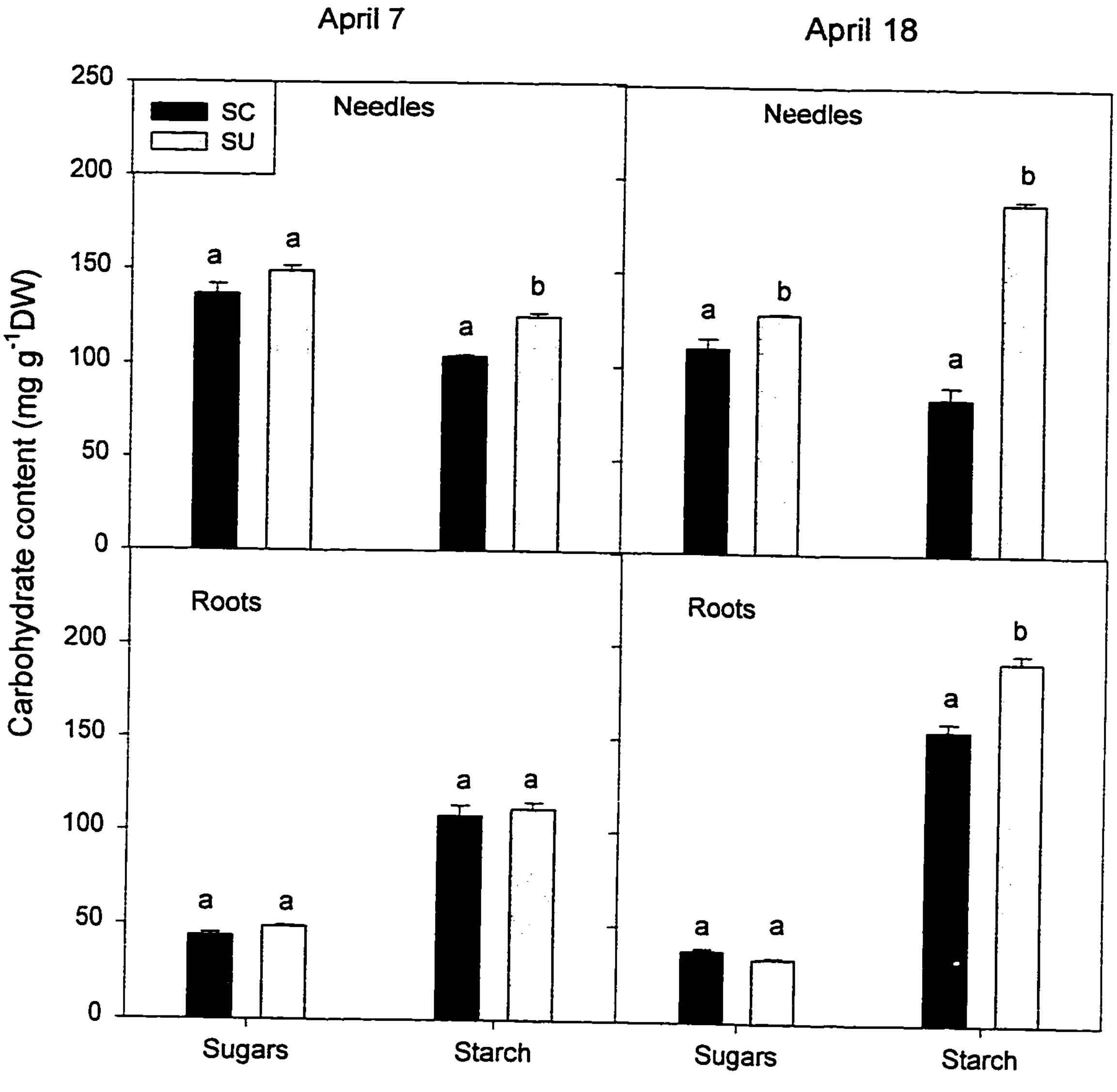

Figure 3.2 Contents of sugars and starch measured on April 7 and 18, 1994, in needles and roots of uncovered (SU) and covered (SC) spring-lifted white spruce seedlings. Bars are means (n=6) + SE. Different letters indicate statistically significant differences at p = 0.05 as determined by t-test.

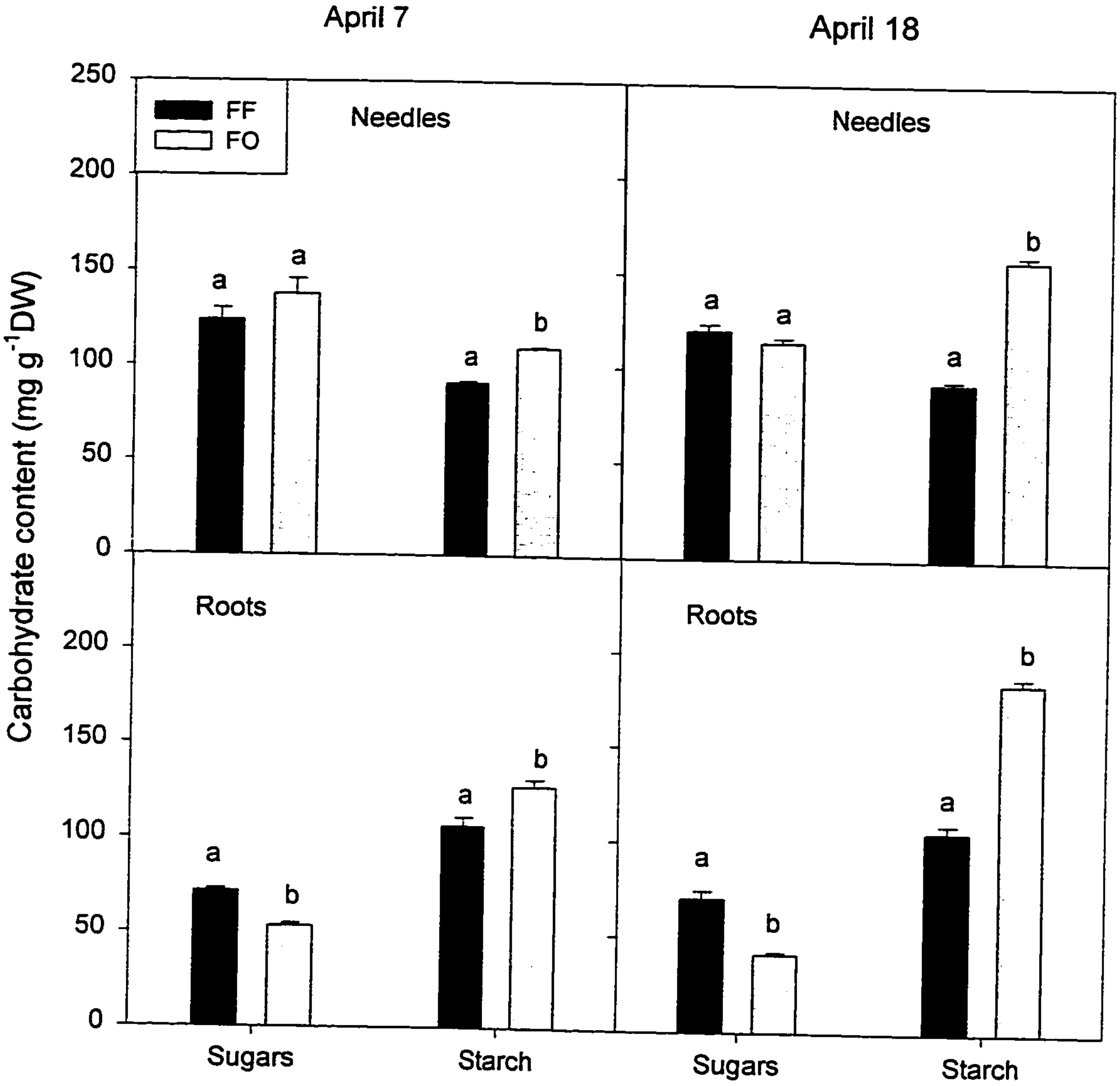

Figure 3.3 Contents of sugars and starch measured on April 7 and 18, 1994, in needles and roots of fall-lifted white spruce seedlings placed outside (FO) and in frozen-storage (FF) in early spring. Bars are means (n = 6) + SE. Different letters indicate statistically significant differences at p = 0.05 as determined by t-test.

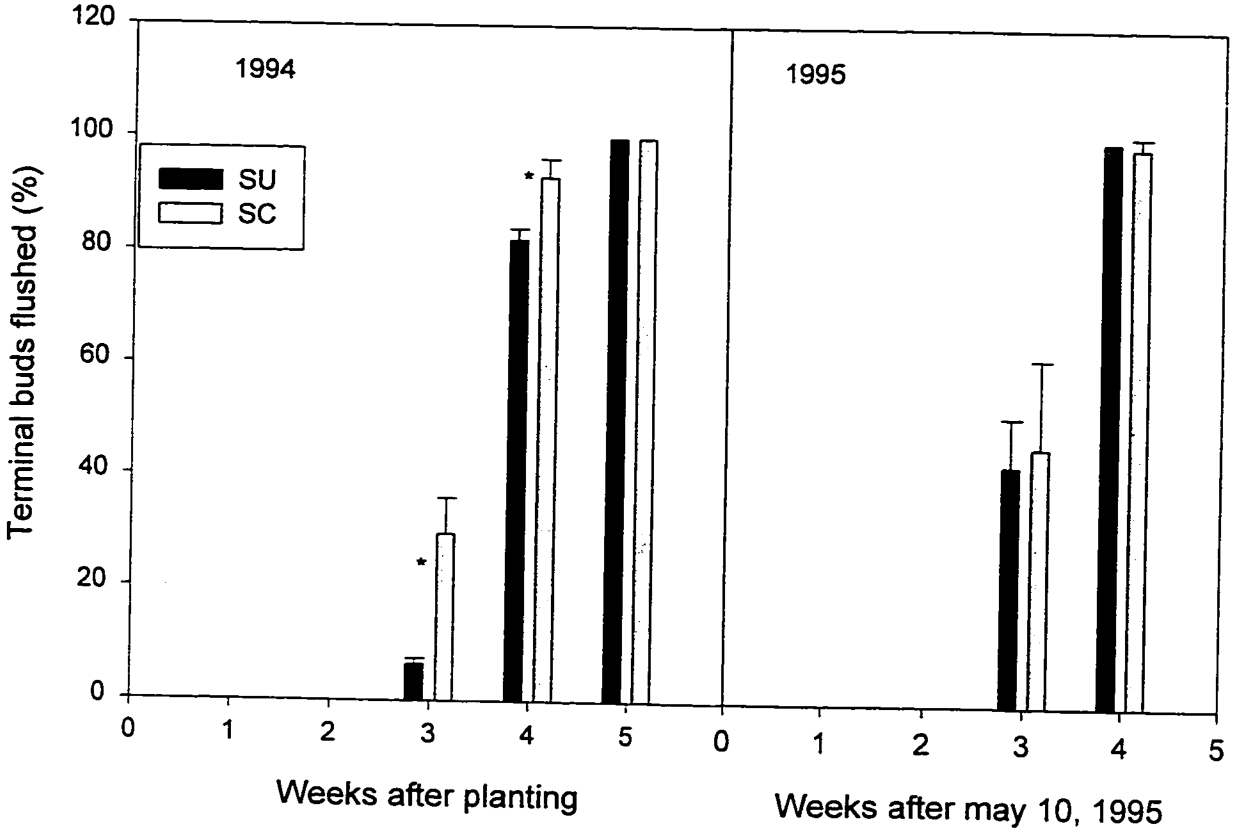

Figure 3.4 Terminal bud flush in white spruce seedlings (Means (n = 80) + SE) following planting in the forest in 1994 and 1995. SC, spring-lifted covered seedlings; SU, spring-lifted uncovered seedlings. * indicates significant difference between covered and uncovered treatments as determined by t-test at $p = 0.05$.

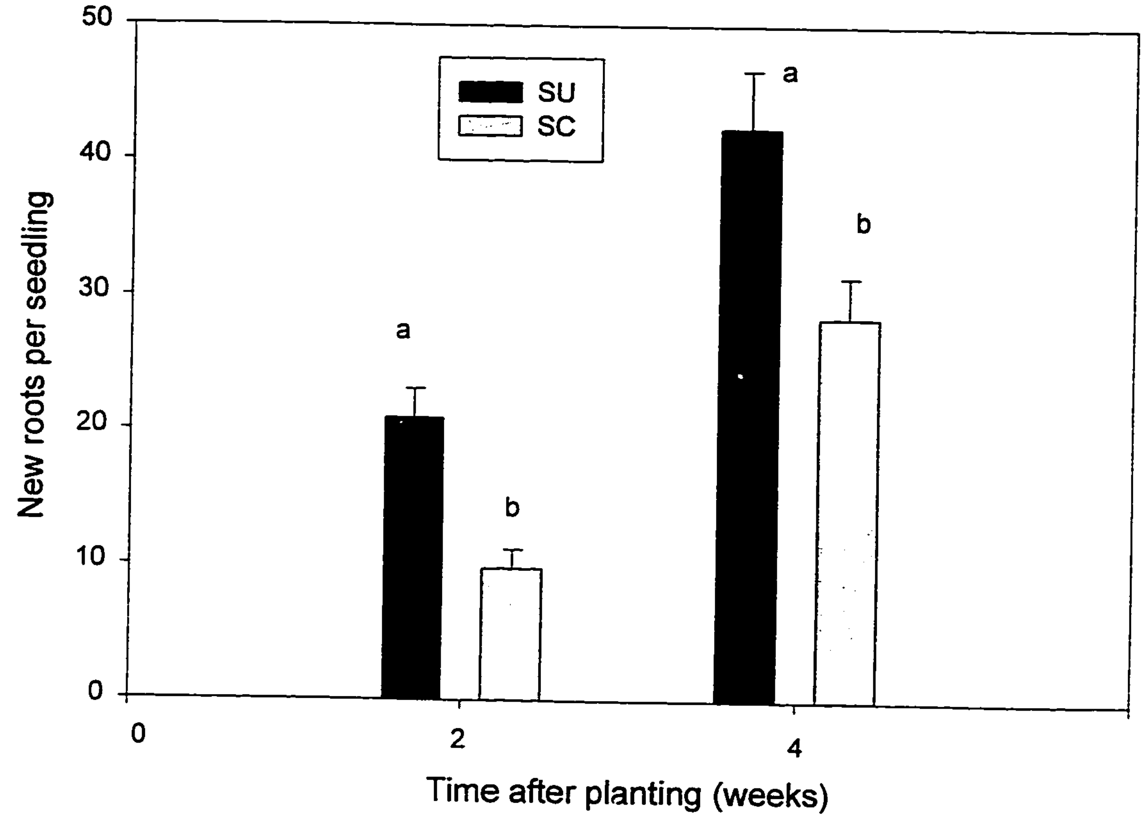

Figure 3.5 New roots > 10 mm produced by seedlings two and four weeks after planting in the forest. Bars are means (n = 20) + SE. Different letters indicate statistically significant differences at p = 0.05 as determined by t-test. SC, spring-lifted covered seedlings; SU, spring-lifted uncovered seedlings.

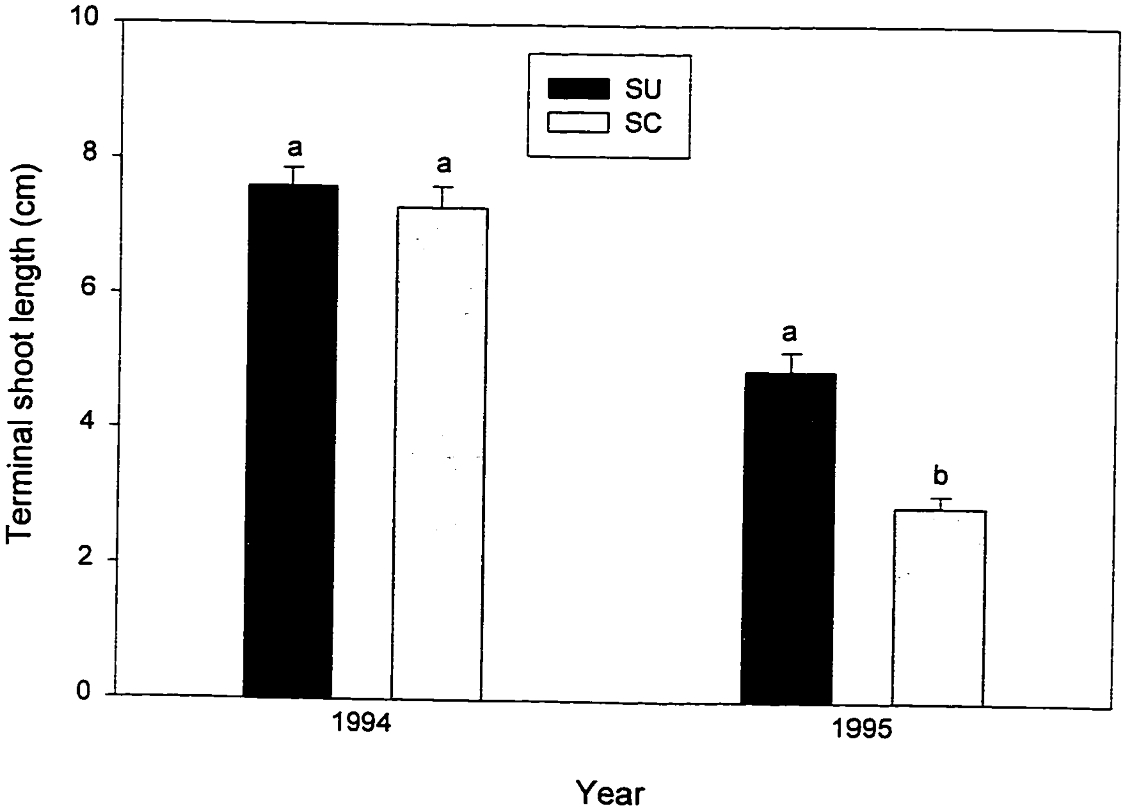

Figure 3.6 The first and second year terminal shoot elongation in white spruce seedlings planted in the forest. Means (n = 80) + SE are shown. Different letters indicate statistically significant differences at p = 0.05 as determined by t-test. SC, spring-lifted covered seedlings; SU, spring-lifted uncovered seedlings.

Chapter IV

Spring Changes in Physiological Characteristics of White Spruce Bareroot Seedlings [1]

4.1 INTRODUCTION

In Alberta, white spruce bareroot seedlings are usually lifted from nursery beds either in fall or in spring. Fall-lifted seedlings are stored frozen until spring planting. Spring-lifted seedlings are planted immediately or stored for a short time. It has been documented that spring-lifted white spruce seedlings have higher starch content, higher rates of shoot and root growth and net assimilation, earlier bud flushing, and are more drought resistant than fall-lifted plants (Jiang et al. 1994, 1995). These characteristics of spring-lifted seedlings may contribute to their quick growth resumption following planting (Turner and Jones 1983, Becker et al. 1987, McCreary and Duryea 1987, Burdett 1990, Jiang et al. 1994). However, in spring, seedlings undergo developmental changes from the state of dormancy to active growth. Non-dormant seedlings are not suitable for early planting in areas that are susceptible to spring frost (Jiang et al. 1994). To extend the planting season, forest tree nurseries usually store spring-lifted seedlings at above-zero temperatures, but prolonged storage at these temperatures may result in molding and bud flushing. These problems could be alleviated by lowering storage temperature. The tolerance to low temperature and prolonged storage depends on physiological characteristics of the seedlings at lifting. However, better understanding of seedling physiological characteristics in early spring is required to optimize lifting time, storage and field operations. The objective of this study was to improve the understanding of seedling physiology in spring. We examined changes of water relations, gas exchange, and carbohydrate content and composition that occurred in spring in white spruce bareroot seedlings.

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[1] A version of this chapter has been submitted for publication. Wang, Y. and Zwiazek, J. J. 1998. Can. J. For. Res.

4.2 MATERIAL AND METHODS

4.2.1 Plant material and experimental design

White spruce [*Picea glauca* (Moench) Voss] seedlings used in this study were grown at the Pine Ridge Forest Nursery, Alberta, Canada. The seeds (seed-lot # 60-20-5-83 SW) were germinated and plants were grown for six months in containers in the greenhouse and then transplanted outside in nursery beds for another 1.5 years (P+1.5). The experiment started on March 30, 1995, when snow had melted, and ended on May 11, 1995, prior to bud swelling but when roots just started to grow. Midday shoot water potentials (ψ) and gas exchange were measured weekly in seedlings grown in nursery beds. Seedlings for shoot pressure-volume (P-V) and needle carbohydrate analysis were collected on each measurement day. Air and soil temperatures for this period were obtained from the Pine Ridge Forest Nursery Weather Station.

4.2.2 Measurements

4.2.2.1 Water relations

Midday shoot ψ were measured using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Seven seedlings were randomly selected for the measurements. After the measurements, 10 seedlings were lifted, placed in plastic bags, and brought to the University of Alberta for the P-V analyses. Seedlings were placed in covered beakers with roots submerged in water and stored overnight at 4°C in the dark to rehydrate. The P-V curves were constructed using the free transpiration method (Hinckley et al. 1980). The shoots were used for P-V curves only when initial shoot ψ was higher than -0.3 MPa. Turgid mass (TM) of shoots was determined immediately following the first measurement and the shoots were allowed to dry at the room temperature. Parallel shoot ψ and fresh mass (FM) were determined at regular intervals. After the last measurement, shoots were dried at 70°C for 48 hours to determine dry mass (DM).

Relative water content (RWC) of each shoot was calculated as:

$$RWC = (FM - DM)/(TM - DM) \qquad (4.1)$$

The P-V curves were plotted as $1/\psi$ vs. $1\text{-}RWC$ (Fig. 4.1).

The osmotic potentials at the full turgor (π_{100}) and at the turgor loss point (π_0) were derived from the points on the linear part of the P-V curves. The data in the linear region were fitted by the least-square method with the linear regression model:

$$1/\psi = a\,(1-RWC) + b \qquad (4.2)$$

The y-intercept of this regression gave an estimate of reciprocal of π_{100}. The turgor loss points were visually estimated from P-V curves to identify RWC at turgor loss point (RWC_{tlp}) and used to estimate π_0 with equation (4.2). The relative water contents in the symplast (RWC_s) and in the apoplast (RWC_a) were obtained from the x-intercept of the regression equation (4.2). The absolute apoplastic water contents (WCa) and symplastic water contents (WCs) were calculated as:

$$WCa = RWC_a(TM - DM)/DM \qquad (4.3)$$

$$WCs = RWC_s(TM - DM)/DM \qquad (4.4)$$

The bulk modulus of cell wall elasticity (ε) was calculated following Roberts et al. (1980) as

$$\varepsilon = (\psi_{P1} - \psi_{P2}) / ((RWC_{s1} - RWC_{s2}) / RWC_s) \qquad (4.5)$$

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 entire range of turgor.

4.2.2.2 Gas exchange

Net assimilation (NA), transpiration (E), and stomatal conductance (g_s) were measured using an infrared gas analyzer LCA-4 (Analytical Development Company Ltd., Hoddesdon, Hertfordshire, UK). Seven seedlings were randomly selected on each measurement day. The measurements were conducted between 12:00 and 2:00 PM on the uppermost lateral shoots of same seedlings for measuring shoot ψ. During cloudy days, light was supplemented to 1000 μmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) using a quartz halogen lamp attached to the leaf chamber. Projected needle areas were measured using a LI 3000 leaf area meter (LICOR Instruments Co., Lincoln, NB). Mesophyll conductance (g_{mes}) was calculated as

$$g_{mes} = NA/C_i \qquad (4.6)$$

where C_i is the intercellular CO_2 concentration.

4.2.2.3 Carbohydrate analysis

Needle carbohydrates were analyzed in 5 randomly selected seedlings. The needles were freeze-dried for 48 hours and sugars were extracted with boiling 85% ethanol for 10 min (0.2 g needles per sample). The extracts were filtered through Whatman No.1 filter paper, and the residues extracted twice with 85% ethanol. The filtered extracts were combined, reduced to the water phase in a vacuum evaporator at 40°C and made up to 20 ml with distilled water. Sugars were colorimetrically measured using the anthrone method (Ashwell 1957). The residues were freeze-dried and used for starch analysis. Starch was hydrolyzed with perchloric acid (Rose et al. 1991) and quantified as glucose equivalents using the anthrone method (Ashwell 1957).

To determine the composition of soluble carbohydrates, 1 ml of the filtered sugar extracts were freeze-dried and the trimethylsilyl (TMS) derivatives of carbohydrates were prepared by dissolving the residues in 0.5 ml pyridine followed by adding 0.1 ml trimethylchlorosilane and 0.1 ml hexamethyldisilazane (Zwiazek and Shay 1988). After 20-26 h, TMS ethers of carbohydrates were analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a model 3396A electronic integrator (Hewlett Packard, Mississugua, ON, Canada). Samples were separated on a 30-m-long, 0.25-mm internal diameter fused silica DB-5 capillary column, 0.25-μm film thickness (J & W Scientific, Folsom, CA, USA). Conditions during the run were as follow: initial oven temperature 180°C, increased to 210°C at the rate of 2°C/min and then from 210°C to 275°C at the rate of 5°C/min and held for 30 min at 275°C; injector and flame ionization detector temperatures 300°C; carrier gas (helium) flow rate 25 ml/min (Tholakalabavi et al. 1994). Carbohydrates were identified by co-chromatography with authentic carbohydrate standards obtained from Sigma and BDH and quantified using manitol as an internal standard.

4.2.3 Statistical analysis

The data were analyzed by analysis of variance (one-way ANOVA) using SAS statistical software package (SAS 1995) with measurement date as the main effect.

Duncan's test was used for a comparison of means with significance set at the $p = 0.05$ level. Linear regressions between E and soil temperature were done using REG procedure.

4.3 RESULTS

4.3.1 Water relations

In early April, air temperature showed large fluctuations with the maximum temperature above 10°C and the minimum reaching -20°C. However, soil temperature was relatively stable and remained below 0°C until April 10, 1995 (Fig. 4.2). Between March 30 and April 6, midday RWC remained unchanged, but midday shoot Ψ decreased from -1.20 ± 0.05 MPa ($n = 6 \pm$ SE) to -1.56 ± 0.15 MPa (Fig. 4.3). From April 6 to May 11, midday RWC gradually increased from 89% to 95%. The midday shoot Ψ increased from -1.56 ± 0.15 on April 6 to -1.22 ± 0.09 MPa on April 21 and remained relatively constant thereafter (Fig. 4.3).

Shoot π_{100} and π_0 decreased from -1.76 MPa and -2.65 MPa, respectively, on March 30 to -2.49 MPa and -3.34 MPa on April 27. Both values increased to -1.88 MPa (π_{100}) and -2.55 MPa (π_0) on May 4 (Table 4.1). Shoot RWC_{tlp} remained relatively constant throughout April, ranging from 75.7% to 77.3%. In May, RWC_{tlp} were significantly higher and measured 84.9% on May 4 and 81.1% on May 11 (Table 4.1). Seedlings shoots had high WCs between April 13 and April 27. However, in May, WCs declined (Table 4.1). Shoot ε_{max} was low on March 30 and April 6 and measured 7.29 ± 0.24 MPa and 6.97 ± 0.59 MPa, respectively, indicating relatively elastic cell walls. After April 6, ε_{max} dramatically increased and reached 15.04 ± 2.39 MPa on April 27 (Table 4.1), suggesting a decrease in cell wall elasticity. The increase of ε_{max} measured after April 27 was not statistically significant.

4.3.2 Gas exchange

Positive values of NA, g_s and g_{mes} were recorded in late March and early April (Fig. 4.4) when the maximum soil temperature was still below zero (Fig. 4.2). NA sharply increased from April 6 to April 27 and then slightly decreased. The rates of g_{mes} followed the same pattern as NA (Fig. 4.4). Stomatal conductance was near zero on March 30 and remained relatively constant throughout April. A large increase in g_s was measured in late April and early May (Fig. 4.4). Transpiration increased gradually in April and early May (Fig. 4.5) and showed a linear relationship with the soil temperature ($r^2 = 0.84$) (Fig. 4.5).

4.3.3 Carbohydrates

The levels of total sugars in needles were relatively constant in April and early May (Fig. 4.6A). The starch content of needles increased from April 6 to May 11 (Fig. 4.6A). Sucrose, the major soluble sugar in needles, increased from March 30 to May 11 while raffinose decreased from about 20 mg g^{-1} DW tissue on March 30 to undetectable level in May (Fig. 4.6B). Glucuronic acid declined from about 33 mg g^{-1} DW tissue on March 30 to 12 mg g^{-1} DW tissue on May 11 and glucose decreased from about 20 mg g^{-1} DW tissue on March 30 to less than 4 mg g^{-1} DW tissue on April 27. Similar patterns to those of glucose were measured for fructose and galactose (Fig. 4.6B).

4.4 DISCUSSION

4.4.1 Water relations

Water relations of woody plants fluctuate seasonally and drastically change during bud burst, shoot elongation, and bud set (Tyree et al. 1978, Ritchie and Shula 1984, Doi et al. 1986, Kubiske and Abrams 1991, Colombo and Teng 1992). In the present study, major changes in seedling water relations occurred during the short period in early spring preceding bud flush and shoot growth. In the field, the gradual increase of midday RWC was likely due to the uptake of water from the soil by seedlings when the soil temperature increased above 0°C (Figs. 4.2 & 4.3). However, changes in midday shoot Ψ did not follow the same pattern as RWC, reflecting changes in shoot π and Ψ_p.

Shoot π_{100} and π_0 gradually decreased in white spruce seedlings throughout April (Table 4.1) when air and soil temperatures were increasing (Fig. 4.2). In the absence of growth, photosynthesis may result in solute accumulation (Turner and Jones 1980, Ritchie and Shula 1984, Teskey et al. 1984, Abrams 1988). Soluble carbohydrates are primarily responsible for osmotic adjustment in plants (Jones et al. 1980, Ackerson and

Herbert 1981, Borowizka 1981, Zwiazek and Blake 1990), however, in the present study spring photosynthates did not increase sugar levels in needles (Fig. 4.6A). It is posssible that osmotic potentials were affected by other solutes such as amino acids (Zwiazek and Blake 1990). Early spring photosynthates were rapidly incorporated into cell wall materials in Scots pine (*Pinus sylvestris*) (Hansen et al. 1996). Changes in cell wall composition regulate ε (Hansen et al. 1996, Renault and Zwiazek 1997). The cell walls of one-year-old conifer needles usually become less elastic before shoot elongation (Colombo 1987, Grossnickle 1989, Colombo and Teng 1992, Renault and Zwiazek 1997). This is consistent with our results that showed a decrease in cell wall elasticity from early to late April (Table 4.1).

Successful adaptation of plants to water stress involves the maintenance of turgor, which, in turn, is largely determined by osmotic potentials and cell wall elasticity (Jones and Turner 1978, Kikuta and Richter 1986, Ritchie and Shula 1984). Shoot π_{100} and π_0 decreased in April with a corresponding decrease in RWC_{tlp} (Table 4.1). The low π_{100} and π_0 and RWCtlp may be associated with high turgor maintenance capacity (Jane and Green 1983). A complex relationship exists between turgor maintenance capacity and cell wall elasticity is complex. Plant cells with elastic walls lose large volumes of symplastic water with a small decline in turgor while maintaining high physiological activity (Ritchie and Shula 1984, Grossnickle 1988). On the contrary, plants with rigid cell walls have a large decrease of water potential with small decrease of RWC. The decline in ψ helps plants absorb water from the soil. In our study, seedlings reduced π_{100} and π_0, εl and RWC_{tlp} in late April. Therefore, the seedlings improved their dehydration avoidance. In May, the seedlings had high π_{100} and π_0 and RWC_{tlp} and therefore were likely more susceptible to drought stress.

4.4.2 Gas exchange

Air temperature is not the sole cause of winter photosynthesis inhibition in northern conifers (Jurik et al. 1988). Low soil temperature limits photosynthesis via stomatal and nonstomatal effects (Turner and Jarvis 1975, Delucia 1986). In our study, the air temperature in late March and early April measured as high as 10°C, but the NA remained relatively low. Since the soil was still frozen, the low photosynthetic rates observed in early April could be due to the effects of low soil temperature on photosynthesis. Most field studies have shown that photosynthetic activity of conifers gradually increases in spring, over the course of a month or two (McGregor and Kramer 1963, Bamberg et al. 1967). However, a rapid recovery of photosynthesis in spring was also reported in eastern white pine (*Pinus strobus* L.) (Jurik et al 1988). In the present study, photosynthesis recovered rapidly from middle to late April (Fig. 4.4), at the time when bareroot seedlings are usually lifted from nursery beds. The recovery of photosynthesis resulted in the accumulation of starch in needles (Fig. 4.6). On May 4 and May 11, the low photosynthetic rates could be due to low g_s and (or) g_{mes}. Also, the high temperatures inside the leaf chamber reached 26°C and 29°C, respectively. These temperatures are above the optimum photosynthetic temperature for white spruce seedlings (Man & Lieffers 1997).

Soil temperature affects the rate of transpiration by altering root conductivity. In cold soil, water uptake is also reduced as the water has a higher viscosity (Kaufmann 1977, Grossnickle and Blake 1985). The increased transpiration rates observed in May likely coincided with the increased seedling hydraulic conductivity as shoot water potentials remained unchanged.

4.4.3 Carbohydrates

Photosynthesis resulted in a rapid accumulation of starch in needles in mid-April (Fig. 4.6A). This increase in starch content suggests that the seedlings were accumulating energy reserves to prepare for growth. Similar observations were recorded for red pine (*Pinus resinosa* A.) (Pomeroy et al. 1970), balsam fir (*Abies balsamea*)(Little 1970a, b), and Scots pine (*Pinus sylvestris*) (Fischer and Holl 1991).

Needle total sugar levels did not change before bud flushing, however, we observed an increase in sucrose and a decrease in other soluble carbohydrates (Fig. 4.6B). Sucrose is the principal translocatable carbohydrate in plants, therefore, the increase in sucrose could be due to an increase in sugar transport processes. Similarly, Fischer and Holl (1991) found that in Scots pine, sucrose content increased and that of other sugars decreased. In our study, raffinose, which is thought to play a role in cold hardiness (Kandler and Hopf 1980, Caffery et al. 1988), decreased in white spruce seedlings from about 20 mg g^{-1} DW in early April to zero in May. The decrease in raffinose may indicate that the seedlings no longer needed protection against cold as the air and soil temperatures increased in May. Changes in sugar composition may reflect the differences in metabolic activities, therefore, future studies should examine the possibility of using specific carbohydrates as indicators of an optimum lifting time.

In conclusion, the results of our study showed that rapid changes in water relations, gas exchange and carbohydrates were taking place in white spruce seedlings in early spring. In late April, seedlings developed relatively low π_{100} and π_0, RWC$_{tlp}$, and high ε and RWCs. Photosynthesis recovered rapidly in April and resulted in an increase of starch in needles. Total sugar levels did not change, but the composition of soluble carbohydrates was altered before bud flushing. These changes took place in April in the present study. However, more work is required to relate these changes to environmental factors.

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Table 4.1. Water relations of white spruce seedlings grown in nursery beds at the Pine Ridge Forest Nursery, Smoky Lake, Alberta. Means (n=7) ± SE are shown. Means followed by different letters indicate significant difference at p=0.05 determined by the Duncan's test. $\pi_{0.}$ osmotic potential at turgor loss point; π_{100}, osmotic potential at full turgor; $\varepsilon_{max.}$ modulus of maximum cell wall elasticity; $RWC_{tlp.}$ relative water content at turgor loss point; WCa, apoplastic water content; WCs, symplastic water content.

Date	π_{100} (MPa)	π_0 (MPa)	RWC_{tlp} (%)	ε_{max} (MPa)	WCa (g H_2O g $^{-1}$ DW)	WCs (g H_2O g^{-1}DW)
Mar. 30	-1.76±0.08 a	-2.65±0.14 a	76.8±1.2 a	7.29±0.24 a	0.306±0.038 a	0.808±0.072 a
Apr. 6	-2.05±0.15 b	-2.93±0.18 b	77.3±1.5 a	6.97±0.59 a	0.205±0.089ab	0.883±0.118 a
Apr. 13	-2.22±0.04 b	-3.00±0.04 b	77.2±1.3 a	9.23±0.63 b	0.149±0.041 b	1.017±0.022 b
Apr. 21	-2.44±0.14 c	-3.21±0.13 c	76.2±1.2 a	13.07±0.85 c	0.195±0.047 b	1.044±0.087 b
Apr. 27	-2.49±0.08 c	-3.34±0.05 c	75.7±1.2 a	15.04±2.39cd	0.260±0.088 a	1.077±0.067 b
May 4	-1.88±0.03 a	-2.55±0.08 a	84.9±1.1 b	16.76±1.85 d	0.383±0.034 c	0.824±0.032 a
May 11	-1.97±0.04 b	-2.77±0.08 ab	81.1±1.1 b	17.25±1.79 d	0.446±0.031 c	0.833±0.034 a

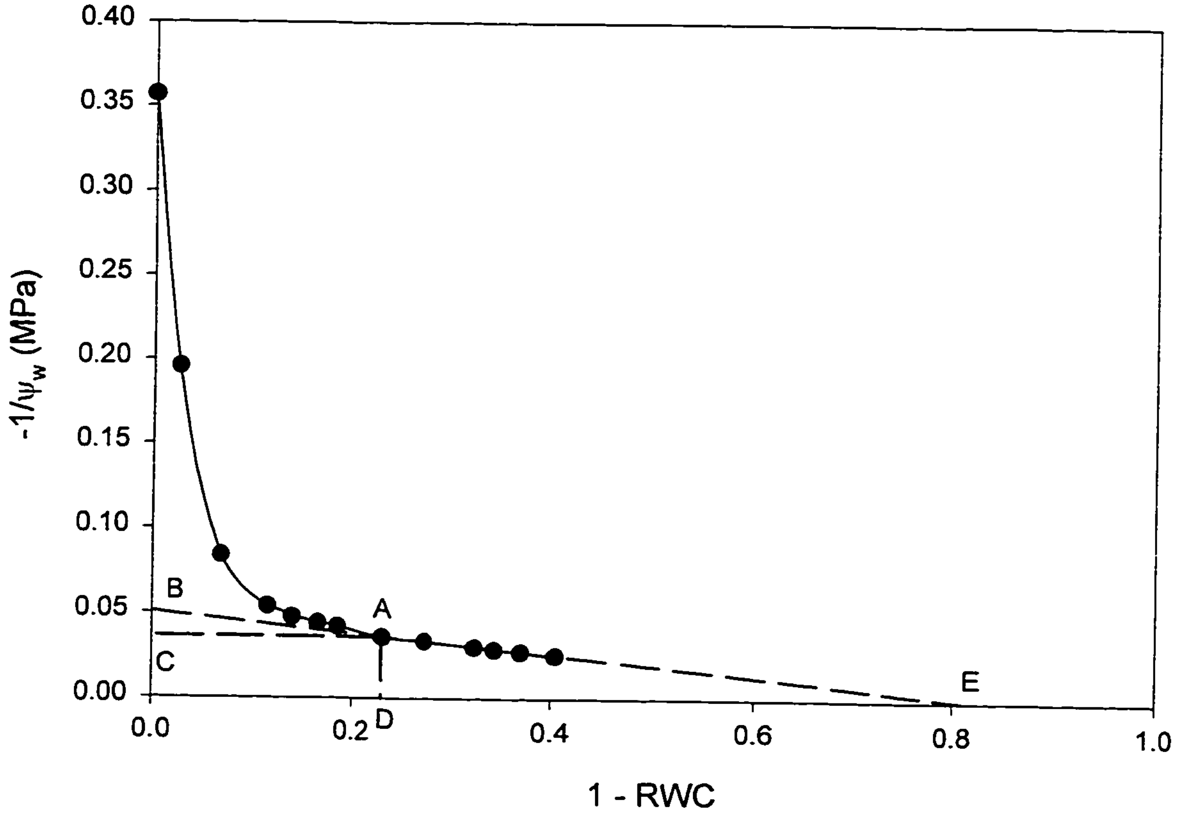

Figure 4.1 A typical Pressure-Volume (P-V) curve showing derivation of tissue-water relation parameters. A is the turgor loss point; B is the inverse of osmotic potential at full saturation; C is the inverse of osmotic potential at turgor loss point; D is the relative water content at turgor loss point; E is the original relative water content of osmotic water.

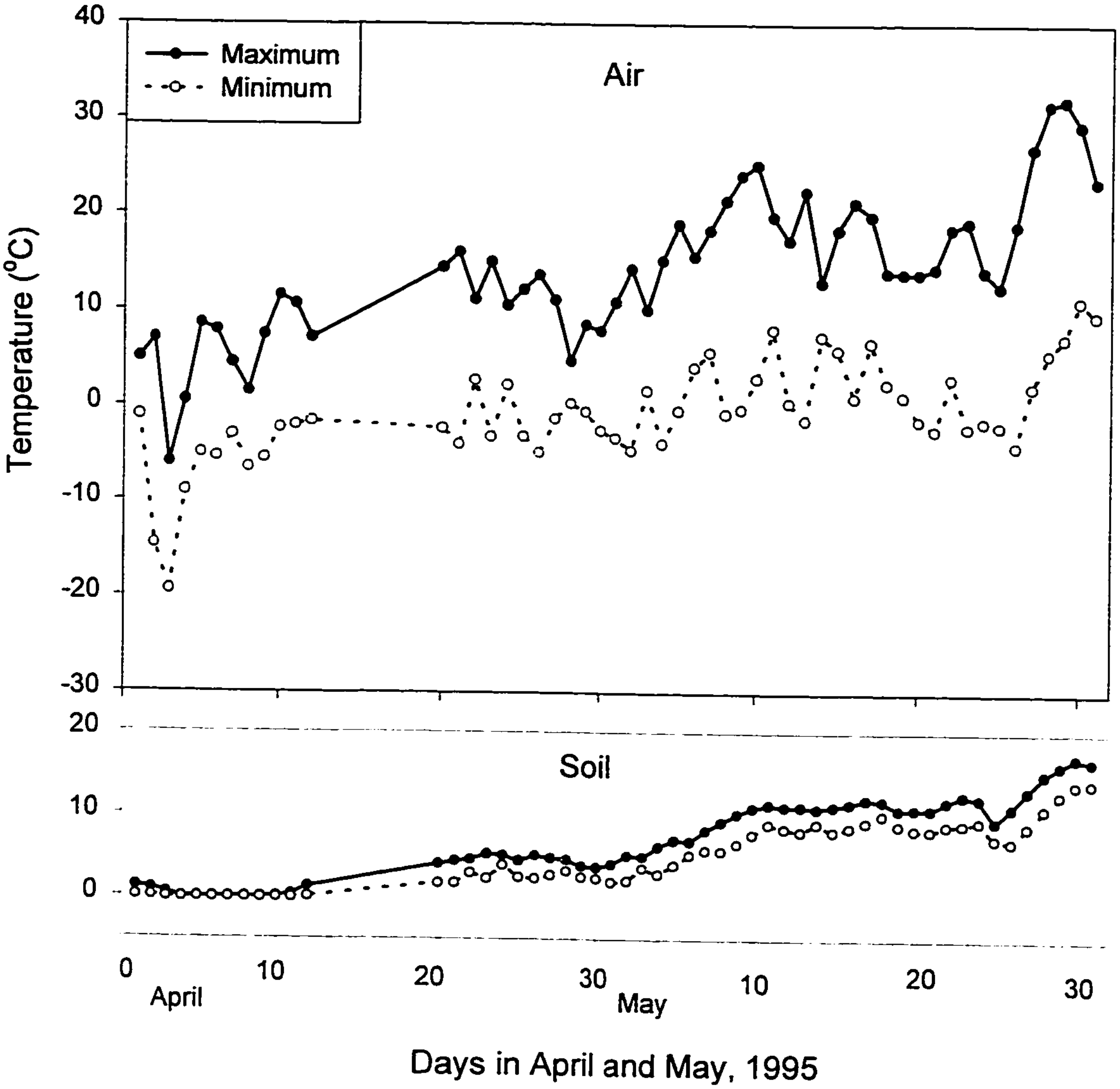

Figure 4.2 Maximum and minimum air and soil temperatures in April and May, 1995, at the Pine Ridge Forest Nursery, Smoky Lake, Alberta. Data were obtained from the Pine Ridge Forest Nursery Weather Station.

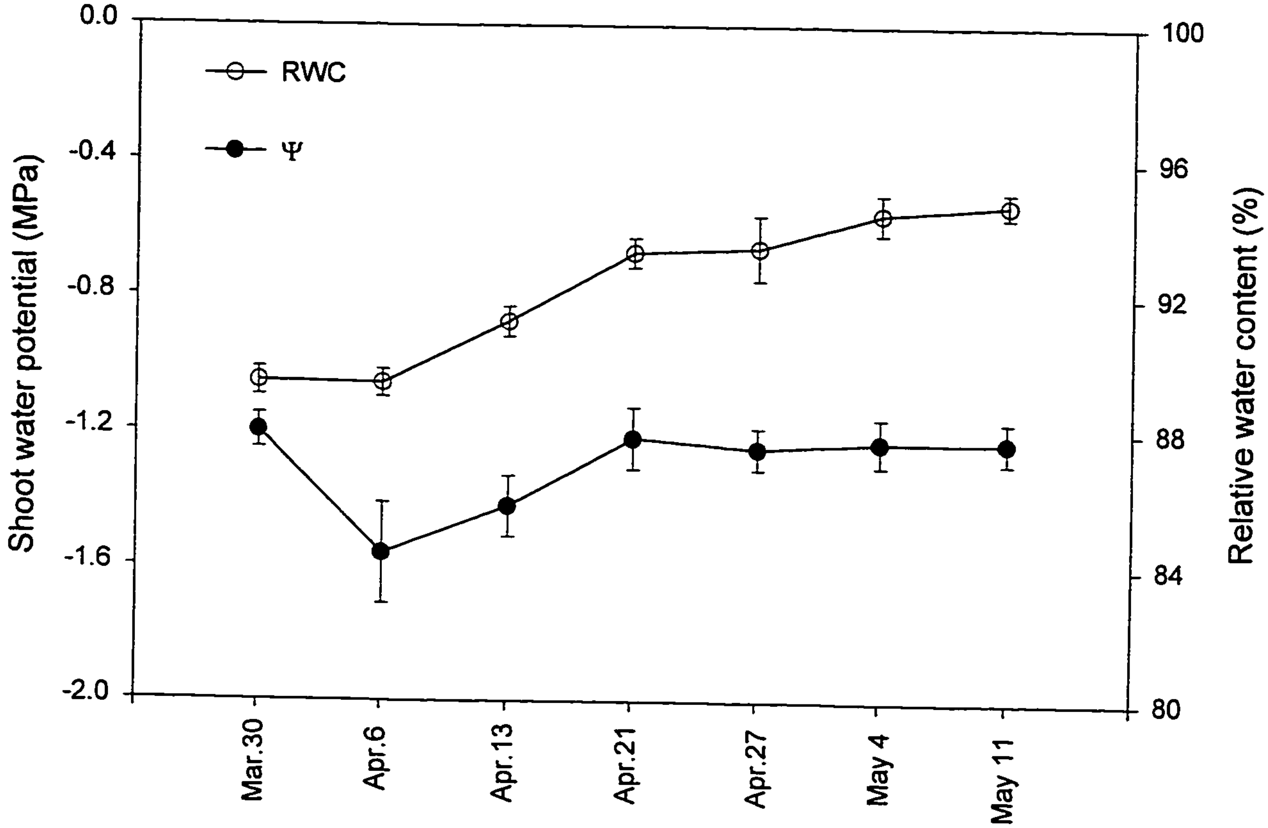

Figure 4.3 Midday shoot water potentials (ψ) and relative water contents (RWC) in white spruce seedlings in spring, 1995. Means (n=7) ± SE are shown.

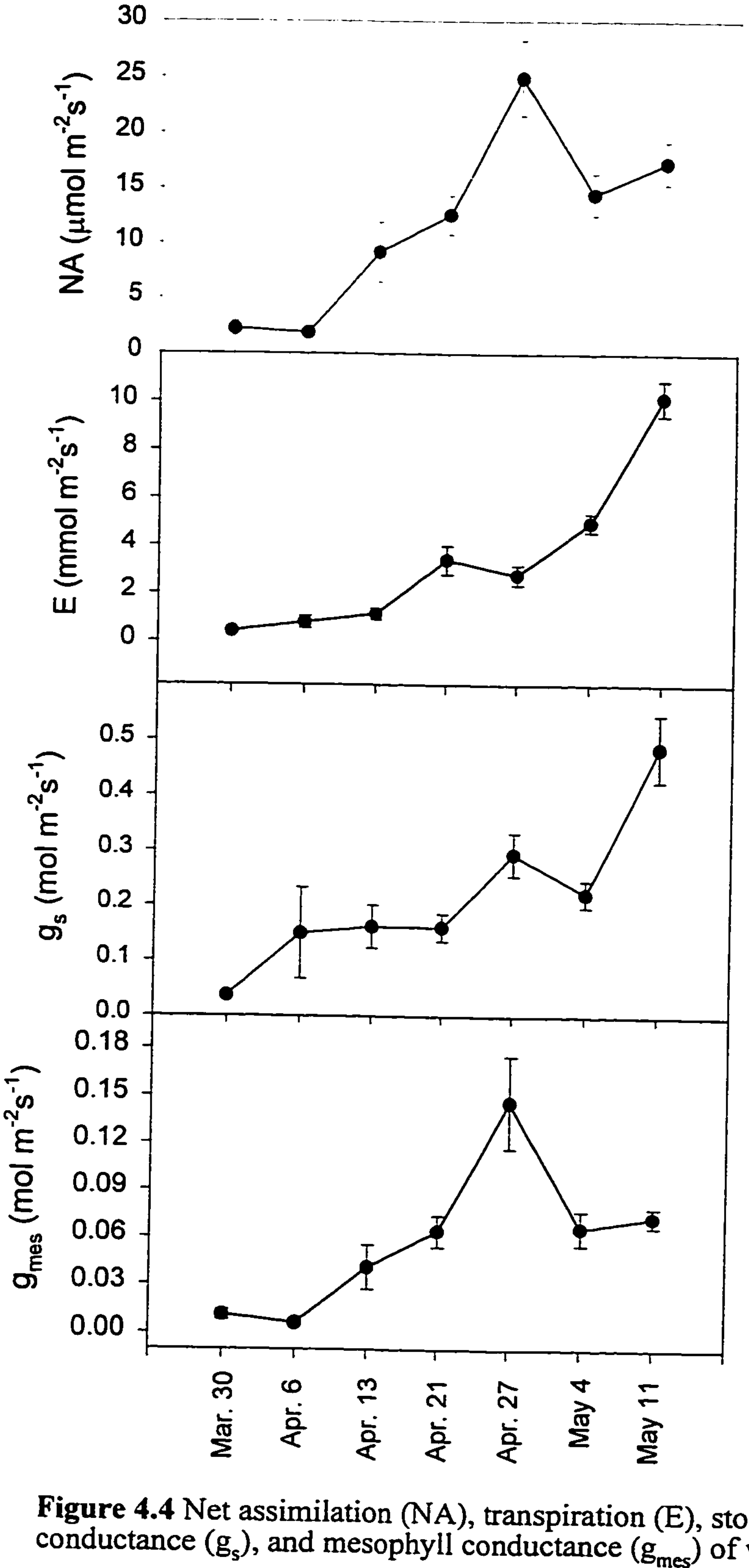

Figure 4.4 Net assimilation (NA), transpiration (E), stomatal conductance (g_s), and mesophyll conductance (g_{mes}) of white spruce seedlings. Measurements were taken in nursery beds in spring, 1995. Means (n = 7) ± SE are shown.

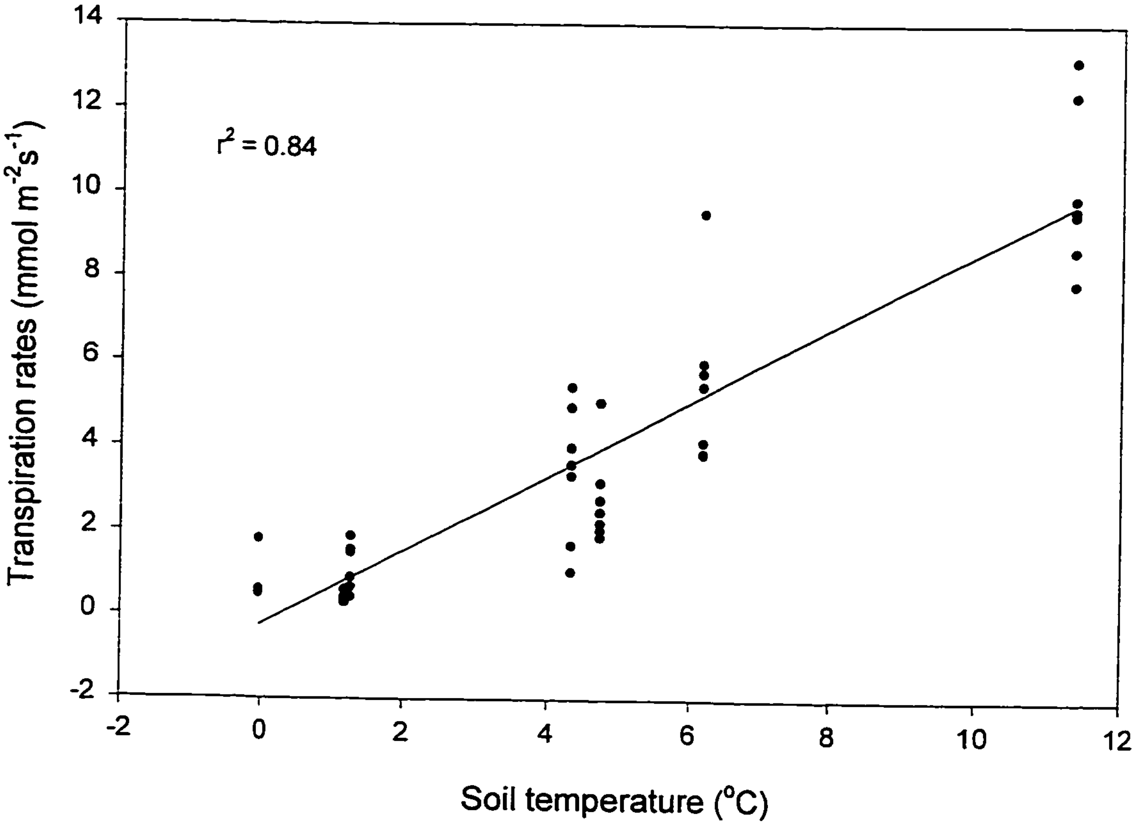

Figure 4.5 Relationship between soil temperature and transpiration of white spruce seedlings in spring, 1995. Transpiration rates were measured in nursery beds and soil temperatures were obtained from the Pine Ridge Forest Nursery Weather Station, Smoky Lake, Alberta.

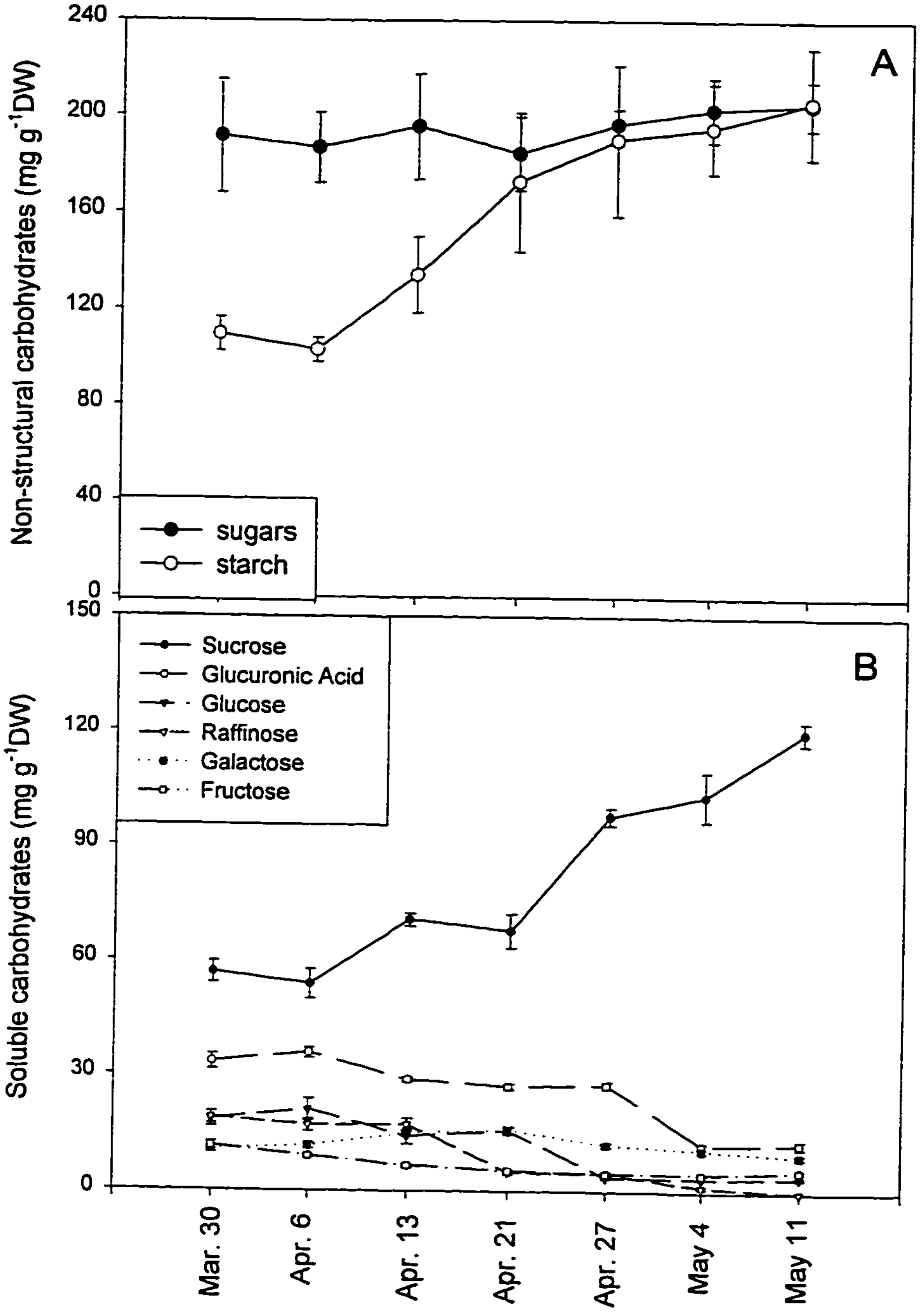

Figure 4.6 Contents of sugars and starch (A) and composition of major soluble carbohydrates (B) in needles of white spruce seedlings in spring, 1995. Means (n = 5) ± SE are shown.

Chapter V

Effects of Lifting Time, Storage Temperature and Storage Duration on Carbohydrate Content, Bud Flush, Shoot Growth and Root Growth Potential of Spring-Lifted White Spruce Bareroot Seedlings

5.1 INTRODUCTION

Cold storage is an important procedure in bareroot seedling production. The success of cold storage depends on seedling physiological status at the time of lifting and on storage environments (Camm et al. 1994). There are numerous studies that have examined effects of cold storage on fall-lifted seedlings (Hocking and Nyland 1971, Camm et al. 1994). However, in Alberta, white spruce bareroot seedlings are also lifted in spring. It has been documented that spring-lifted white spruce seedlings have higher rates of shoot and root growth, higher net assimilation rates and starch content, earlier bud flushing, and are more drought resistant than fall-lifted seedlings (Jiang et al. 1994, 1995). It may be advantageous to lift white spruce seedlings in spring, but spring-lifted seedlings are not suitable for early spring planting in areas susceptible to spring frost (Jiang et al. 1994). To extend the planting season, cold storage of spring-lifted seedling is also necessary. However, effects of low temperature storage on spring-lifted seedlings are largely unknown.

Cold storage has been found to affect seedling carbohydrate levels (Ritchie 1982, Cannell et al. 1990, Omi et al. 1994, Jiang et al. 1994), dormancy status (DeWald and Feret 1985, Ritchie et al. 1985, Rietveld 1989), root growth potential (Duryea and McClain 1984, Omi et al. 1994), gas exchange (Omi et al. 1991), and stress resistance (Ritchie 1982, Ritchie 1986, Faulconer 1988). The root growth potential (RGP) is considered to be an important estimate of seedling quality because it integrates numerous morphological and physiological factors. However, RGP tests do not reliably predict field performance (Simpson and Ritchie 1997) because they are carried out under optimum growth conditions that do not represent those in the field. Seedlings planted in the forest grow new roots and achieve positive carbon balance later compared with those planted in the greenhouse (Marshall 1985, Vapaavouri et al. 1992). The measurement of bud flush, shoot growth and carbohydrate content may be more reliable in evaluating seedling quality after cold storage. Bud flushing and shoot growth are strongly related to seedling water relations (Kaufmann 1968), which are altered by planting stress (Sands 1984, Caldwell et al. 1986, Sutton 1995). Reserve carbohydrates are important for the maintenance respiration and growth resumption following outplanting (McNabb 1985, van den Driessche 1987). Tissue carbohydrate content has been correlated with seedling field growth and survival of *Picea abies* (Venn 1980), *Pinus elliottii* (McNabb 1985) and *Pinus sylvestris* (Puttonen 1986).

In the present study, we hypothesized that lifting time, storage temperature and duration would affect seedling carbohydrate levels and, consequently, their post-planting performance. The principal objective was to examine the effects of lifting time, storage temperature and storage duration on carbohydrate levels, bud flushing, growth of terminal shoots and root growth potentials in spring-lifted white spruce bareroot seedlings.

5.2 MATERIAL AND METHODS

5.2.1 Plant material and experimental design

White spruce [*Picea glauca* (Monech) Voss] seedlings (seed lot # DP-88-SW) were grown at the Pine Ridge Forest Nursery, Smoky Lake, Alberta, Canada. The seeds were germinated and grown in a greenhouse for six months and then transplanted and grown in nursery beds for another 1.5 years. The seedlings were lifted on April 20 (designated A20 seedlings), April 27 (designated A27 seedlings), and May 4 (designated M4 seedlings), 1994, and were packed in cardboard boxes and placed in storage at -2°C and at 4°C at the University of Alberta. After 3, 5, 7, 9, and 11 weeks, seedlings were removed from each storage and planted in two forest sites near Devon (53°22' N, 113°45' W), Alberta. The planting sites were 20-year-old aspen and white spruce mixed-wood forest and were located within a 2-km distance. The major shrub species in the planting sites, *Rosa acicularis* L., was sparsely scattered on both sites. The dominant grasses were *Bromus inermis ssp pumpellianus* and *Agropyron trachycaulum* (Link) Mailte. The annual precipitation from 1964 to 1994 averaged 468 mm, with June and July being the wettest months. The average slope on both sides was less than 2% (south-facing). The planting design was a completely randomized block design. Due to the cooler temperature control failure, some storage treatments were terminated early.

Root growth potentials were tested in the second experiment conducted in 1996, when the seedlings were lifted on April 20, April 27, and May 4, 1996. Following lifting, seedlings were stored at -2°C and 4°C. After 3, 6, 9, and 12 weeks of storage, seedlings were planted in 4-liter pots (one seedling per pot) containing a mixture of peat moss and sand (3:1 by volume). Seedlings were grown in the greenhouse at 23/18°C (day/night) temperature with 18-h photoperiod and were watered every three days. Twenty seedlings per treatment were planted. RGP was tested 4 weeks after planting.

5.2.2 Measurements

5.2.2.1 Carbohydrate contents

Five seedlings were randomly selected from each storage temperature after 3, 5, 7, 9, and 11 weeks of storage. The seedlings were thoroughly washed and roots and needles separated. The tissue was lyopholized for 48 hours and sugars were extracted from 0.2 g dry weight of needles and 0.4 g dry weight of roots for 10 min with 85% boiling ethanol. The extracts were filtered through Whatman No.1 filter paper, and the residues extracted two more times with 85% ethanol. The filtered extracts were combined and reduced to water phase in a vacuum evaporator at 40°C and made up to 20 ml with distilled water. Sugars were measured colorimetrically using the anthrone method (Ashwell 1957). The residues were freeze-dried and used for starch analysis. Starch was extracted with perchloric acid (Rose et al. 1991) and quantified as glucose equivalents using the anthrone method (Ashwell 1957). Total non-structural carbohydrates (TNC) were calculated by adding starch and sugars.

5.2.2.2 Terminal bud flushing and shoot growth

Bud flushing was recorded weekly in forest-planted seedlings. Buds were recorded as flushed when the bud scales opened and the green tissue was visible. The terminal shoot lengths were measured in mid-October, 1994 and 1995. In 1996, bud flushing was recorded every three days in the greenhouse-planted seedlings.

5.2.2.3 Root growth potentials

Root growth potentials were measured in seedlings lifted in 1996. After four weeks growing in the greenhouse, twenty seedlings were carefully removed from the pots and new roots > 10 mm counted.

5.2.3 Data analysis

The carbohydrate and root growth potential data were analyzed by the analysis of variance procedure (three-way ANOVA) using SAS software package (Version 6.10). The main effects were lifting time, storage temperature and storage duration with interactions. Shoot growth data were analyzed by GLM model of SAS statistical package (Version 6.10). Planting sites served as randomized blocks, with the model of lifting time, storage temperature, and storage duration as fixed effects with interactions. Bud flushing was analyzed by repeated measures analysis of variances. Carbohydrate contents were regressed with storage duration using REG procedure of SAS (version 6.10). Significant differences between equations of storage temperature –2°C and 4°C were tested.

5.3 RESULTS

5.3.1 Carbohydrate contents

Seedlings stored at–2°C had significantly higher needle sugar levels than seedlings stored at 4°C (Tables 5.1 & 5.2, Fig. 5.1). Needle sugar levels increased with duration of –2°C storage, but needle sugar levels remained relatively constant or slight decrease with duration of 4°C storage (Fig. 5.1). Needle starch content decreased with time in both 4°C and –2°C-stored seedlings (Fig. 5.1). During the first 7 weeks of storage, there was a drastic decrease in needle starch content, after which the levels remained relatively constant. Needle starch contents of A20 seedlings were not significantly different between –2°C and 4°C-stored seedlings (Table 5.2, Fig. 5.1). However, A27 and M4 seedlings stored at –2°C had higher needle starch content than seedlings stored at 4°C

(Table 5.2, Fig. 5.1). The total non-structural carbohydrates (TNC) in needles decreased with storage duration and the decrease rate was higher in seedlings stored at 4°C than in seedlings stored at –2°C (Fig. 5.1). A27 and M4 seedlings had higher needle TNC at the time they were placed in storage compared with A20 seedlings (Fig. 5.1).

In roots, –2°C storage resulted in a significant increase in sugar levels in seedlings from all three lifting dates (Fig. 5.2). 4°C storage maintained sugar levels similar to those at the time of lifting (Fig. 5.2). Storage duration significantly interacted with storage temperature to affect root sugar levels (Table 5.1, Fig. 5.2). At the time of lifting, M4 seedlings had higher root starch content than that in A27 seedlings, which, in turn, was higher compared with A20 seedlings (Fig. 5.2). In the first 3 weeks of –2°C storage, root starch content drastically decreased and later steadily declined in seedlings from all three lifting dates (Fig. 5.2). Seedlings stored at 4°C maintained relatively constant root starch content during the 7 weeks of storage (Fig. 5.2). Overall, seedlings stored at 4°C had significantly higher root starch content compared with seedlings stored at –2°C (Table 5.1, Fig. 5.2). Root TNC decreased slightly with storage duration. Root TNC levels in M4 seedlings were higher than those in A27 seedlings, which, in turn, were higher than in A20 seedlings (Fig. 5.2).

5.3.2 Bud flushing

In the first year following planting in the forest, terminal bud flushing was significantly affected by lifting time, storage temperature and storage duration (Table 5.3). A20 seedlings stored at 4°C had a higher percentage of flushed buds in a shorter time compared with A20 seedlings stored –2°C (Fig. 5.3). The duration of 4°C storage also influenced the timing of bud flush as seedlings stored for 5 and 7 weeks flushed earlier than those stored for 3 weeks (Fig. 5.3). The duration of 4°C storage did not have drastic effect on timing of terminal bud flush in A27 seedlings (Fig. 5.3). However, in M4 seedlings, long duration of storage at 4°C speeded up bud flushing (Fig. 5.3F). The duration of –2°C storage significantly reduced bud flush rates in M4 seedlings and only 86%, 42% and 31% of the seedlings stored for 7, 9 and 11 weeks, respectively, flushed their terminal buds (Fig. 5.3C). However, these effects were not observed in A20 and A27 seedlings (Fig. 5.3A, B).

In the second growing season, the timing of terminal bud flushing was still affected by storage duration (Table 5.3). M4 seedlings did not flush buds after 11 weeks of –2°C and 4°C storage (Fig. 5.4 C, F). Other seedlings stored at 4°C had similar bud flushing rates (Fig. 5.4 D, E, F). However, bud flush in seedlings stored at –2°C from all three lifting dates was significantly affected by storage duration (Fig. 5.4 A, B, C). Terminal bud flush in A20 seedlings stored at –2°C was 90% when stored for less than 9 weeks and below 40% when stored for 11 weeks (Fig. 5.4 A). Bud flush was greater than 90% in A27 seedlings when stored for 7 weeks or less, 77% in seedlings stored for 9 weeks, and 21% in seedlings stored for 11 weeks (Fig. 5.4 B). Bud flush in M4 seedlings stored at –2°C was greater than 90% after 7 weeks, less than 40% after 9 weeks, and 0% after 11 weeks of storage (Fig. 5.4 C).

When planted in the greenhouse, M4 seedlings stored for 12 weeks at –2°C did not break their terminal buds following planting. Other groups of seedlings flushed within 14 days after planting in the greenhouse. The buds that did not open were damaged by frozen storage. On the other hand, 10% of M4 seedlings stored at 4°C for 12 weeks had flushed buds before planting.

5.3.3 Terminal shoot growth

The elongation of terminal shoots was affected by lifting time and storage duration but not by storage temperature in the first and second years following planting (Fig. 5.5, Table 5.4). In all treatments, terminal shoot length was considerably reduced in the second year following planting (Fig. 5.5). However, A20 seedlings had longer terminal shoots than A27 and M4 seedlings (Fig. 5.5). Longer storage reduced terminal shoot length in the second year from all three lifting times (Fig. 5.5 D, E, F). There was no shoot growth in M4 seedlings after 11 weeks of storage at –2°C and 4°C since the terminal buds did not open (Fig. 5.5 F).

5.3.4 Root growth potentials

Storage temperature and duration significantly affected RGP (Table 5.5). The RGP values were similar in seedlings from all lifting times and storage temperatures after 3 weeks of storage (Fig. 5.6). For all lifting dates, RGP declined with the duration of 4°C storage (Fig. 5.6). Lifting time interacted with storage temperature and duration to affect RGP (Table 5.5).

With the exception of M4 seedlings following 12 weeks of –2°C storage, RGP declined with the duration of –2°C storage (Fig. 5.6). The difference in RGP between –2°C and 4°C stored seedlings also varied with lifting time and storage duration (Fig. 5.6). The statistical analysis showed that storage temperature had significant effects on RGP (Table 5.5). However, the effects were not significant in A20 seedlings (Fig. 5.6A). After 9 weeks, A27 seedlings stored at 4°C had significantly higher RGP than seedlings stored at –2°C (Fig. 5.6B). On the contrary, M4 seedlings after 12 weeks of –2°C storage showed significantly higher RGP than seedlings stored at 4°C (Fig. 5.6C).

5.4 DISCUSSION

5.4.1 Carbohydrate contents

TNC content was different in seedlings from the different lifting dates (Figs. 5.1 & 5.2). Needle TNC content in A27 seedlings was 20% and 8% higher than that in A20 and M4 seedlings, respectively (Fig. 5.1). On the other hand, root TNC content in M4 seedlings was 15% higher than that in A20 seedlings and 3% higher than that in A27 seedlings (Fig. 5.2). The spring increase in carbohydrate contents was reported for white spruce in Chapters III and IV and for other conifer species including red pine (*Pinus resinosa*) (Pomeroy et al. 1970), balsam fir (*Abies balsamea*) (Little 1970 a, b), red spruce (*Picea rubens*) (Snyder 1990), and Scots pine (*Pinus sylvestris*) (Fischer and Holl 1991). In the present study, needle and root TNC decreased during –2°C and 4°C storage. These results are in agreement with previous reports (McCracken 1978, Ritchie 1982, and Cannell et al. 1990, Jiang et al. 1994). In our study, –2°C stored white spruce seedlings accumulated sugars in both needles and roots (Figs. 5.1 & 5.2). These sugars were likely derived from starch since there was a corresponding decrease of starch levels. In 4°C storage, sugar contents of needles and roots were relatively stable. Soluble sugars are used for osmotic adjustment by many plant species (Jones et al. 1980, Ackerson and Herbert 1981, Borowizka 1981, Zwiazek and Blake 1990). In black spruce, the accumulation of solutes helps plants adapt to osmotic stress (Zwiazek and Blake 1989, 1990). In the present study, –2°C stored seedlings had higher sugar levels, suggesting higher resistance to drought and freezing. Frozen storage preserved frost tolerance of roots in Norway spruce and Scots pine (Lindstorm and Stattin 1994) and helped seedlings avoid transplanting shock (Blake 1983). However, in our study, there was no correlation between sugar or TNC levels and bud flushing or shoot growth in white spruce seedlings.

5.4.2 Bud flushing

Timing of bud break depends upon fulfillment of the chilling requirement and on environmental conditions preceding bud break (Nienstaedt 1966, Perry 1971). In the present study, 4°C storage promoted bud flushing in A20 seedlings. It is plausible that 4°C was sufficiently high to induce bud flush. Additionally, seedlings from different storage durations were planted in the forest at different times. Therefore, they likely experienced different environmental conditions after planting. Storage at –2°C did not speed up bud flushing in A20 and A27 seedlings (Fig. 5.4 A, B), probably because -2°C inhibited metabolism processes in buds.

M4 seedlings responded differently to storage temperature compared with A20 and A27 seedlings. Bud flush in M4 seedlings was significantly reduced by the duration of –2°C storage (Fig. 5.4). It is possible that the decline of bud flushing was caused by frost injury at –2°C. The bud flushing of 4°C-stored seedling in storage was probably due to the metabolic activity in buds before storage. The bud injury of –2°C-stored seedlings and bud flushing in 4°C storage were also observed in seedlings planted in the greenhouse in 1996.

The timing of bud flushing may be an indicator of overall seedling vitality, as delayed bud break may result in failure to overcome environmental stress (Lavender 1985). Bud flushing in the second growing season was reduced by –2°C storage (Fig. 5.4). The effect of –2°C storage was aggravated by late lifting (Fig. 5.4 A, B, C).

However, the duration of 4°C storage did not have an effect on the bud flushing in the second growing season with the exception of seedlings lifted on May 4 and stored for 11 weeks (Fig. 5.5 D, E, F). Since seedlings experienced similar winter and growth conditions, the difference in timing of bud flush between 4°C and –2°C-stored seedlings was likely due to the overall seedling vigor.

5.4.3 Terminal shoot growth

In the first growing season, the length of terminal shoots in A20 and A27 seedlings was not significantly affected by 4°C and –2°C storage duration (Fig. 5.5). However, prolonged 4°C and –2°C storage significantly reduced terminal shoot length of M4 seedlings (Fig. 5.5). The reason for this reduction may be that the seedlings lifted at the later date had poor low temperature tolerance (Chapter IV). It is also possible that shoot growth was affected by a shorter growth season due to late planting. The reduced shoot growth has also been shown in late planted Scots pine and Norway spruce seedlings (Ericsson et al. 1983).

The growth of terminal shoots was considerably lower in the second growing season than that in the first season. This reduced shoot growth referred to as "check" is common in white spruce in the second growing season following outplanting (Mullin 1963, Burdett et al. 1984, Sutton 1992) and may persist for decades (Sutton 1995). Planting "check" is considered to be due to the restricted uptake of water and (or) mineral nutrients. The decreased shoot growth with increased storage duration and lifting time may reflect differences in seedling vigor at the time of bud set in the planting year. Poor tolerance of long-term frozen or cold storage may affect seedling bud structure and result in reduced shoot growth the following year (Fig. 5.5).

5.4.4 Root growth potentials

Root growth potential is inversely related to seedling dormancy and cold hardiness (Ritchie and Shula 1984, Ritchie 1986, Tinus et al. 1986). It peaks with the fulfillment of chilling requirement and the development of cold hardiness and declines abruptly at the time of bud break and loss of cold hardiness (Stone et al. 1963, Ritchie and Dunlap 1980, Ritchie 1986, Tinus et al 1986). Many conifer seedlings show a decrease in RGP when placed in cold storage before the attainment of dormancy or after the fulfillment of chilling requirement (Stone and Jenkinson 1971, van den Driessche 1977, Nelson and Lavender 1979, Ritchie and Stevens 1979, Jenkinson 1984, Ritchie 1984, Ritchie et al. 1985). In our study, the seedlings from all lifting times and storage temperatures showed a decline in RGP with storage duration (Fig. 5.6). This decline may reflect loss of seedling dormancy. Ritchie et al. (1985) speculated that the decrease in RGP of cold-stored western conifer species in spring was due to increased metabolism in the buds once the chilling requirement had been met.

The effect of storage temperature on RGP of white spruce seedlings was influenced by lifting time (Table 5.5). Seedlings lifted in late spring are more physiologically active and closer to the active growth stage (Chapter IV) and, therefore, are susceptible to low temperatures (Levitt 1972) and injured by frozen storage (Parker 1959, Pellett and White 1969, Smit-Spink et. al. 1985). It is possible that root injury in frozen storage is responsible for the decline of RGP in seedlings. It is interesting that the seedlings lifted on May 4 and stored –2°C for 12 weeks had relatively high RGP. Since the buds in these seedlings did not flush following planting, it is possible that more energy was available for root growth.

In conclusion, we found that spring-lifted white spruce bareroot seedlings could be safely stored at –2°C or 4°C for limited time. Storage temperature affected seedling carbohydrate levels but did not have a significant effect shoot growth after planting. Seedling carbohydrate levels at planting were not correlated with bud flushing and shoot growth. Both bud flushing and shoot growth were affected by storage duration and (or) planting season. Storage temperature and duration significantly affected RGP. The RGP of –2°C-stored seedlings declined with storage duration. In 4°C storage, relatively high RGP was maintained for 6 weeks in seedlings lifted on April 20, 9 weeks in seedlings lifted on April 27, and 3 weeks in seedlings lifted on May 4.

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Table 5.1 ANOVA - probability values for sugars, starch and total non-structural carbohydrates (TNC) in needles and roots of white spruce seedlings.

Sources	Needles			Roots		
	Sugars	Starch	TNC	Sugar	Starch	TNC
Lifting time (L)	0.0001	0.0001	0.0001	0.0283	0.0001	0.0001
Storage temperature (T)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0519
Storage duration (D)	0.0783	0.0001	0.0001	0.1167	0.0001	0.0001
L*T	0.0001	0.0001	0.0001	0.0011	0.0003	0.0001
L*D	0.0013	0.0001	0.0001	0.5952	0.0001	0.0001
T*D	0.0001	0.0003	0.0001	0.0128	0.0004	0.0001
L*T*D	0.2701	0.0001	0.0053	0.0610	0.0001	0.0001

Table 5.2 Probability values for comparing regression equation differences between seedlings stored at –2°C and 4°C.

Lifting time	Needles			Roots		
	Sugars	Starch	TNC	Sugars	Starch	TNC
April 20	0.0001	0.6234	0.0534	---	---	0.0022
April 27	0.0001	---	---	---	---	0.0466
May 4	0.0001	0.5031	0.0001	---	---	0.5484

Note: --- comparison was not done because of different types of curve.

Table 5.3 ANOVA - probability values for terminal bud flushing of white spruce seedlings in the first year and second year following planting.

Sources	Terminal bud flushing	
	First year	Second year
Site	0.1001	0.3254
Lifting time (L)	0.0001	0.4298
Storage temperature (T)	0.0001	0.2524
Storage duration (D)	0.0001	0.0001
L*T	0.0051	0.8823
L*D	0.0001	0.7901
T*D	0.0001	0.5914
L*T*D	0.2301	0.8530

Table 5.4 ANOVA - probability values for terminal shoot length of white spruce seedlings in the first year and second year following planting.

Sources	Terminal shoot length	
	First year	Second year
Site	0.2157	0.1946
Lifting time (L)	0.0001	0.0043
Storage temperature (T)	0.3149	0.2110
Storage duration (D)	0.0001	0.0002
L*T	0.5140	0.6109
L*D	0.0001	0.1646
T*D	0.1974	0.7360
L*T*D	0.0330	0.5704

Table 5.5 ANOVA - probability values for root growth potential (RGP) of white spruce seedlings.

Sources	RGP
Lifting time (L)	0.2660
Storage temperature (T)	0.0098
Storage duration (D)	0.0001
L*T	0.0001
L*D	0.0032
T*D	0.0098
L*T*D	0.0007

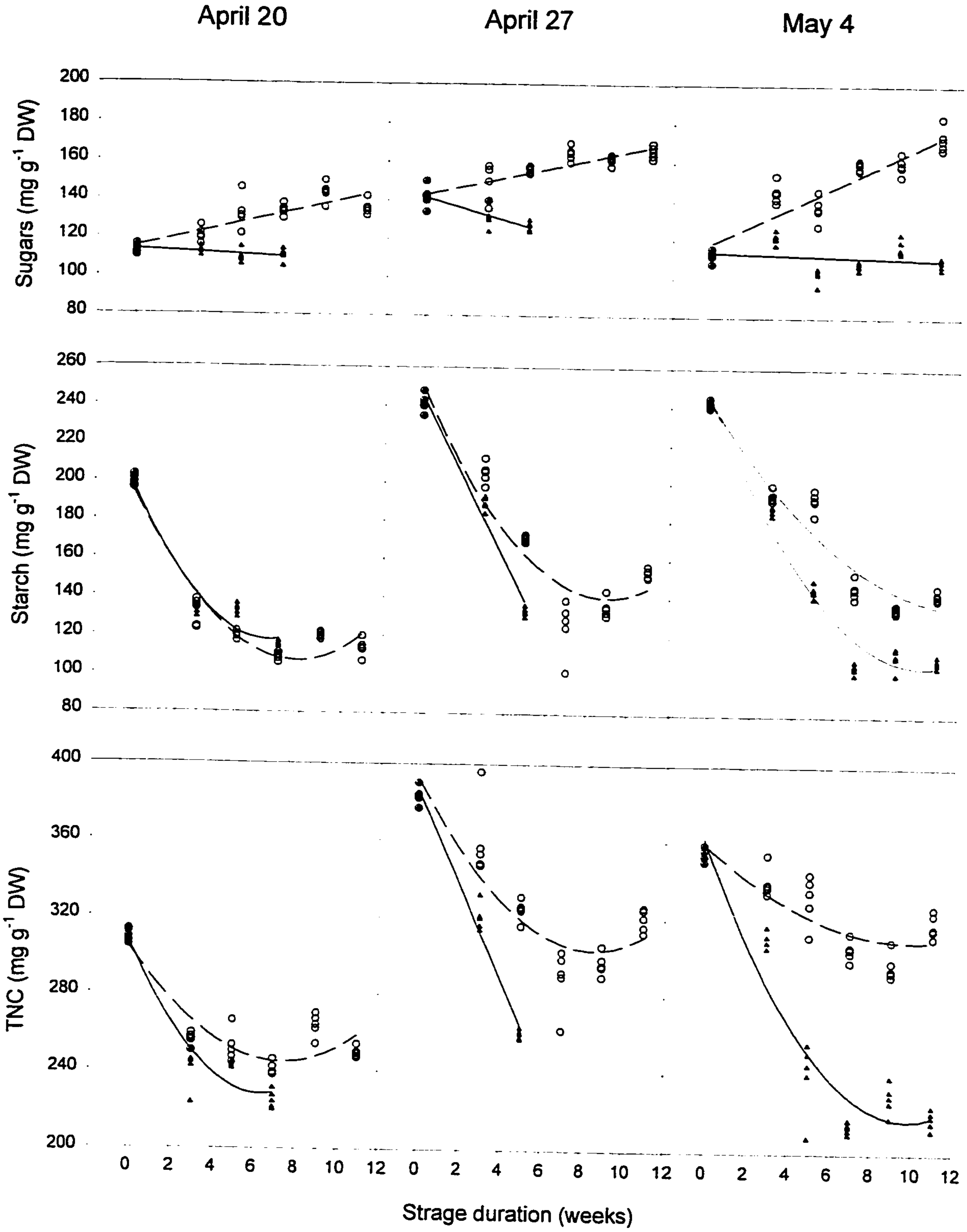

Figure 5.1 Changes of sugar, starch and total non-structural carbohydrate (TNC) contents in needles of white spruce seedlings lifted on April 20, April 27 and May 4, 1994, during 11 weeks of storage at -2°C (open circle and dash line) and 4°C (filled triangle and solid line).

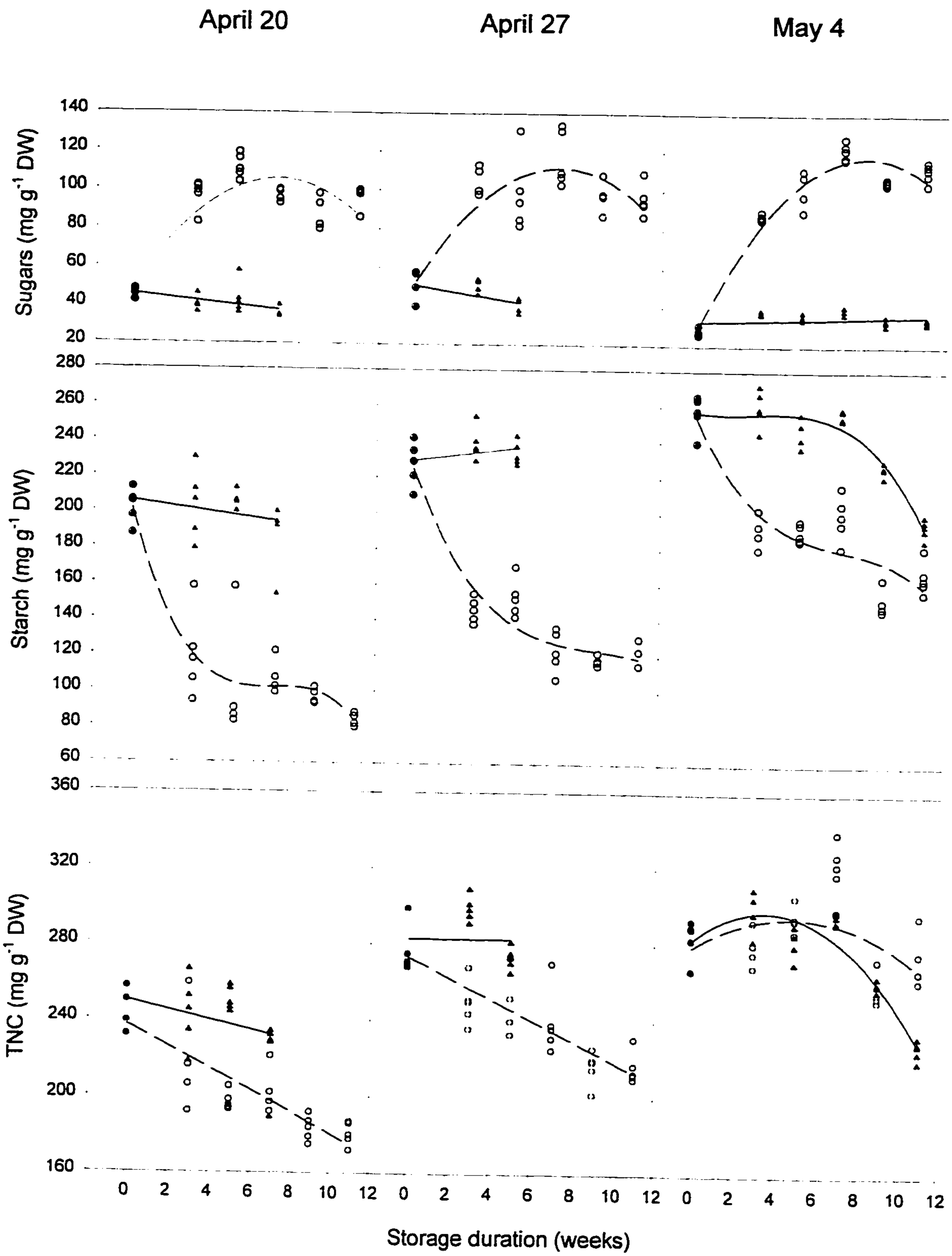

Figure 5.2 Changes of sugar, starch and total non-structural carbohydrate (TNC) contents in roots of white spruce seedlings lifted on April 20, April 27 and May 4, 1994, during 11 weeks of storage at -2°C (open circle and dash line) and 4°C (filled triangle and solid line).

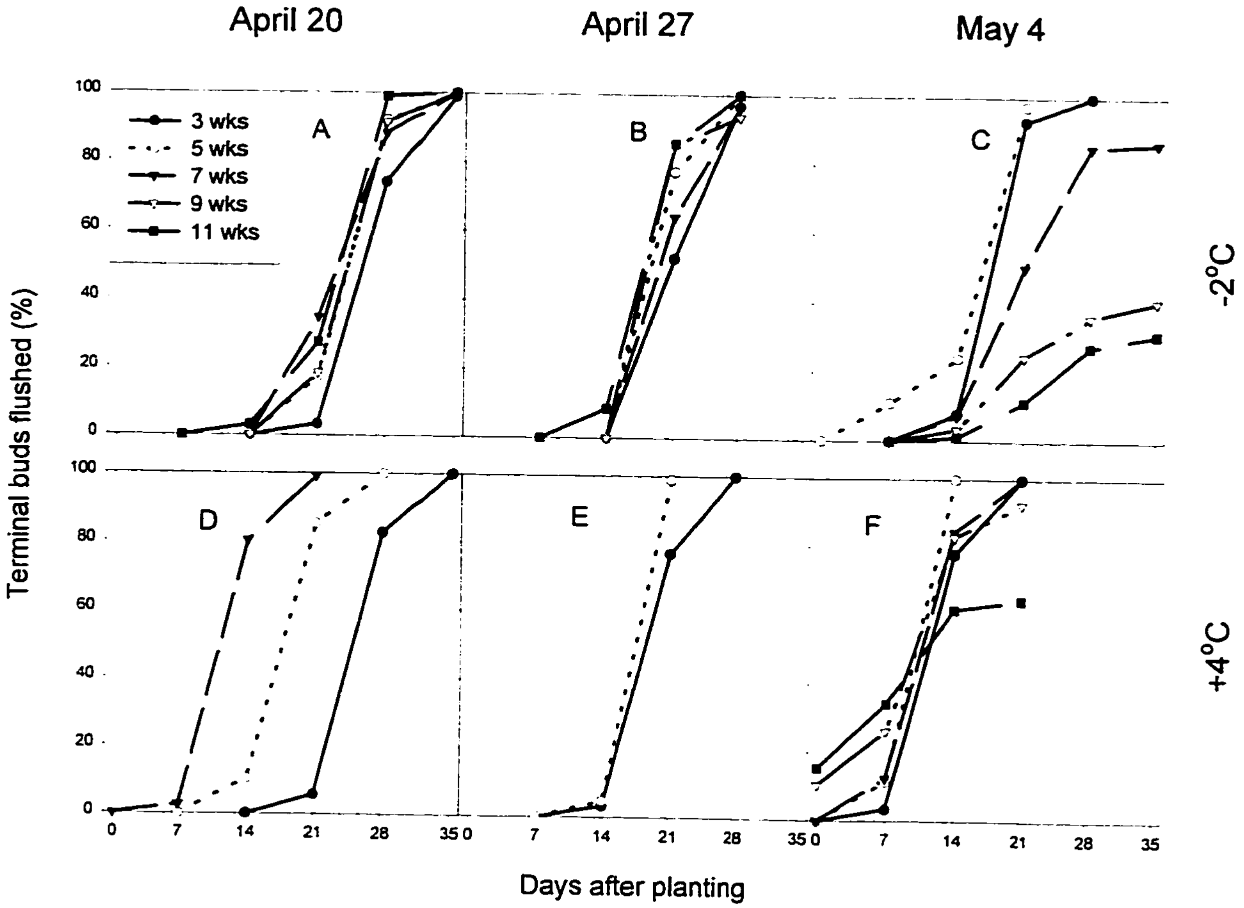

Figure 5.3 Timing of terminal bud flush in white spruce seedlings during the first year following planting in the forest. Seedlings were lifted on April 20 (A, D), April 27 (B, E) and May 4 (C, F) 1994, and stored at -2°C (A, B, C) or at 4°C (D, E, F) for 3, 5, 7, 9 and 11 weeks before planting (n=80 seedlings).

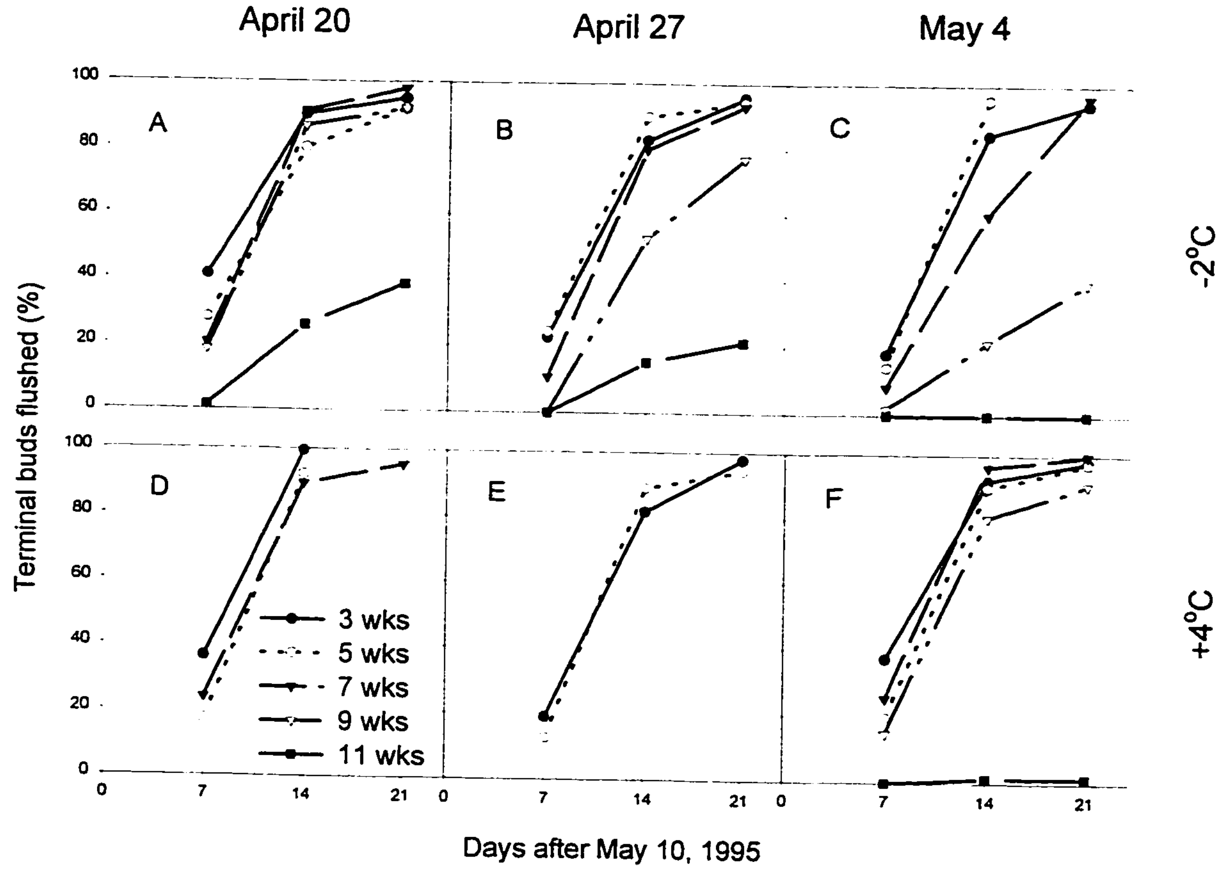

Figure 5.4 Timing of terminal bud flush in white spruce seedlings during the second year following planting in the forest. Seedlings were lifted on April 20 (A, D), April 27 (B, E) and May 4 (C, F), 1994, and stored at -2°C or 4°C for 3, 5, 7, 9 and 11 weeks before planting in the forest (n=80 seedlings).

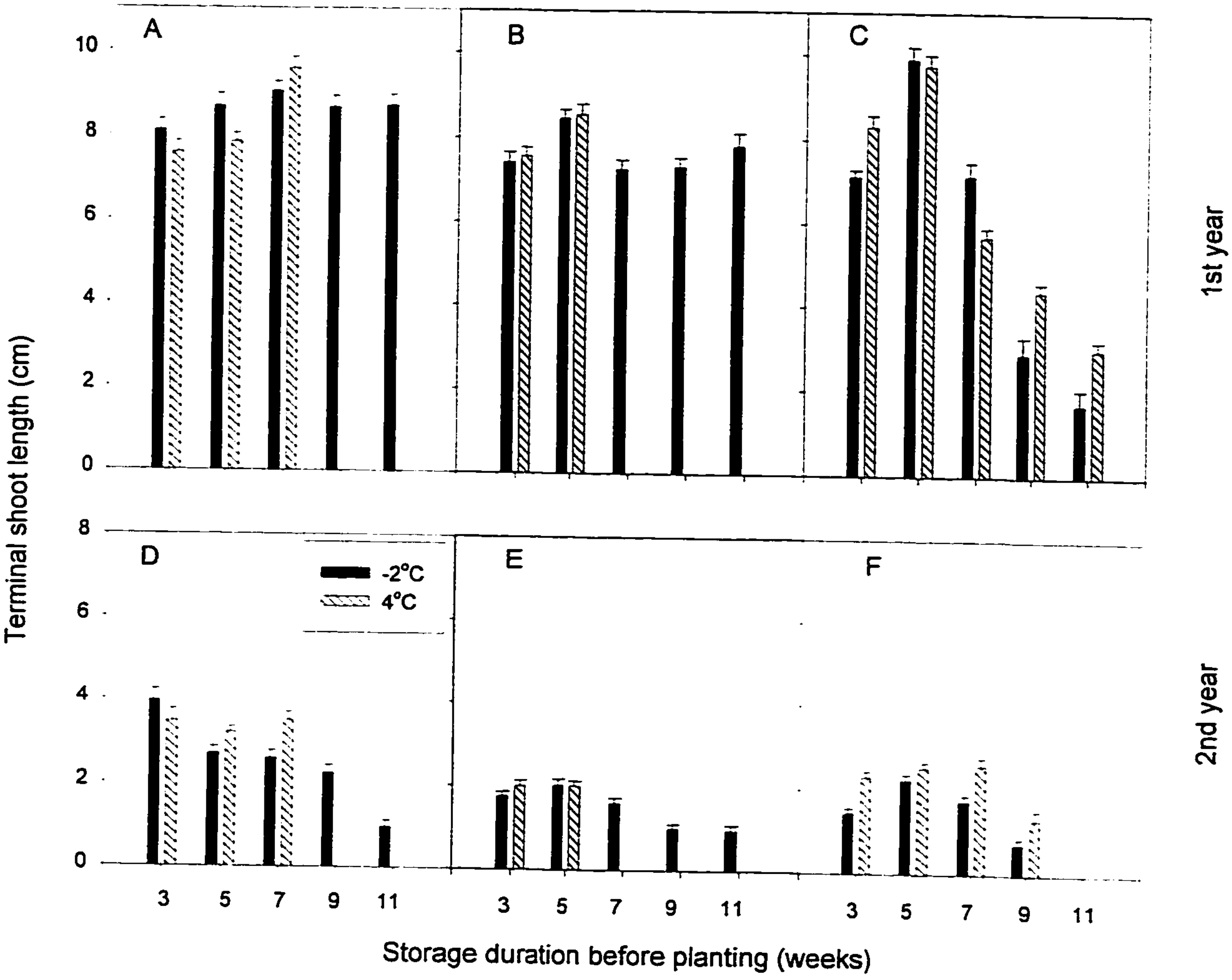

Figure 5.5 Terminal shoot length in the first year (A, B, C) and second year (D, E, F) following planting in the forest. Seedlings were lifted on April 20 (A, D), April 27 (B, E) and May 4 (C, F) and stored at -2°C or at 4°C for 3, 5, 7, 9 and 11 weeks before planting. Means (n = 80) + SE are shown.

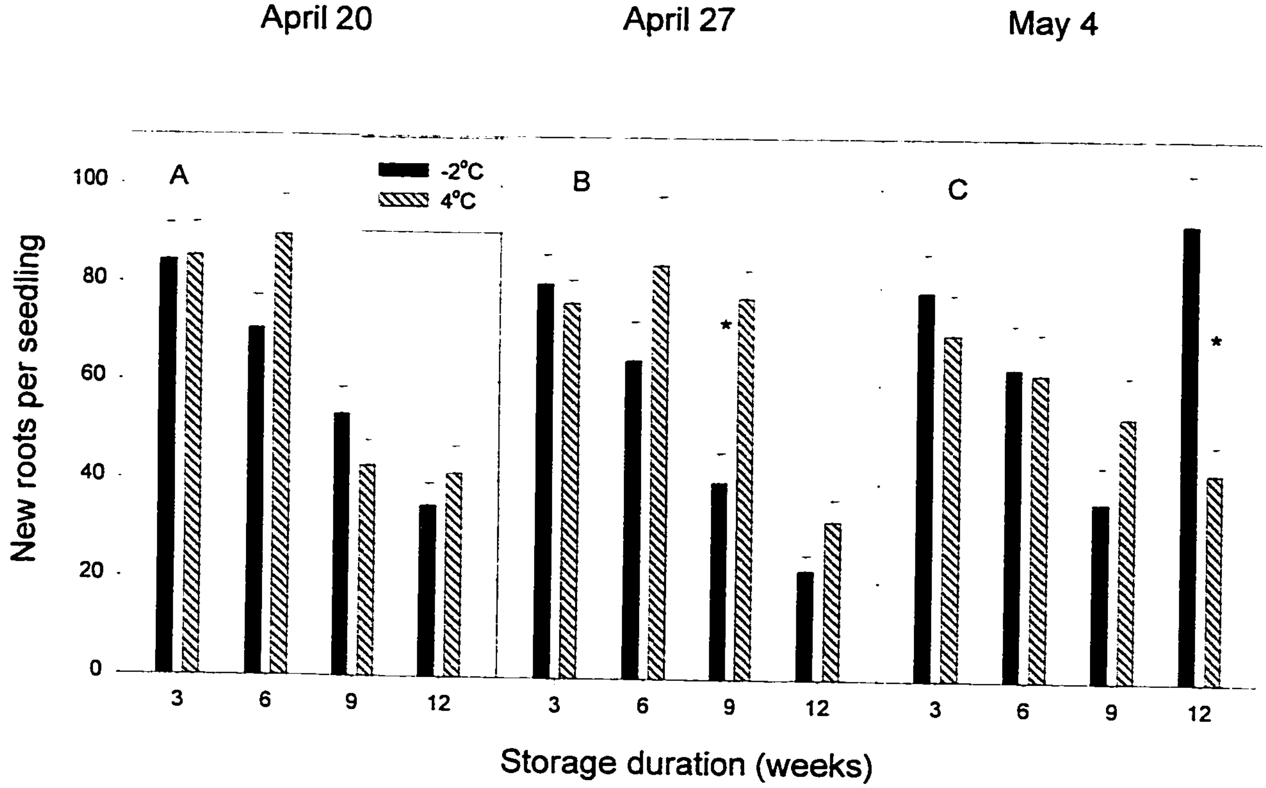

Figure 5.6 Root growth potentials of white spruce seedlings lifted on April 20 (A), April 27 (B) and May 4 (C) and after 3, 6, 9 and 12 weeks of -2°C and 4°C storage. Data are means (n=20) + SE. * indicates significant difference between -2°C and 4°C stored seedlings at p = 0.05 as determined by t-test.

Chapter VI

Effects of Storage Temperature on Physiological Characteristics of Fall-Lifted White Spruce Bareroot Seedlings[1]

6.1 INTRODUCTION

Fall-lifted white spruce bareroot seedlings are usually winter-stored at -2°C. During storage, seedling carbohydrate reserves may be reduced (Cannell et al. 1990, Jiang et al. 1994). Newly-planted seedlings depend on root growth to overcome planting stress (Burdett 1990) and the energy for root growth comes from carbohydrate reserves (Philipson 1988, Binder et al. 1990, Omi et al. 1991) and (or) current photosynthates (van den Driessche 1987, 1991, Ritchie and Tanaka 1990, Noland et al. 1997). Since photosynthesis is usually inhibited in newly-planted seedlings, sufficient carbohydrate reserves may help seedlings survive planting stress. The depletion of carbohydrates was correlated with poor survival and growth (Venn 1980, Puttonen 1986) and delayed bud flushing (Jiang et al. 1994).

Carbohydrate consumption in frozen storage can be reduced by lowering storage temperature (Ritchie 1987). Temperatures lower than -5°C, however, have been shown to be damaging to a range of species including southern pine (Williston 1974); Douglas fir (van den Driessche 1977); Colorado spruce and Scots pine (Cram and Lindquist 1981). However, the effects of low temperature storage on the survival and physiology of white spruce seedlings have not been throughly studied. To consider the use of lower storage temperatures in maintaining high tissue carbohydrate content, these effects must be understood. We examined the effects of different storage temperatures on electrolyte leakage, carbohydrate content, gas exchange, water relations, bud flushing, and root growth potentials in fall-lifted white spruce [*Picea glauca* (Moench) Voss] bareroot seedlings.

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[1] A version of this chapter has been submitted for publication. Wang, Y. and Zwiazek, J. J. 1998. Can. J. For. Res.

6.2 MATERIAL AND METHODS

6.2.1 Plant material and experimental design

White spruce (*Picea glauca* (moench) Voss) bareroot seedling (P+1.5) (seedlot # DS-86-SW) were obtained from the Pine Ridge Forest Nursery, Smoky Lake, Alberta. The seedlings were lifted on October 20, 1994, packed in cardboard boxes and stored at -2°C, -4°C and -6°C. Once a month, five seedlings were randomly selected from boxes from each storage temperature and carbohydrate contents and root and shoot injury were measured. After 4 and 7 months, 20 seedlings were removed from each storage temperature, thawed for 4 days at 4°C, and planted in a greenhouse under 18-h photoperiod and 23/18°C day/night temperatures. Seedlings were planted in 4-L pots (one seedling per pot) containing a mixture of peat moss and sand (3:1 by volume) and were watered every three days. Seedlings stored for 7 months were also planted On May 29, 1995, in two forest sites near Devon (53°22'N, 113°45'W), Alberta. The sites were 20-year-old aspen and white spruce mixed-wood forest and were about two kilometers apart. The major shrub species present in the planting sites was *Rosa acicularis* L., which was about 1-m tall and sparsely scattered. The dominant grasses were *Bromus inermis ssp pumpellianus* and *Agropyron trachycaulum* (Link) Mailte. The annual precipitation averaged from 1964 to 1994 by a nearby weather station was 468 mm, with June and July the wettest months. The average slope on both sides was less than 2% (south-facing). Seedlings were planted using a randomized complete block design, with two blocks (planting sites) and three treatments (-2°C, -4°C and -6°C). There were 40 seedlings per treatment planted in each of the two sites.

6.2.2 Measurements

6.2.2.1 Shoot and root injury

Shoot and root injury was measured using electrolyte leakage method (Zwiazek and Shay 1988). Briefly, one lateral shoot or root segment, about 2-cm-long, was excised from each of the five seedlings, washed in deionized water and immersed in 10 ml deionized water in a test tube. After four hours, the conductivity (C) of the solution was measured using a conductivity meter (Md1 C33, Fisher Scientific, Edmonton, AB). After the measurement, the tissue segments were frozen in liquid nitrogen and incubated in 10 ml deionized water for one hour to obtain the total electrolytes (C_{total}). Electrolyte leakage (% total electrolytes) was calculated as:

$$E = C/C_{total}*100 \tag{6.1}$$

6.2.2.2 *Carbohydrates*

Soluble sugars were extracted from 0.2 g dry weight (DW) needles and 0.4 g DW roots with 85% boiling ethanol for 10 min (Jiang et al. 1994). The extracts were filtered through Whatman No.1 filter paper and the residue extracted two more times with 85% ethanol. The filtered extracts were combined, reduced to water phase in a vacuum evaporator at 40°C and made up to 20 ml with distilled water. The residues were freeze-dried and used for starch analysis. Sugars were measured using the anthrone method (Ashwell 1957). Starch was extracted with perchloric acid (Rose et al. 1991) and quantified as glucose equivalents using the anthrone method (Ashwell 1957).

6.2.2.3 *Gas exchange*

Gas exchange was measured using an infrared gas analyzer (IRGA) LCA-4 (Analytical Development Company Ltd. Hoddesdon, UK). Field measurements were taken between 10:00 and 12:00 in the morning. The measurements in the greenhouse were taken in the afternoon. When the photosynthetically active radiation (PAR) was lower than 1000 μmol $m^{-2}s^{-1}$, artificial light was supplied to supplement PAR to 1000 μmol $m^{-2}s^{-1}$. Uppermost lateral shoots from seven seedlings from each storage temperature were selected for the measurements. The measurements were taken one day after planting and thereafter once every week for four weeks. Projected leaf areas were measured using a LI 3000 leaf area meter (LAMBDA Instruments Co., Lincoln, NB).

6.2.2.4 *Water relations*

Water relations were measured in seedlings stored for 7 months. On May 24, 1995, 10 seedlings were removed from each storage temperature, and placed in plastic bags in a dark cold room at 4°C for two days. Then seedlings were placed in covered beakers with roots submerged in water and stored overnight at 4°C in the dark to rehydrate. The P-V curves were constructed using the free transpiration method (Hinckley et al. 1980). The shoots were used for P-V curves only when initial shoot water potential (ψ) was higher than -0.3 MPa. Turgid mass (TM) of shoots was determined immediately following the first measurement and the shoots were allowed to dry at the room temperature. Parallel shoot ψ and fresh mass (FM) were determined at regular intervals. After the last measurement, shoots were dried at 70°C for 48 hours to determine dry mass (DM).

Relative water content (RWC) of each shoot was calculated as:

$$\mathrm{RWC} = (\mathrm{FM} - \mathrm{DM})/(\mathrm{TM} - \mathrm{DM}) \tag{6.2}$$

The P-V curves were plotted as $1/\psi$ vs. 1-RWC (Fig. 4.1).

The osmotic potentials at the full turgor (π_{100}) and at the turgor loss point (π_0) were derived from the points on the linear part of the P-V curves. The data in the linear region were fitted by the least-square method with the linear regression model:

$$1/\psi = a\,(1\text{-}\mathrm{RWC}) + b \tag{6.3}$$

The y-intercept of this regression gave an estimate of reciprocal of π_{100}. The turgor loss points were visually estimated from P-V curves to identify RWC at turgor loss point (RWC_{tlp}) and used to estimate π_0 with equation (6.3). The relative water contents in the symplast (RWC_s) and in the apoplast (RWC_a) were obtained from the x-intercept of the regression equation (6.3).

The bulk modulus of cell wall elasticity (ε) was calculated following Roberts et al. (1980) as

$$\varepsilon = (\psi_{P1} - \psi_{P2}) \,/\, ((\mathrm{RWC}_{s1} - \mathrm{RWC}_{s2}) \,/\, \mathrm{RWC}_s) \tag{6.4}$$

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 entire range of turgor. The maximum ε (ε_{max}) was obtained.

6.2.2.5 Bud flushing

Terminal bud flushing was recorded once a week in field-planted seedlings and twice a week in greenhouse-planted seedlings. The seedlings were recorded as flushed when bud scales of the terminal buds opened and the green tissue was visible. Bud flushing was recorded for 20 seedlings per storage temperature in the greenhouse and 80 seedlings per storage temperature in the field.

6.2.2.6 Root growth potential

Root growth potential was measured in seedlings grown for 4 weeks in the greenhouse (one seedling per pot). The seedlings were carefully lifted, their roots thoroughly washed and new roots > 10 mm were counted in 20 seedlings from each storage temperature (Ritchie 1985).

6.2.3 Statistical analysis

All data were analyzed by analyses of variance using general linear model (GLM) of SAS software package (Version 6.10). The main effects are storage temperature and storage duration with interaction. The contrast procedure was used to test the difference for electrolyte leakage and carbohydrates during storage with the values before storage. Duncan's test ($p = 0.05$) was used for comparisons of means of storage temperatures at each measurement date.

6.3 RESULTS

6.3.1 Electrolyte leakage

There was no increase in electrolyte leakage from shoots as a result of -2°C storage (Fig. 6.1). Electrolyte leakage from shoots of seedlings stored at -4°C increased after one month of storage but later decreased to the control (pre-storage) level (Fig. 6.1). Seedlings stored at -6°C showed significantly increased electrolyte leakage from shoots after one, two and six months of storage (Fig. 6.1).

At all storage temperatures, the electrolyte leakage from roots was significantly higher after one month of storage than that from control. The extent of leakage increased with the decreasing storage temperature (Fig 6.1). After the first month, the electrolyte leakage from roots decreased in all storage temperatures. The electrolyte leakage from roots of seedlings stored at -4°C was similar to that in control for the last 6 months of storage. During the same time, seedlings stored at -2°C had significantly lower root electrolyte leakage compared with controls. Seedlings stored at -6°C had increased electrolyte leakage from roots after all examined storage durations (Fig. 6.1).

6.3.2 Carbohydrates

All storage temperatures resulted in an increase in sugar levels of needles and roots (Fig. 6.2). After seven months of storage, seedlings stored at –4°C and –6°C had significantly higher sugar levels in both needles and roots than those in seedlings stored at –2°C (Fig. 6.2). Starch contents of needles and roots decreased in all storage temperatures (Fig. 6.2). The starch content of needles decreased in the first month in frozen storage and then remained relatively constant. The starch content of roots decreased with storage duration. After seven months of storage, seedlings stored at all temperatures had similar starch contents both in needles and roots (Fig. 6.2). Total non-structural needle carbohydrates (TNC) did not significantly change during 7 months of storage at –4°C and –6°C. However, the needle TNC in seedlings stored at –2°C for 7 months was significant lower than that before storage (Fig. 6.2). In all storage temperatures, root TNC decreased with storage duration (Fig. 6.2). However, after 7 months of storage, seedlings stored at -4°C and -6°C had higher root TNC levels compared with those stored at -2°C.

6.3.3 Gas exchange

In greenhouse-planted seedlings, positive NA were achieved one day after planting (Fig. 6.3). NA and g_s of –2°C-stored seedlings reached their maximum one week earlier than that from –4°C and –6°C-stored seedlings. Seedlings stored –6°C had significantly reduced NA and g_s compared to other storage temperatures (Fig. 6.3). In forest-planted seedlings, storage temperature had a significant effect on NA on days 1 and 28 after planting and on g_s only on day 1 after planting (Fig. 6.4).

6.3.4 Water relations

Seedlings stored at -4°C and -6°C had lower osmotic potentials at full hydration and at the turgor loss points and higher modulus of cell wall elasticity compared with seedlings stored at -2°C (Table 6.1). However, the relative water contents of shoots at turgor loss point were not significantly different (Table 6.1).

6.3.5 Bud flushing

Seedlings stored for 7 months flushed buds earlier compared with those stored for 4 months (Fig. 6.5). Lower storage temperatures resulted in delayed bud flushing both in greenhouse and forest-planted seedlings (Fig. 6.5). In all storage temperatures, greenhouse-planted seedlings flushed terminal buds within 3 weeks following planting. Forest-planted seedlings started opening terminal buds 17 days after planting and some seedlings flushed as late as 6 weeks after planting (Fig. 6.5).

6.3.6 Root growth potential

Seedling root growth potentials were higher after 7 months of storage compared to those after 4 months of storage (Fig. 6.6). Storage temperature had no significant effect on RGP in seedlings stored for 4 months. However, after 7 months of storage, seedlings stored at -6°C had lower root growth potentials compared with those stored at -2°C and -4°C (Fig. 6.6).

6.4 DISCUSSION

Freezing damage to plants is usually accompanying by loss of selective permeability of cell membranes (Levitt 1972). With the loss of membrane selective permeability, electrolyte leakage from cells increases. Our results demonstrated an increase in electrolyte leakage from shoots and roots of seedlings stored at -6°C (Fig. 6.1). The roots of seedlings in all storage temperatures showed high electrolyte leakage after one month of storage. The reason for this increase may be due to the initial injury to young roots. These roots were probably killed during the first month of storage and only resistant roots remained. The decrease of electrolyte leakage thereafter were probably due to chilling response which subsequently induce acclimation.

Sugar levels in plants increase in response to low temperature (Levitt 1972). In our results, the sugar levels in both needles and roots increased after seedlings were placed in frozen storage (Fig. 6.2). The high sugar levels were maintained until the end of storage in seedlings stored at -4°C and -6°C. The increased sugar levels were probably due to starch breakdown. Contrary to other reports (Ronco 1973, McCracken 1979, Ritchie 1982, Forry and Zaerr 1988, Cannell et al. 1990, Jiang et al. 1994), we did not find a significant decline in sugar levels in needles and roots during frozen storage. However, the levels of starch decreased in needles and roots (Fig. 6.2). The decreased levels of starch and sugars during cold storage may be due to respiration (Camm et al. 1994). Therefore, the decrease in root but not needle TNC in our study may indicate higher root respiration rates during frozen storage. This also explains a greater loss of TNC from roots stored at -2°C compared with those at -4°C and -6°C (Fig. 6.2).

Seven months of frozen storage altered water relations in seedlings. The lower osmotic potentials at full turgor and at turgor loss point of seedlings stored at lower temperatures could be due to higher sugar content (Fig. 6.2) or changes in carbohydrate composition in response to lower temperature (Halmer and Berley 1982, Ritchie 1982). Another factor contributing to changes in water relations is cell wall elasticity. The cell wall elasticity of seedlings decreased in lower storage temperatures (Table 6.1). This decrease was probably due to changes in cell wall composition in response to different temperatures (Renault and Zwiazek, 1997). Cell wall elasticity in conifer seedlings decreases in late fall with decreasing temperatures (Ritchie and Shula 1984, Grossnickle 1989, Colombo and Teng 1992). Due to the low osmotic potentials at turgor loss point and relatively rigid cell walls, seedlings stored at -4°C and -6°C were likely more drought resistant compared with those stored at –2°C. This is consistent with Blake's (1983) result that frozen-stored white spruce seedlings had better drought stress resistance compared with freshly-lifted seedlings.

The higher rates of stomatal conductance in seedlings stored for 7 months compared with those in seedlings stored for 4 months could be due to higher rates of root growth following planting. Longer storage duration resulted in higher root growth potentials (Fig. 6.6). New root growth reduces water flow resistance (Grossnickle and Blake 1985, Rietveld 1986) and increases needle water potential (Nambiar et al. 1979, Rietveld 1986) and stomatal conductance (Grossnickle 1988). It is also possible that root growth resulted in increased cytokinin synthesis in roots which, in turn, increased stomatal opening (Schulze 1986). Harper and Camm (1993) also reported an increase of stomatal conductance in white spruce seedlings after 22 weeks of storage, but this increase was associated with a decrease of RGP. The decline in NA after cold and frozen storage has been demonstrated in mountain pine (*Pinus mugo*), montery pine (*Pinus radiata*) (McCracken 1978) and white spruce (*Picea glauca*) (Harper and Camm 1993). Both disruption of photosynthetic system (McCracken 1978, Camm et al. 1993) and low stomatal conductance (Mattsson and Treong 1986) could result in a decline of photosynthesis. If stomatal conductance was the limiting factor for photosynthesis of white spruce seedlings, the increase of NA after 7 months of storage was probably due to the increased stomatal opening. The period of photosynthetic recovery following planting in frozen-stored seedlings depends on storage temperature and storage duration (Mattsson and Treong 1986, Harper and Camm 1993). In the present study, after 7 months of storage at -6°C, seedlings had lower rates of NA and g_s when planted in the greenhouse. However, in forest-planted seedlings, significant differences in NA between seedlings stored at different temperatures were noticeable only on days 1 and 28 following planting (Fig. 6.4). The differences in stomatal conductance between seedlings stored at different temperatures were observed only on day 1 following planting (Fig. 6.4). Forest-planted seedlings likely experienced water deficit stress shortly after planting due to poor root to soil contact. Seedlings stored at -4°C and -6°C had lower osmotic potentials and cell wall elasticity and it is possible that they were less affected by planting stress. It is also possible that the higher rates of NA and g_s in seedlings stored at -4°C were due to their higher drought stress resistance (Table 6.1).

The timing of bud flushing depends upon fulfillment of the chilling requirement during winter and subsequent environmental conditions in spring (Nienstaedt 1966, Perry 1971). Frozen storage could fulfill the chilling requirement although subfreezing temperature is less effective than temperatures of 4 to 6°C (Hinsley 1982, Ritchie 1984, Ritchie et al. 1985). Our results also showed that the time required for bud flushing was shortened by long storage duration (Fig. 6.5). The delayed bud flushing of seedlings stored at -4°C and -6°C suggests that the lower storage temperature was less effective in dormancy release. Forest-planted showed delayed bud flushing compared with those in the greenhouse. This delay was probably caused by the water stress that was induced by planting (Marsden et al. 1996).

Root growth potential is considered to be closely related to seedling dormancy status (Lathrop and Mecklenburg 1971, Ritchie and Dunlap 1980). In the present study, the increase of RGP with storage duration could be due to bud dormancy release during storage. The low RGP in seedlings stored at –6°C for 7 months was likely due to root injury at this storage temperature. Roots of woody plants are more sensitive to low temperature than shoots (Parker 1959, Pellett and White 1969, Smit-Spinks et al. 1985). In the present study, low RGP values correlated with high root electrolyte leakage. Seedlings stored at -6°C had lower RGP values and higher electrolyte leakage compared with those stored at -2°C and -4°C. Seedlings stored at -2°C and -4°C had similar electrolyte leakage and RGP values (Figs. 6.1 and 6.6).

The results of this study showed that storage temperature affected physiological characteristics and post-planting performance of fall-lifted white spruce bareroot seedlings. White spruce seedlings stored at –4°C had superior stress resistance characteristics including low π_{100} and π_0 and high cell wall elasticity, carbohydrate content and root growth potential. However, the effect of storage temperature on seedling physiology may be affected by factors such as seedling stock type, seed source and lifting time. Clearly, more research will be necessary to address these issues.

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Table 6.1 The effects of storage temperature on osmotic potentials at full hydration (π_{100}) and at turgor loss point (π_0), relative water content at turgor loss point (RWC_{tlp}), apoplastic water content (AWC), and maximum cell wall elasticity (ε) of white spruce seedlings stored for 7 months at –2°C, -4°C and –6°C. Means (n = 7) and standard error are shown. Means followed by different letters indicate significant difference at 0.05 level as determined by Duncan's test.

Temperature	π_{100} (MPa)	π_0 (MPa)	RWC_{tlp} (%)	AWC (%)	ε (MPa)
-2°C	-1.62 ± 0.06 (b)	-2.28 ± 0.07 (b)	80.0 ± 0.91	30.1 ± 3.36	0.41 ± 0.05 (b)
-4°C	-1.84 ± 0.06 (a)	-2.51 ± 0.05 (a)	79.4 ± 0.76	25.7 ± 3.50	0.75 ± 0.08 (a)
-6°C	-1.81 ± 0.07 (a)	-2.61 ± 0.08 (a)	78.7 ± 0.79	26.0 ± 4.48	0.67 ± 0.11 (a)

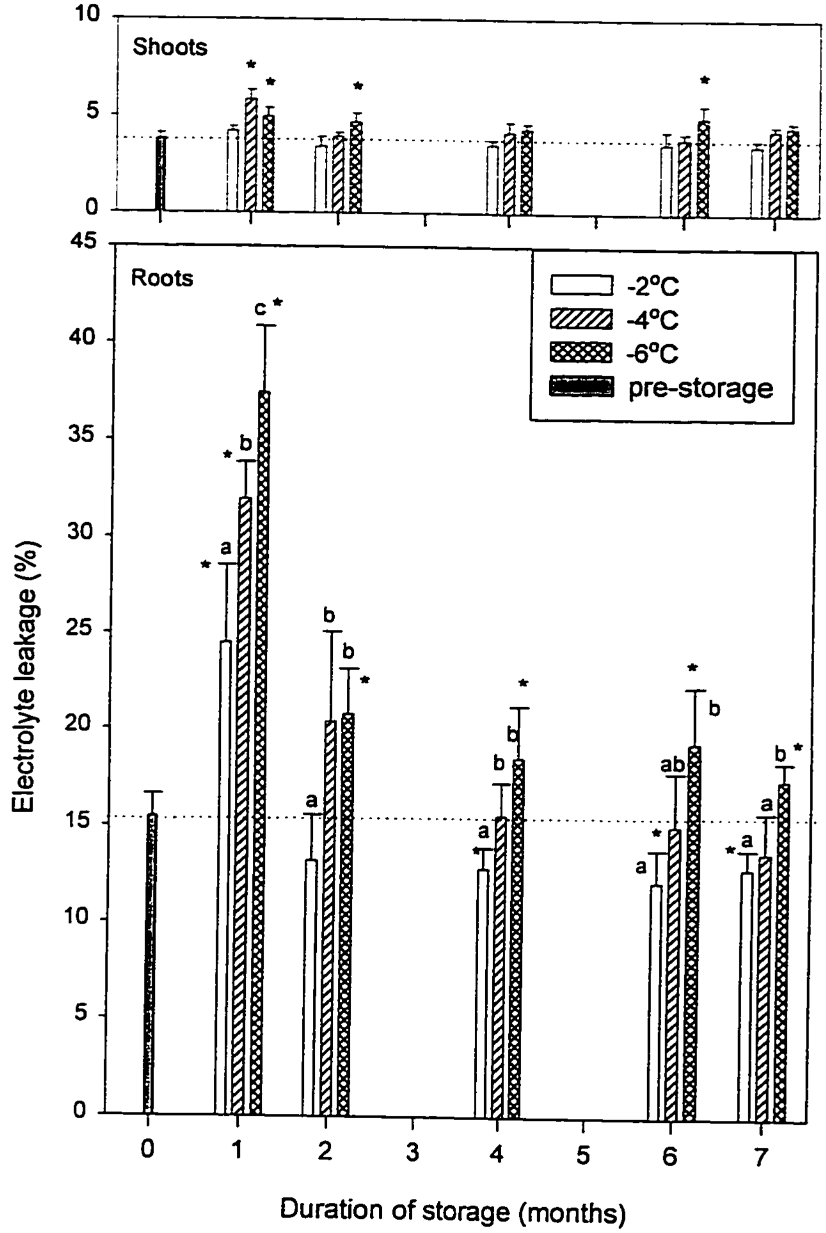

Figure 6.1 Effects of storage temperature on electrolyte leakage from shoots and roots of fall-lifted white spruce bareroot seedlings. Seedlings were stored for seven months. * indicates significantly different values (p=0.05) from those before storage as determined by contrast. On each measurement day, bars with different letters indicate significant (p=0.05) differences between storage temperatures determined by Ducan's test.

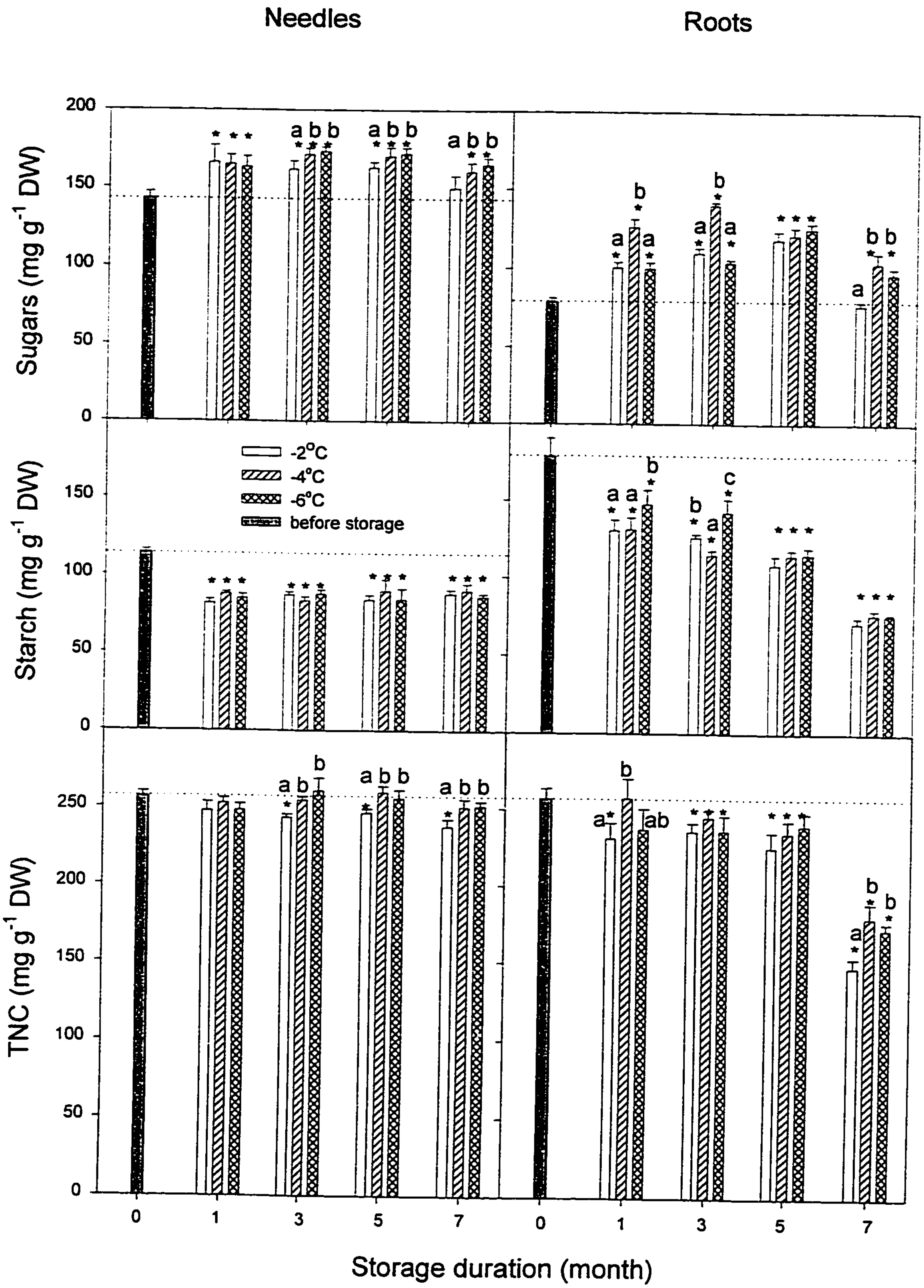

Figure 6.2 Soluble sugar, starch and total non-structural carbohydrate contents (TNC) in needles and roots of white spruce bareroot seedlings stored at -2°C, -4°C and -6°C for seven months. Bars are means (n=5) + SE. * indicates significantly different values (p=0.05) from those before storage as determined by contrast. On each measurement day, bars with different letters indicate significant (p=0.05) difference between storage temperatures as determined by the Ducan's test.

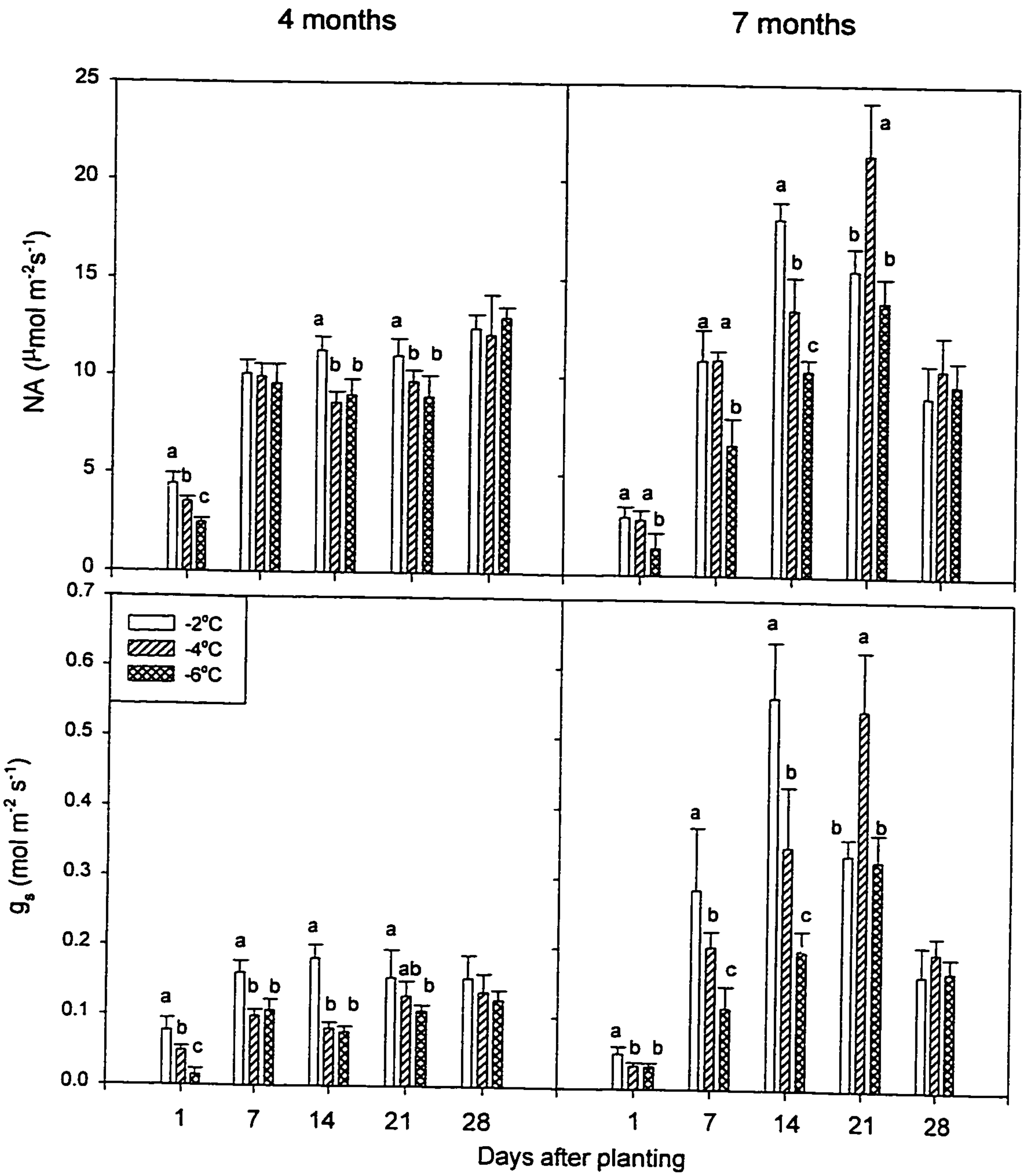

Figure 6.3 Net assimilation (NA) and stomatal conductance (g_s) during 28 days of growing in the greenhouse of fall-lifted white spruce seedlings after 4 and 7 months of storage at -2°C, -4°C and -6°C. Means (n=7) + SE are shown. On each measurement day, bars with different letters indicate significant (p=0.05) differences between storage temperatures determined by the Ducan's test.

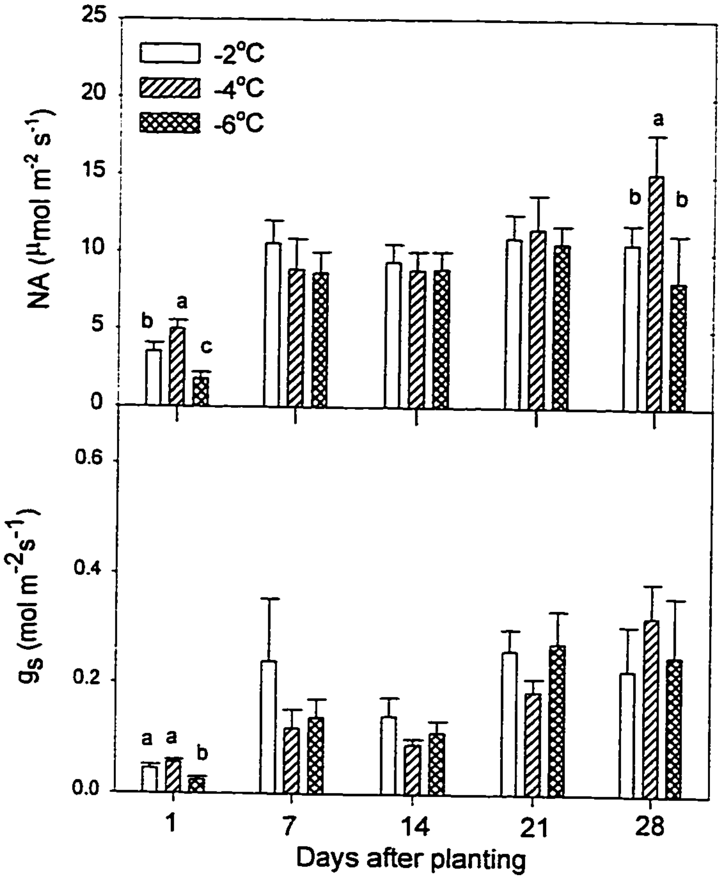

Figure 6.4 Net assimilation (NA) and stomatal conductance (g_S) of forest-planted white spruce bareroot seedlings after 7 months of storage at -2°C, -4°C and -6°C. Means (n = 7) + SE are shown. On each measurement day, bars with different letters indicate significant (p = 0.05) differences between storage temperatures as determined by Duncan's test.

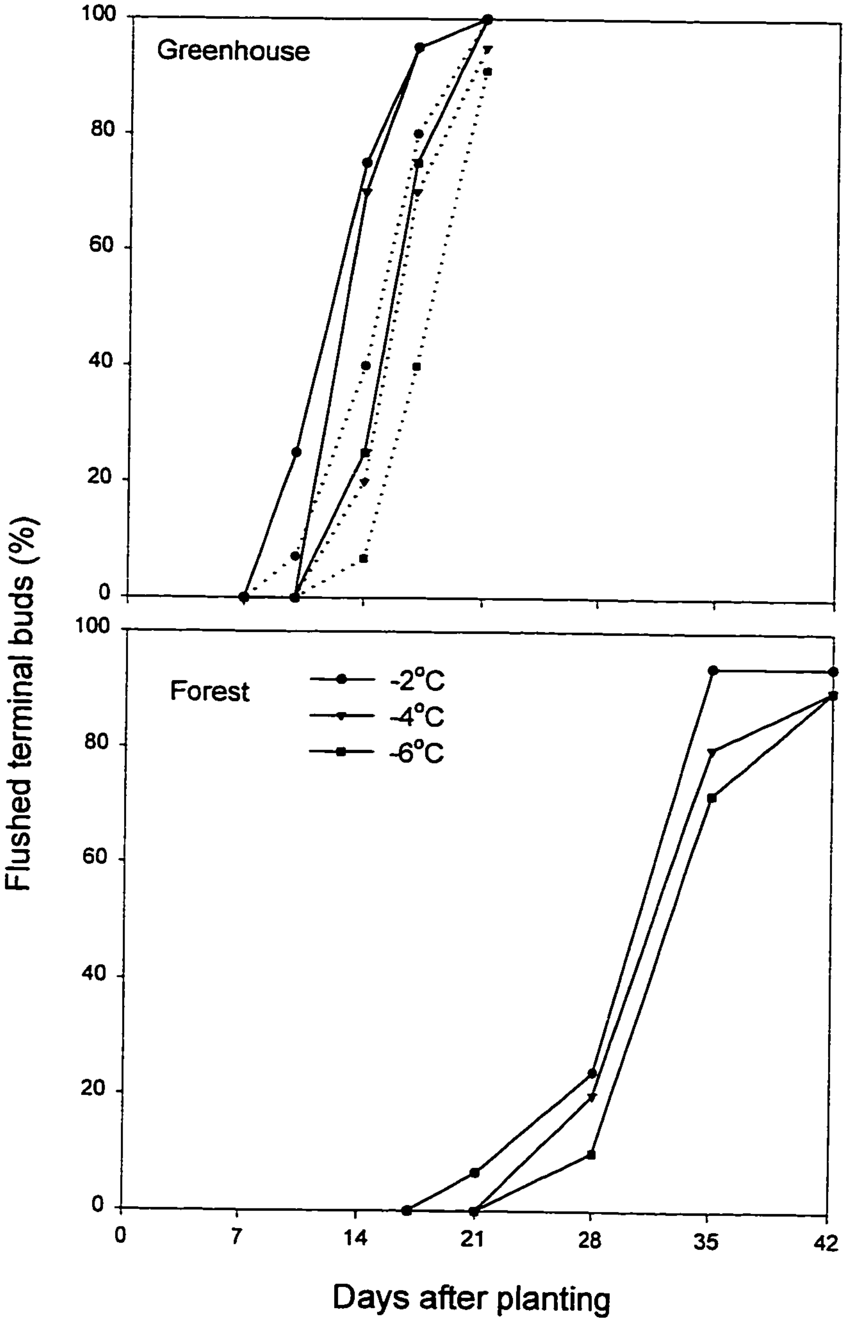

Figure 6.5 The timing of terminal bud flushing in greenhouse (n = 20) and forest-planted (n = 80) white spruce seedling after 4 months (dotted line) and 7 months (solid line) of storage at -2°C, -4°C and -6°C.

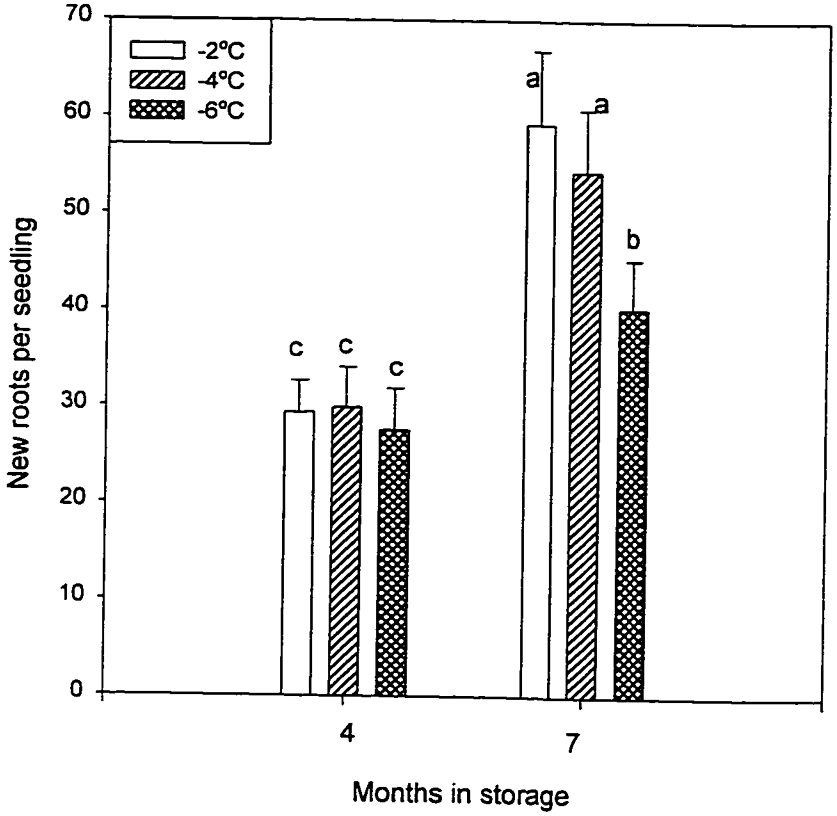

Figure 6.6 Root growth potentials of fall-lifted white spruce seedlings after 4 and 7 months of storage at -2°C, -4°C and -6°C. Means (n=20) + SE are shown. Bars with different letters indicate significant difference at p = 0.05 as determined by the Duncan's test.

Chapter VII

Synthesis

7.1 General Discussion and Conclusions

The overall objective of this series of studies was to advance the knowledge of cold and frozen storage effects on white spruce bareroot seedlings that is needed to improve the establishment of planted seedlings. Chapter III addressed the effects of early spring photosynthesis on carbohydrate levels and seedling field performance. Chapter IV focused on seedling physiological characteristics before spring-lifting. Chapter V examined the effects of cold and frozen storage on spring-lifted seedlings. Finally, Chapter VI discussed the effects of different frozen storage temperatures on the physiology of fall-lifted seedlings.

Although a positive correlation between carbohydrate content and survival has been demonstrated in many species (Hellmer 1962, Hocking and Ward 1972, McCracken 1979, Puttonen 1980), other studies found no effects of seedling carbohydrate content on growth and survival (Ritchie and Dunlap 1980, Duryea and McClain 1984). Root growth is essential to the survival of planted seedlings. It has been long thought that high carbohydrate content increases root growth potential (RGP). However, some studies had documented that there may be no correlation between carbohydrate levels and RGP (Ritchie 1982). The present study found no correlation between RGP and carbohydrate levels in white spruce bareroot seedlings (Chapters III, V, VI). Numerous studies suggested that in conifers root growth depends on current photosynthates rather than carbohydrate reserves (Carlson 1976, 1977, Ritchie and Dunlap 1980, van den Driessche 1987, 1991, Ritchie and Tanaka 1990, Noland et al. 1997). We also observed a quick recovery of photosynthesis following planting in the greenhouse (Chapters IV, VI). It is possible that white spruce seedlings use current photosynthates to grow new roots in the conditions used for RGP tests. However, in the field-planted seedlings, positive carbon balance may not be achieved for several weeks following planting since seedlings experience water deficit stress (Marshall 1985, Burdett 1990, Vapaavuori et al. 1992).

Therefore, forest-planted seedlings must initially depend on carbohydrate reserves for respiration and, probably, for the resumption of new root growth (Carlson 1976, 1977, Philipson 1988, Binder et al. 1990, Omi et al. 1991). When planting site is not favourable for seedling photosynthesis, carbohydrate reserves could be used to initiate root growth (Mcnabb 1985, van den Driessche 1987). Our results (Chapter III) showed that the growth of new roots in the first four weeks following planting in the forest correlated with carbohydrate levels. Therefore, carbohydrate reserves may be a measure of capacity to maintain tissue function under stress, especially during seedling establishment.

4°C, -2°C, -4°C and –6°C storage tends to reduce carbohydrate levels (Chapter V, VI). Lowering storage temperature better preserves seedling carbohydrates (Chapters V, VI), but the difference is relatively small. Carbohydrate levels can be increased by exposing seedlings to natural conditions in early spring (Chapters III, IV). In this study, the seedlings regained photosynthetic capacity rapidly in early spring and accumulated carbohydrates in needles and roots (Chapters III, IV). Other seedling physiological characteristics also changed in spring and the seedlings gradually lost stress tolerance (Chapter IV). Low frozen storage temperatures resulted in higher sugar content, mostly in roots (Chapter V). The increase of sugar levels likely resulted in a decrease of osmotic potential (Levitt 1972) which may help increase stress resistance. The results in Chapter VI showed that storage at -4°C and –6°C also altered seedling water relations and affected bud flushing (Chapters IV, V). Seedling RGP and dormancy were affected by storage temperature (Chapters IV, V). Following planting, fall-lifted seedlings took longer to open their buds compared to spring-lifted seedlings (Chapter IV, V). Hence, fall-lifted seedlings may be more suitable for planting in early spring or in these areas that are susceptible to early spring frost.

Spring-lifted seedlings could be safely stored at 4°C or -2°C for a limited time. The duration of storage depended on lifting time. The maximum storage duration recommended is 9 weeks. Seedlings stored for longer than 9 weeks showed either bud flushing or terminal bud injury in storage and poor survival and shoot growth (Chapter IV). Our results suggest that an increase of apoplastic water content and depletion of raffinose in spring should be considered as indicators of seedling tolerance of cold storage.

In conclusion, high seedling carbohydrate content was not reflected by high RGP, but correlated with the root growth following planting in the field. Lowering storage temperatures from 4°C to –2°C and from -2°C to –4°C and –6°C reduced carbohydrate consumption, however, the effects of these storage temperatures on carbohydrate contents in both spring- and fall-lifted seedlings were relatively small. Early spring photosynthesis dramatically increased seedling carbohydrate levels. Subzero storage retarded dormancy release and altered water relations in fall-lifted seedlings. Spring-lifted seedlings could be safely stored at 4°C or -2°C for a limited time with storage duration depending on lifting time.

7.2 Suggestions for Future Studies

Several areas of research pertaining to these studies still require more work. The present results showed that the decline in needle carbohydrates of fall-lifted seedlings was rather small during the long-term storage, unlike the decline reported by Jiang et al. (1994). We also found that fall-lifted white spruce seedlings could be safely stored at -6°C. However, the present study was conducted on seedlings from one seed source. Due to provenance variations in frost tolerance of conifer seedlings (Binder and Fielder 1996), more studies are required to examine seedlings from a variety of seed sources. It is also important to examine more closely the effects of lifting time on seedling storage tolerance (Hallgren and Tauer 1989, Hallgren et al. 1993).

A better understanding is required of seedling readiness for spring frozen storage. We demonstrated that seedling water relations and carbohydrate composition changed in early spring. Some characteristics such as apoplastic water content and raffinose may serve as the indicators of a seedling's ability to tolerate cold storage. Raffinose was shown to be related to cold hardiness in several conifers (Parker 1959). When seedlings undergo a transition from dormancy to growth, their metabolism changes and different sets of proteins are produced, as each growth stage requires different set of enzymes

(Kozlowski et al. 1991). These proteins could also be potentially useful in identifying different stages of hardiness.

More studies of root water relations are required. Water stress is the primary stress in newly-planted seedlings (Burdett 1990), and this stress is considered to arise due to reduced water uptake (Sands 1984, Caldwell et al. 1986, Sutton 1995). Root systems often severely limit water movement from soil to leaves (Kramer 1983). However, there have been relatively few studies of root water relations (Kandiko et al. 1980, Ritchie and Shula 1984). Osmotic adjustment can assist in maintaining physiological activity by helping extract water from soil (Turner 1986). It has been shown that the osmotic adjustment of roots was important in maintaining root growth in drought-stressed pea (Greacen and Oh 1972) and maize (Sharp and Davies 1979). Soluble sugar accumulation has been correlated with osmotic adjustment (Levitt 1972, Osonubi and Davies 1978). Our findings demonstrated that root sugar levels increased in response to lower storage temperature (Chapters V, VI), but it is not clear if this increase represented osmotic adjustment in roots. Osmotic potentials of roots affect root hydraulic conductivity which, in turn, controls the rates of water flow from soil to leaves. Timmis (1980) suggested using root hydraulic conductivity to determine seedling quality. Compared to root growth potentials, measurements of root hydraulic conductivity are not time consuming. However, we need to known more how lifting, storing, and handling affect root water relations and hydraulic conductivity in seedlings. A better understanding of these processes will aid in a production of high quality seedlings for reforestation.

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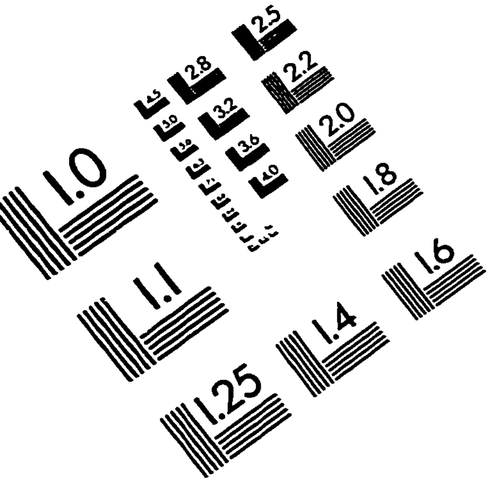
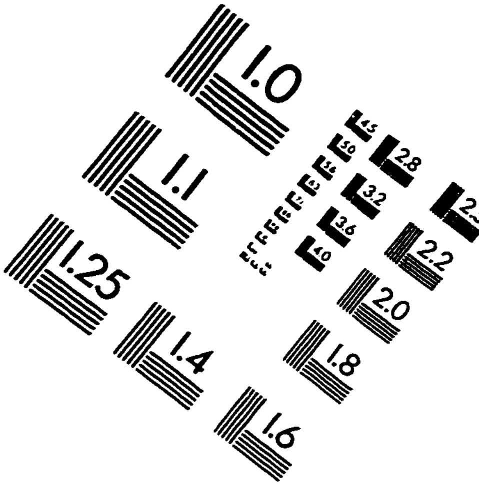
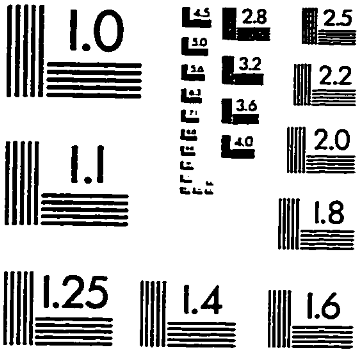
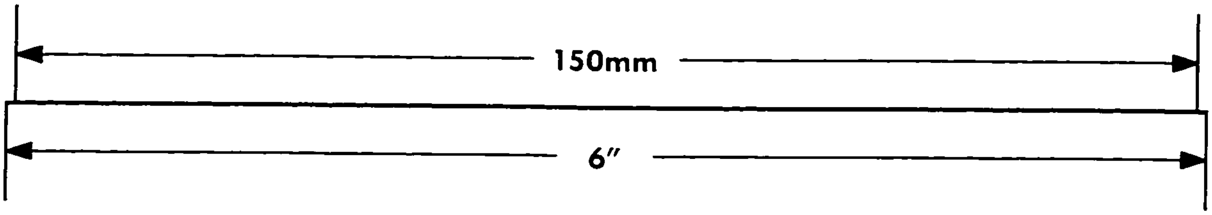
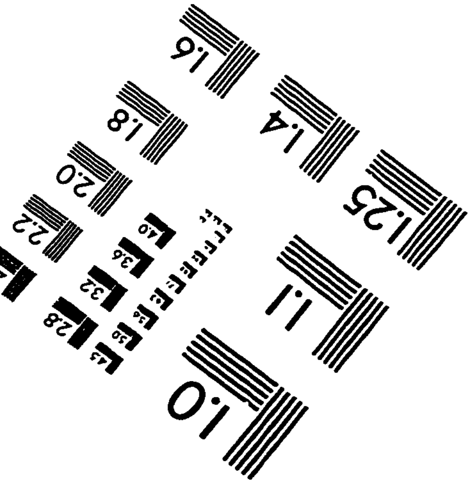
IMAGE EVALUATION TEST TARGET (QA-3)











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