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# EFFECTS OF PROLONGED COLD STORAGE ON PHYSIOLOGY AND FIELD PERFORMANCE OF WHITE SPRUCE (*PICEA GLAUCA*) SEEDLINGS

BY

**YONG JIANG** ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

**DEPARTMENT OF FOREST SCIENCE**

EDMONTON, ALBERTA

SPRING, 1993

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **EFFECTS OF PROLONGED COLD STORAGE ON PHYSIOLOGY AND FIELD PERFORMANCE OF WHITE SPRUCE (*PICEA GLAUCA*) SEEDLINGS** submitted by **YONG JIANG** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**.

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# Abstract

This study was conducted to assess the impact of changes in seedling physiology during cold storage on outplanting performance and stress tolerance of white spruce (*Picea glauca*) bareroot seedlings.

Three-year-old bareroot white spruce seedlings of three seedlots were either lifted in the fall of 1990 and stored for the winter in cold storage at -2°C or lifted in the spring of 1991. The soluble sugar, starch and protein contents in needles, stems and roots were measured in fall-lifted seedlings during cold storage and in spring-lifted seedlings 10 days before outplanting. Soluble sugar content of the seedlings decreased significantly in all tissues during cold storage. Sugar and protein levels were similar in fall- and spring-lifted seedlings, but starch content was about two-fold higher in spring-lifted seedlings than that in fall-lifted seedlings at the time of planting. After outplanting in spring, spring-lifted seedlings flushed and resumed root growth sooner than the fall-lifted, cold stored seedlings. Spring-lifted seedlings also had initially higher photosynthetic rates and water potentials compared with the fall-lifted seedlings. However, both types of seedlings had similar survival and first year shoot growth in the field. The responses of seedlings to cold storage were similar among seedlots.

In January, 1992, 1.5 + 1.5 (planted in container for 1.5 years and then in nursery beds for 1.5 years) white spruce seedlings, which were lifted either in the fall of 1991 or in January 1992 were placed in nutrient solution and subjected to -1.1 MPa or -2.7 MPa osmotic stress induced by PEG 3350 for nine days. Lifting time did not have a significant effect on seedling net assimilation, symplastic volume, osmotic potentials at full turgor and at turgor loss point. With the exception of osmotic potentials at turgor loss point, all of the above parameters were significantly affected by osmotic stress.

In May of 1992, spring-lifted and fall-lifted seedlings were planted in pots which were either exposed to gradual water stress or well watered in the greenhouse. Higher values of gas exchange parameters were observed in spring-lifted seedlings compared with fall-lifted seedlings in both well-watered and in moderate water stress conditions. This suggests that prolonged cold storage may retard the photosynthetic recovery of seedlings. Relatively high mortality rates observed in cold-stored seedlings subjected to severe water stress was possibly due to the inadequate energy supply required for water stress resistance including osmotic adjustment.

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# CHAPTER ONE

## General Introduction

The increasing demand for forest products and the pressure to ensure sustainability of forest resources have made reforestation an important issue for the forestry industry. In some instances, natural regeneration, with or without advanced stocking, may be adequate for reestablishing appropriate forest types. However, in many instances, artificial regeneration, which may involve direct seeding and seedling planting, is required (Brown 1984). Seedling planting is a widely used method of artificial regeneration. A key to success in seedling planting is seedling quality, which is defined as seedling growth and survival potential on a particular forest site (Ritchie 1984; Duryea 1985). Seedling quality can be affected by many factors during seedling production, such as seed collection and storage, aspects of the nursery cultural practices including water, nutrition, temperature, light and $CO_2$ regimes, and post-cultural processes, such as cold storage and handling. These factors must all be properly managed in order to optimize seedling quality and ultimately ensure good growth and survival of outplanted seedlings.

Cold storage of conifer seedlings is an important process in seedling production which affects seedling quality (Ritchie 1984; Cannell et al. 1990). Cold storage is a common practice in many nurseries in Canada and the United States (Hoching 1972, Ritchie 1984). Tree nurseries often store spring-lifted bareroot seedlings for short periods at 0-4°C before shipping them to planting sites. Alternatively, bareroot seedlings may be lifted in the fall and placed in cold storage for the winter at temperatures below zero (Hoching and Nyland 1971). Cold storage is often necessary for one or many of the following reasons: 1) Nurseries often supply planting stock to a range of forest sites spread over a wide geographical area; 2) Readiness of the site for planting often does not coincide with the lifting time at the nursery. In this case, cold storage can be helpful to adjust lifting and shipping schedules (Hoching and Nyland 1971). 3) Cold storage can also provide a buffer for meeting planting quotas when bad weather or other delays affect normal lifting schedules (Slayton 1970); 4) Cold storage helps ease spring workloads when labour is often difficult to recruit (Hee 1987); 5) Finally, cold storage can alleviate winter losses due to rodents, desiccating winds, snow scald or extreme temperatures (Brown 1971). In general, if seedlings are handled and protected carefully, and cold storage conditions are properly controlled, seedlings can be stored successfully for 6-8 months (Hoching and Nyland 1971; Hoching and Ward 1972; Tung et al. 1985; Hee 1987).

While cold storage can provide many benefits, it also may cause some problems (Ritchie 1987) and unsuccessful overwinter cold storage of spruce, pines, and other conifers has been reported (Venn 1980; Mason and McKay 1990). When seedlings are placed in cold storage they are exposed to environmental conditions that are different from those experienced by seedlings in nursery beds during winter. Cold-stored seedlings do not experience normal seasonal and diurnal fluctuations in air temperature, light and atmospheric humidity. Also, their roots do not contact soil like those of seedlings in nursery beds and this may lead to root desiccation (Venn 1980). These factors can generate changes in physiological processes which, in turn, may influence subsequent field performance (Stone 1955; Ritchie 1984; Duryea and McClain 1984). Previous studies (Venn 1980; Ritchie 1987; Harper et al. 1989) identified the following problems as being responsible for poor performance of cold-stored seedlings:

1. Mould,
2. Loss of carbohydrate reserves,
3. Reduction in root growth capacity,
4. Delay of bud dormancy release,
5. Reduced frost hardiness.

Even with these problems, cold storage is still recommended by many researchers because spring lifting, initially implemented to minimize length of storage, is complicated by the increasing sensitivity of seedlings to physiological stresses after the end of the safe lifting window (Ritchie and Dunlap 1980) and the increased risk of root damage by lifting when soils are frozen or soggy in the early spring (Hoching and Nyland 1971).

The present study was conducted to determine the effects of prolonged cold storage on physiological processes in white spruce (*Picea glauca* (Moench) Voss) seedlings and determine whether the differences in performance between spring-lifted and cold-stored seedlings are due to differences in seedling physiology caused by cold storage. The objectives were approached by: 1) examining both energy reserves and other physiological parameters in cold stored, fall-lifted and spring-lifted seedlings, which are important for survival and growth of newly planted seedlings, 2) comparing the field performance, such as root and shoot growth and survival of fall-lifted and spring-lifted seedlings, and 3) testing resistance of the two types of seedlings to water stress, which is known to be a major factor affecting seedling survival and growth following outplanting.

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# Chapter 2

# Carbohydrate and Protein Contents of Needles, Stems and Roots in Fall- and Spring-Lifted White Spruce Seedlings and Their Field Performance

## 2.1 Introduction

Many seedling transplants fail to survive and others grow slowly following outplanting because of transplanting shock. The response of seedlings to transplanting largely depends on their physiological status when they are transplanted (Blake 1983, Grossnickle and Blake 1985). Harsh environmental conditions on many boreal sites make it particularly important to ensure that the seedling transplants are highly resistant to environment stresses and capable of resuming growth shortly after growth can be supported by the environment (Grossnickle and Blake 1985). Outplanted bareroot seedlings have an additional task of establishing root to soil contact before enough water and mineral nutrients can be absorbed from the soil to support early rapid shoot extension (van den Driessche 1983).

Early root growth is dependent on energy reserves stored by the seedlings in previous growing seasons (Philipson 1988) or on current photosynthesis (Glerum and Balatinecz 1980; van den

Driessche 1987). When the photosynthetic rate of newly-planted seedlings is decreased by water or temperatures stresses, more reserve energy may be required to sustain root growth. If the seedlings are debilitated by one or more processes in seedling production before outplanting the threshold energy level may be too high for some seedings to grow and survive after outplanting (Burdett 1990).

Sugars, starch and proteins are important forms of stored energy in trees (Ronco 1973, Glerum 1980). Previous studies have demonstrated that energy reserves, especially sugars and starch, of seedlings can be reduced during cold storage (Hoching and Nyland 1971; Ritchie 1982). Such depletion of energy reserves has been correlated with poor seedling survival and root growth (Venn 1980; Puttonen 1986).

Blake (1983) found that white spruce (Picea glauca (Moench) Voss) seedlings which had experienced prolonged cold storage appeared to avoid transplanting shock by delaying bud flushing. However, lengthy delays of bud flushing may influence subsequent growth and the induction of bud dormancy in the fall. Field observations of white spruce bareroot seedlings indicated that this may be one of the reasons for poor seedling survival on many planting sites in Alberta where the growing season is relatively short and the summer is dry.

The present study was conducted to investigate how energy reserves in foliage, stems and roots of fall-lifted white spruce bareroot seedlings change during cold storage, and how fall-lifted and cold-stored seedings perform after planting as compared to spring-lifted seedlings.

## 2.2 Materials and Methods

### 2.2.1 Plant material and experimental design

White spruce bareroot seedlings of three seed lots, DF (101-10-5-79) from Footner Lake forest, northern Alberta, DL (72-21-4-83) from Lac LaBiche forest and DW (62-22-5-83) from Whitecourt forest, both in central Alberta were grown in Pine Ridge Forest Nursery, Smoky Lake, Alberta for three years until October 1990 when the seedlings were randomly divided into two groups for the experiment. Seedlings of one group were lifted, culled, packed in plastic bags and placed in cold storage at -2°C in the dark and with no humidity control. The temperature in cold storage was changed to 2°C on April 15. Seedlings of the second group were left in the nursery beds for the winter and lifted on April 20, 1991 before their buds flushed. These spring-lifted seedlings were culled, packed and placed in cold storage until May 8 and 9 when they were outplanted together with fall-lifted seedlings.

### 2.2.2 Carbohydrate and protein estimation

Ten seedlings per seedlot were selected randomly at each lifting date (spring-lifted seedlings were sampled on May 2, 1991) as well as in about two-month intervals over the period of cold storage. Their carbohydrate and protein contents were analyzed as follows. The seedlings were thoroughly washed and then separated into roots, stems and needles before the tissues were lyophilized for about 36 hours. Samples containing 0.2 g of needles, 0.4 g of roots and 0.4 g of stems were extracted with approximately 50 ml of 85 % ethanol and boiled for 10 min. After homogenization at 20,000 rpm for 3 min, the extracts were filtered through Whatman No. 1 filter paper and the residues were extracted again with 50 ml of 85 % ethanol. This procedure was repeated two more times, and then filtered extracts were combined, reduced to water phase in a vacuum evaporator at 40°C and made up to 20 ml with distilled water. After extraction the residues were freeze-dried and stored at -20°C for starch analysis as described below. Soluble sugars were quantified as $\beta$-D-glucose equivalents using the anthrone method (Ashwell 1957) and tissue protein content was estimated using Folin-Ciocalteau procedure (Lowry et al. 1951).

Starch extraction was carried out as described by Carpita and Kanabus (1987). Briefly, samples (25 mg) containing lyophilized tissue residues were suspended in 8 ml of dry dimethyl sulfoxide (DMSO) in 25-ml flasks, and the suspensions were stirred vigorously for 36 h at ambient temperature. The undissolved material was pelleted by centrifugation at 2,400g and 1 ml of the DMSO solution was pipetted into a vial in which DMSO was evaporated under nitrogen. Starch in the dried residue was hydrolysed with glucoamylase in a mixture of 200 $\mu$l 20 % DMSO solution, 550 $\mu$l distilled water, 230 $\mu$l 220 mM Na acetate, pH 5.0, in a water bath for 12 hours at 40°C. Glucose released from starch through the action of the glucoamylase was measured by the anthrone method. Total non-structural carbohydrates (TNC) were estimated by combining soluble sugar and starch levels.

### 2.2.3 Field experiments

In early May of 1991, 200 spring-lifted and 200 fall-lifted seedlings, of DL and DW seedlots were planted on forest cutblocks at Calling Lake (55°10', 113°05') and Fort Assiniboine (54° 30', 115°05'), Alberta in the vicinity of the areas where seeds were collected. The experiment was conducted as a nested design. There were eight plots in each planting site. Four plots were randomly selected to be planted with 50 fall-lifted seedlings and the remaining 4 plots were planted with spring-lifted seedlings. Therefore, there were four plots for each type of seedlings (fall- and spring-lifted). Field measurements of water potentials and gas exchange parameters were taken two days, two weeks, four weeks, six weeks and eight weeks after planting. Sixteen seedlings per treatment (4 from every plot) were randomly selected for measurement at each time. Gas exchange was not measured in DL seedlot six weeks after planting because of continuous rain. After gas exchange and water potential measurements taken 2, 4 and 6 weeks after planting, the seedlings were carefully lifted and the number of new roots, equal to or longer than 5 mm, was recorded. The number of flushed terminal buds in 40 seedlings per treatment was recorded at each planting site 4 and 6 weeks after planting. Survival and shoot length were measured one year after planting in the spring of 1992.

Gas exchange parameters, stomatal conductance to water vapour ($g_s$, mmol $m^{-2}$ $s^{-1}$), mesophyll conductance ($g_{m,}$ mmol $m^{-2}$ $s^{-1}$) to $CO_2$ net assimilation (NA, $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$), and water-use efficiency (WUE, $\mu$mol $CO_2$ fixed mmol $H_2O^{-1}$ transpired) were measured using an open system consisting of a portable infrared gas analyzer (LCA-3), air pump, and cuvette (PLC-C) (Analytical Development Corporation, Hoddesdon, England). The air was pumped through two columns of silica desiccant which reduced RH to 2-3%. Measurements were made rapidly to reduce the probability of stomatal closure. Airflow through the cuvette was maintained at 6.7 ml $s^{-1}$. On cloudy days artificial light was supplied by quartz-halogen battery providing 1200

$\mu$mol m$^{-2}$ s$^{-1}$, which is well above the light intensity for saturation of photosynthesis in white spruce (approximately 700 $\mu$mol m$^{-2}$ s$^{-1}$). In the third measurement (4 weeks after planting), when the number of flushed buds varied between the treatments, the previous year's shoots were used to measure gas exchange. In the later two measurements both newly flushed needles and previous year's needles were selected. After the measurement, the shoot was excised and needles were separated for needle area determination. Projected leaf area was measured by using a LI 3100 leaf area meter (LAMBDA Instruments Corporation) and the mean of two measurements was used in calculating net assimilation rates. NA, $g_s$, $g_m$, and WUE were determined using standard calculations (Caemmerer and Farquhar 1981).

Water potentials were measured in excised terminal shoots (6-8 cm in length) of the seedlings, after measurement of gas exchange, using a Scholander-type pressure chamber (Scholander et al, 1965).

All chemicals used in this study were purchased from Sigma.

### 2.2.4 Data analysis

All the data analysis was accomplished with the aid of the SAS computer package (SAS Institute Inc. 1985) and a significant level of $p \leq 0.05$ was used in all instances. Duncan's test was used to compare carbohydrate and protein of fall-lifted seedlings before and after cold storage, between fall- and spring-lifted seedlings and among seedlots prior to outplanting in May, 1991. Duncan's test was also used to compare shoot length between fall- and spring-lifted seedlings. Gas exchange and water potential data for each measurement day were analyzed using analysis of variance (ANOVA) according to following model:

$$y=\mu+S_i+P_{j(i)}+\epsilon \qquad (2.1)$$

Where

$Y$ = observed mean;

$\mu$ = population mean;

$S$ = stock types: $i=1,2$;

$P$ = plots within S: $j=1,2,3,4$;

$\epsilon$ = random error.

## 2.3 RESULTS

### 2.3.1 Total non-structural carbohydrates

Total non-structural carbohydrates (TNC) decreased during cold storage. In the all three seedlots examined, TNC consisted of approximately 86% sugars and 14% starch in fall, and 60% sugars and 40% starch in spring in whole seedlings. The spring-lifted seedlings had a higher TNC content compared with the fall-lifted seedlings about one week before the seedlings were outplanted in May 8 or 9, 1991. There was little difference in TNC among seedlots (Fig. 2-1).

### 2.3.2 Sugar content

In the autumn of 1990, the level of sugars in needles was twice as high as those in roots and stems. Both roots and stems had similar concentrations of sugars (Fig. 2-2). Sugar content in needles, stems, and roots declined significantly during cold storage in all seedlots examined, but patterns of decline differed among different tissues. In the needles the decline was rapid during the first three months in cold storage and then the concentrations remained relatively constant. In stem and roots, the rate of decline was always constant. After six months of cold storage, sugar content of needles declined by about 29% in DF, 27% in DL and 40% in DW seedlings. In stems, sugar levels were lowered by 34% in DF, 33% in DL and 46% in DW, and in roots by 28% in DF, 25 % in DL and 39% in DW (Fig. 2-2). Sugar contents of fall- and spring-lifted seedlings were similar before planting with the exception of sugar content which was higher in the needles of spring-lifted DW seedlings compared with those of fall-lifted DW seedlings (Fig. 2-2).

### 2.3.3 Starch content

The content of starch in roots was higher than that in needles and stems both in fall, 1990 when seedlings were lifted for cold storage and in spring, 1991 when the seedlings were planted (Fig. 2-3). During cold storage, starch content of stems had little change while that in the needles and root increased during the first three months and then either declined or remained relatively constant. After cold storage starch contents of seedlings were either higher or equal to those before cold storage in all of the analyzed tissues. From October to April, there was a significant accumulation of starch in those seedlings which were left in the nursery beds for the winter. The highest accumulation occurred in needles and was followed by roots and stems. This accumulation resulted in more than 100% higher starch content in the spring-lifted seedlings compared with the fall-lifted seedlings at the time of outplanting in spring, 1991. Starch content of the seedlings was similar among the three seedlots before outplanting.

### 2.3.4 Protein content

The protein contents of needles were more than three times higher than those in roots and stems. Cold storage had little effect on total protein content of seedlings and no significant differences were found in protein content of the fall- and spring-lifted seedlings of DW, DL, and DF seedlots before outplanting (Fig. 2-4).

### 2.3.5 Gas exchange

Two days after planting in forest cutblocks, net assimilation (NA) of both fall- and spring-lifted seedlings in seedlots DW and DL was nearly zero and there was no significant difference in NA between stock types (Fig. 2-5 and Fig. 2-6). Two weeks after planting, spring-lifted DW and DL seedlings had a higher NA compared with fall-lifted seedlings, but the difference was statistically significant only for DW seedlot. Four, six and eight weeks after planting, there were either no differences in NA between fall- and spring-lifted seedlings or fall-lifted seedlings showed a significantly higher NA (6 weeks after planting in DW and 4 weeks in DL) (Fig. 2-5 and Fig. 2-6).

The mesophyll conductance ($g_m$) in both DW and DL seedlings and water use efficiency (WUE) in DW seedlings showed a similar pattern to NA (Fig. 2-5 and Fig. 2-6). WUE of fall-lifted DL seedlings was higher four and eight weeks after planting (Fig. 2-5). Significantly higher stomatal conductance ($g_s$) was found two weeks after planting in spring-lifted and four weeks after planting in fall-lifted DW seedlings (Fig. 2-6). No significant differences in $g_s$ were found in measurements taken two days, 4 and 8 weeks after planting (Fig. 2-5 and Fig. 2-6).

### 2.3.6 Water potentials

Two days after planting, water potentials of seedlings were about -1.8 MPa in Fort Assiniboine (DW seedlot) site and about -2.3 MPa in Calling Lake (DL seedlot) site (Fig 2-5 and Fig. 2-6). The spring-lifted seedlings had significantly higher water potentials two days and two weeks after planting in DW seedlot and two weeks after planting in DL seedlot. In the subsequent measurements, water potentials increased and there were no significant differences in water potentials between fall-and spring-lifted seedlings seedlots of either seedlots (Fig 2-5 and Fig. 2-6).

### 2.3.7 Root and shoot growth, bud flushing and survival

Spring-lifted seedlings of DW and DL had more new roots two weeks after planting. However, four and six weeks after planting, the number of new roots longer than 5 mm was similar in both spring- and fall-lifted seedlings (Fig. 2-7 and Fig. 2-9). About 50% of spring-lifted and less than 10% of fall-lifted seedlings flushed within the first 4 weeks after planting. Six weeks after planting, about 95% of spring-lifted seedlings and 90% of fall-lifted seedlings flushed (Fig. 2-7 and Fig. 2-9). Survival and shoot elongation of the surviving seedlings after the first year were similar in fall- and spring-lifted seedlings (Fig. 2-8 and Fig. 2-10) and both fall- and spring-lifted seedlings had similar survival (about 85%).

## 2.4 Discussion

Total nonstructural carbohydrates (TNC) decreased in white spruce seedlings during cold storage. TNC is considered to be the principal food reserve for woody plants (Glerum and Balatinecz 1980). When seedling photosynthesis ceases during cold storage, the decline of TNC represents the use of stored resources to meet the remaining respiration requirement. This phenomenon has been observed in Douglas-fir (Winjum 1963; Ritchie 1982; Cannell et al. 1990), Sitka spruce (Cannell et al. 1990), mugo pine and radiata pine (McCracken 1979), ponderosa pine (Hellmers 1962), and Englemann spruce (Ronco 1973). The carbohydrate loss in cold-stored seedlings is thought to be affected by the rate of respiration. This rate varies depending on species (van den Driessche 1979), temperature of storage (Hoching and Nyland 1971; Puttonen 1986) and the type of packaging (Venn 1980).

Figure 2-2 and Figure 2-3 show that the loss of TNC during cold storage is mainly due to the loss of soluble sugars rather than starch. The sugar content of needles, stems and roots of seedlings in all examined seedlots declined during cold storage. The largest decline in sugars occurred during the first 3 months of cold storage in needles. Similar patterns of decline of sugars during cold storage were found in the foliage of mugo and radiata pine (McCracken 1979) and Douglas-fir (Ritchie 1982). McCracken (1979) suggested that this early high rate of consumption of sugars during cold storage could be due to stimulated respiration or translocation from needles to other tissues at the relatively high temperatures of the packing shed during grading and packaging or potting. However, in the present study, translocation of sugars between needles, stems and roots during the first period of cold storage was not observed in white spruce seedlings. The early high sugar loss in needles of white spruce seedlings in the three examined seedlots did not accompany an increase in sugar contents of stems and roots. A decline in sugars coinciding with an increase in starch during the first three months of cold storage (Fig. 2-2 and Fig. 2-3) indicates that some sugars may be converted to starch during this period.

Starch-sucrose conversions in both vegetative and reproductive tissues occur commonly (Kramer and Kozlowski 1979). Starch can be converted to soluble sugars under cold conditions to increase cold hardiness (Marvin et al. 1971; Nelson and

Dickson 1980; Delucia 1986), and under drought conditions to increase the resistance to water stress (Jones et al. 1981). On the other hand, starch can be derived from sugars when the rate of photosynthesis exceeds that of respiration (Gholz and Cropper 1991). In a study on root carbohydrate dynamics of loblolly pine, Hallgern et al. (1991) found that loblolly pine seedlings have the capacity to continue transport of sugar into fine roots and to increase the starch concentration long after root formation. During winter when plants were dormant, interconversion of starch and soluble carbohydrates in the trunk wood and 1- and 2-year-old shoots was reported in sweet cherry (Keller and Loescher 1989). The starch-sucrose conversion has not been observed in cold-stored seedlings before. However, it may be reasonably postulated that when seedlings are placed in cold storage under a relatively constant temperature of approximately -2°C, the high concentration of sugars which accumulated in late fall to facilitate tolerance of extremely low winter temperatures may convert to starch.

It was previously observed that the soluble sugar levels of many conifers peaked in December and January, and then declined gradually to the lowest point in early summer after rapid shoot growth started (Krueger and Trappe 1967; Pomeroy et al. 1970; Little 1970b; Fischer and Holl 1991). In these studies the maximum accumulation of starch always took place in spring before shoot growth started and starch content reached the lowest point in late autumn or early winter. Although in the present study the winter levels of sugars and starch were not measured in the seedlings left in nursery beds for the winter, the significant decrease in sugars and increase in starch from fall to spring in those seedlings are in agreement with the above studies. The fall accumulation of sugars in trees was thought to be the result of reduced phloem transport and respiration (Krueger and Trappe 1967; Watson 1980) or of increased starch hydrolysis (Nguyen et al. 1990; Fischer and Holl 1991) due to low temperature. The green parts of deciduous trees (Pearson and Lawrence 1958), conifers (Helms 1965) and evergreen trees (Kozlowski and Keller 1966) are able to carry on photosynthesis whenever the soil temperatures are above freezing. Therefore, the spring increase in starch in the spring-lifted seedlings before bud break is likely to be the result of photosynthetic activity in early spring when low night temperatures keep dark respiration at low levels, and active sinks (shoot and root growth) are lacking (Fischer and Holl 1991). This increase in spring starch content could also result from the conversion of sugars and other photosynthates produced in the previous year (Eifert and Eifert 1963; Little 1970a).

In addition to starch and sugars, proteins have also been considered to be a form of storage energy (Glerum and

Balatinecz 1980). Lack of significant changes in protein contents of needles, stems and roots suggests that proteins do not play a major role as energy reserves in white spruce seedings during cold storage. Similarly, no reduction in protein content was found in Engelmann spruce after 4 months in cold storage at 1°-2°C (Ronco 1973). It is also difficult to relate the protein content of white spruce seedlings to their field performance following outplanting because both spring- and fall-lifted seedlings contained similar levels of proteins at the time of planting.

It is well known that chilling is required for bud break of perennial woody plants and the optimum chilling temperature for most temperate zone trees is near 5°C (Perry 1971). An additional exposure to cold in December and January, warmer temperatures following full chilling, as well as high irradiance and long photoperiod also aid in bud flushing of some species (Ninestaedt 1966; Perry 1971; Falusi and Calamassi 1990). The cumulative hours of chilling required to overcome dormancy vary with species (Ninestaedt 1967; Nelson and Lavender 1979) and provenance (Bongarten and Hanover 1986). The chilling requirement for breaking seedling dormancy can be satisfied both in nature and in cold storage (Carlson 1985). However, several studies have shown that the chilling from cold storage is not as effective as that fulfilled in nature because of the delay of bud break in cold-stored seedlings (Norvell and Moore 1982; Ritchie 1984). The reasons for this delay have been attributed to non-optimum chilling temperature, the lack of fluctuation in temperature and the absence of photoperiod in cold storage (Ritchie et al. 1985; Cannell et al. 1990). Data from the present study provide further support that bud break of seedlings can be delayed by cold storage. However, this delay of bud flushing in cold-stored white spruce seedlings may not be only due to the ineffective chilling.

White spruce seedlings subjected to 4 to 8 weeks of chilling at 1 to 4°C (Ninestaedt 1966) or 16 to 25 weeks of chilling at -2 to -5°C (Rose et al. 1992) flushed after 18-22 days when planted in a greenhouse. In the present study, about 50% of spring-lifted seedlings and 7% of fall-lifted seedlings flushed 4 weeks after outplanting on the two forest sites. In a separate experiment and using the same cold-stored seedlings as used in this study, Macdonald (1992) found that most seedlings flushed during the first 15 days after planting in a greenhouse. This difference in time required for bud flushing may suggest that, besides cold storage, environmental conditions after planting can also greatly affect the speed of bud flushing. The impacts of cold storage and environmental conditions on bud flushing have always been studied separately and the interaction between these factors remains unclear.

The delay of bud break of cold-stored white spruce seedlings in spring has been thought to be an adaptive response to help the seedlings avoid water stress (Blake 1983). However, rapid bud break can also serve as a good indicator of seedling vigour and the delay of bud break can be a result of failure to overcome environmental stresses (Lavender 1985). Larsen et al. (1986) confirmed that the number of active buds of loblolly pine has a positive linear relation to field survival and the authors recommended that seedlings with dormant buds should not be planted on sites where rapid root growth is needed for survival. It is not clearly understood whether the delay in bud break of cold-stored seedlings in spring indicates that these seedlings are in deeper dormancy and therefore more resistant to environment stresses (Ritchie 1986; Colombo 1990) or it just reflects the poor physiological conditions of the seedlings. However, in the present study, this delay correlated with lower starch content, slower new initial root growth and lower photosynthesis. This could support the hypothesis that the delay in bud break of cold-stored seedlings is caused by their reduced ability to acclimate to new environmental conditions following planting.

The photosynthesis rates in both fall- and spring-lifted seedlings of two seedlots were nearly zero two days after planting. This is probably due to severe water stress in these newly planted seedlings which results in stomatal closure and high mesophyll resistance to $CO_2$. Water stress is very common in newly planted seedlings because of the lack of contact between soil and roots (Grossnickle 1988). Even if seedlings are planted in well-watered soil, water stress can occur (Sands 1984). Two weeks after planting, cold-stored seedlings showed a lower net assimilation, coinciding with lower water potentials. Lower NA in cold-stored seedlings has also been observed in Pinus species (McCracken 1978; Mattsson and Troeng 1986). The speed of resumption of photosynthesis is related to the reorganization of the photosynthetic apparatus in the chloroplast and absorptive capacity of seedlings to water after planting (Zelawski and Kucharska 1967; McCracken 1978). Therefore, the lower NA in cold-stored seedlings could be explained by their exposure to a long period of darkness, which could be detrimental to the photosynthetic apparatus thus leading to an extended period of acclimation in seedlings when exposed to light at planting (McCracken 1978) or by their inferior ability to absorb water. Since NA of cold-stored seedlings was equal to or in some cases higher than that of spring-lifted seedlings in the subsequent measurements, it can be concluded that cold-stored seedlings are capable of adjusting to the new environment but need more time for this adjustment. Fluctuating environmental conditions in field study may make the integration between photosynthesis and environment very complex. Obviously, more research is needed to test how photosynthesis of seedlings in these two types responds to environmental conditions.

The importance of root development of seedlings for their shoot growth and survival after planting has been documented for several species of conifers (Ritchie and Dunlap 1980; Burdett et al. 1983; Feret and Kreh 1985). Root growth potential (RGP), measured as the ability of seedling to regenerate new roots under standard environmental conditions, has been used as an indicator of seedling quality (Ritchie 1985). RGP of cold-stored seedlings can be affected by the lifting time (Carlson 1985; Ritchie et al. 1985), storage duration and conditions (Webb 1976; Harper et al. 1989) and post cold storage handling (Deans et al. 1990). A faster initial root growth of spring-lifted seedings compared with fall-lifted, cold-stored seedlings in the field suggests that prolonged cold storage might have some negative effects on the initiation of new roots. Rapid new root growth in newly planted seedlings can diminish their water stress (Grossnickle and Reid 1984), one of the most common stresses affecting the survival of the seedlings (Hinckley et al. 1978; Kramer 1983). Therefore, the fall-lifted seedlings could be expected to show poor survival, especially if the time for root initiation is shortened by early summer drought or the seedlings are held for later planting.

The root growth in newly planted seedlings depends on their current photosynthesis (van den Driessche 1987), carbohydrate reserves (Philipson 1988), or both (Johnson-Flanagan and Owens 1985). Energy reserves should be particularly important for resumption of growth when the photosynthesis is limited by suboptimal site conditions. In addition to energy reserves, growth regulators from growing shoots (Ritchie and Dunlap 1980; Philipson 1987) and other factors, such as the status of bud dormancy (Webb 1976; Johnsen et al. 1989), may also play a role in root growth. Therefore, slow growth of new roots in cold-stored white spruce seedlings can be due to any of the above factors or their combination.

It is interesting to note that, despite higher starch levels, faster bud break, faster growth of new roots and higher photosynthesis, spring-lifted seedlings did not show higher survival or shoot growth in the first growing season compared with fall-lifted seedlings. This indicates that the physiological attributes of seedling quality must be used in connection with environmental factors of the planting sites to predict reforestation success.

In summary, it is plausible that sugars are the main energy resource consumed by white spruce seedlings during cold storage. Cold storage inhibits bud flushing, photosynthesis and root initiation of white spruce seedlings. However, these changes in cold-stored seedlings may be not critical enough to influence their subsequent growth and survival in the field. More information is needed to understand how cold-stored seedlings survive and grow after planting on a variety of sites, and how seedling stress resistance is affected by physiological changes resulting from cold storage.

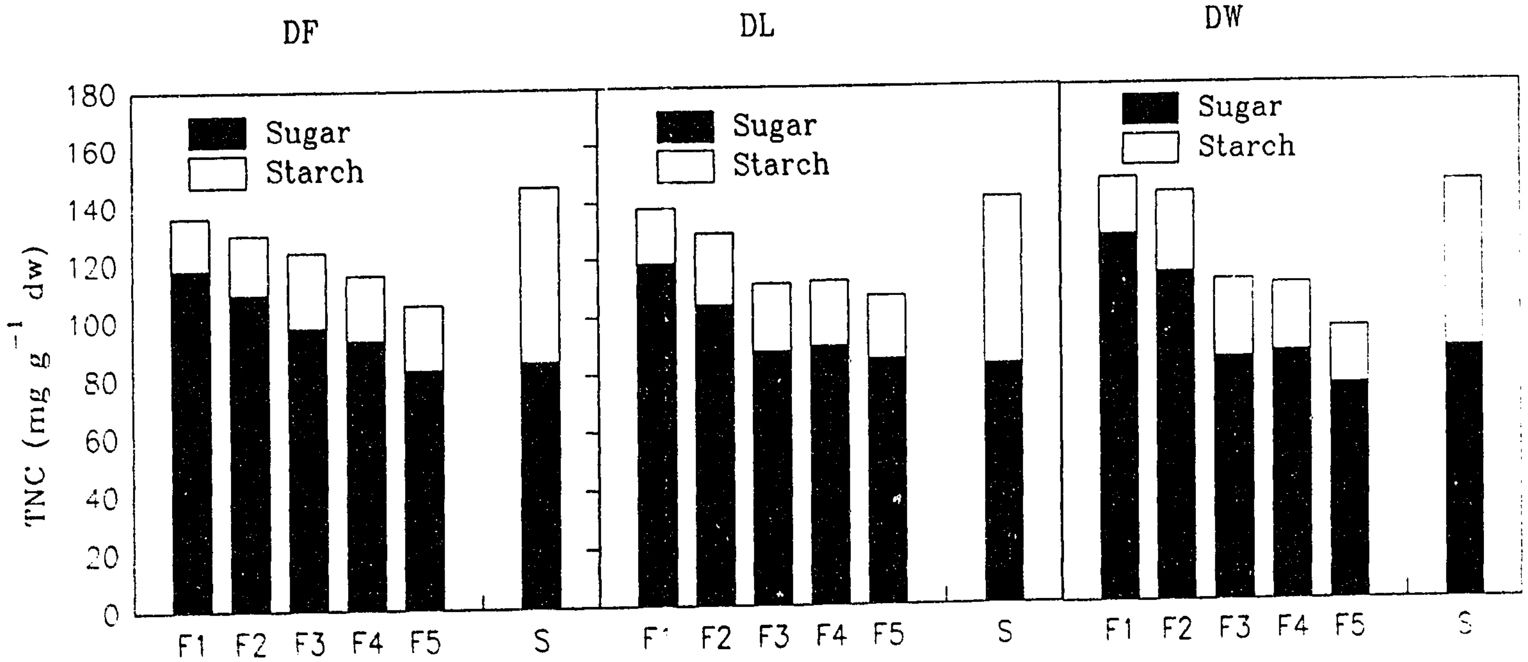

Figure 2-1. Total non-structural carbohydrates (TNC) in spring-lifted (S) and fall-lifted white spruce seedlings before (F1=Oct.), during (F=Nov., F3=Jan., F4=Mar.) and after cold storage (F5=May).

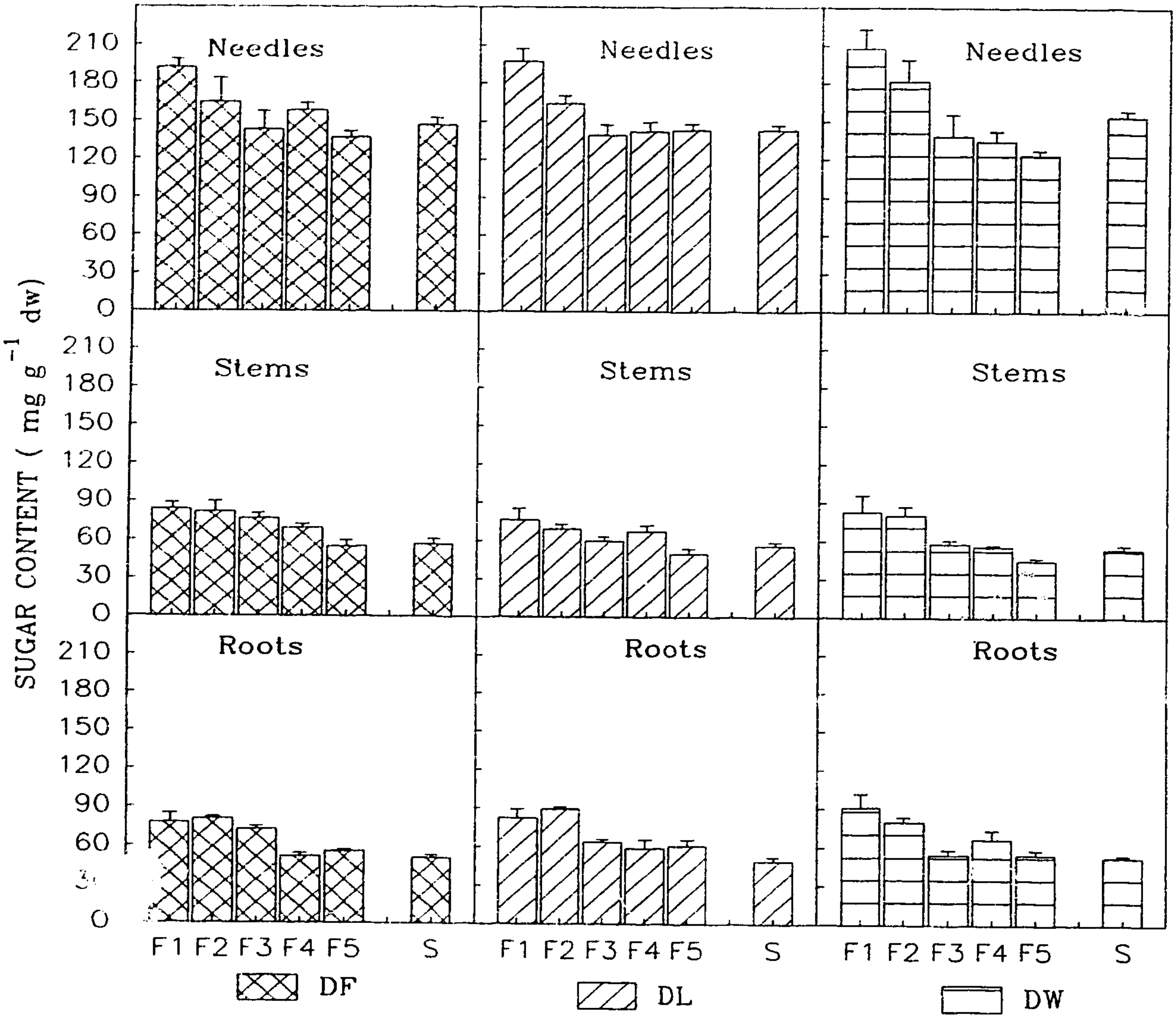

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Figure 2-2. Sugar content of spring-lifted (S) and fall-lifted white spruce seedlings before (F1=Oct.), during (F2=Nov., F3=Jan., F4=Mar.) and after (F5=May) cold storage. Means (n=5) and standard errors are shown.

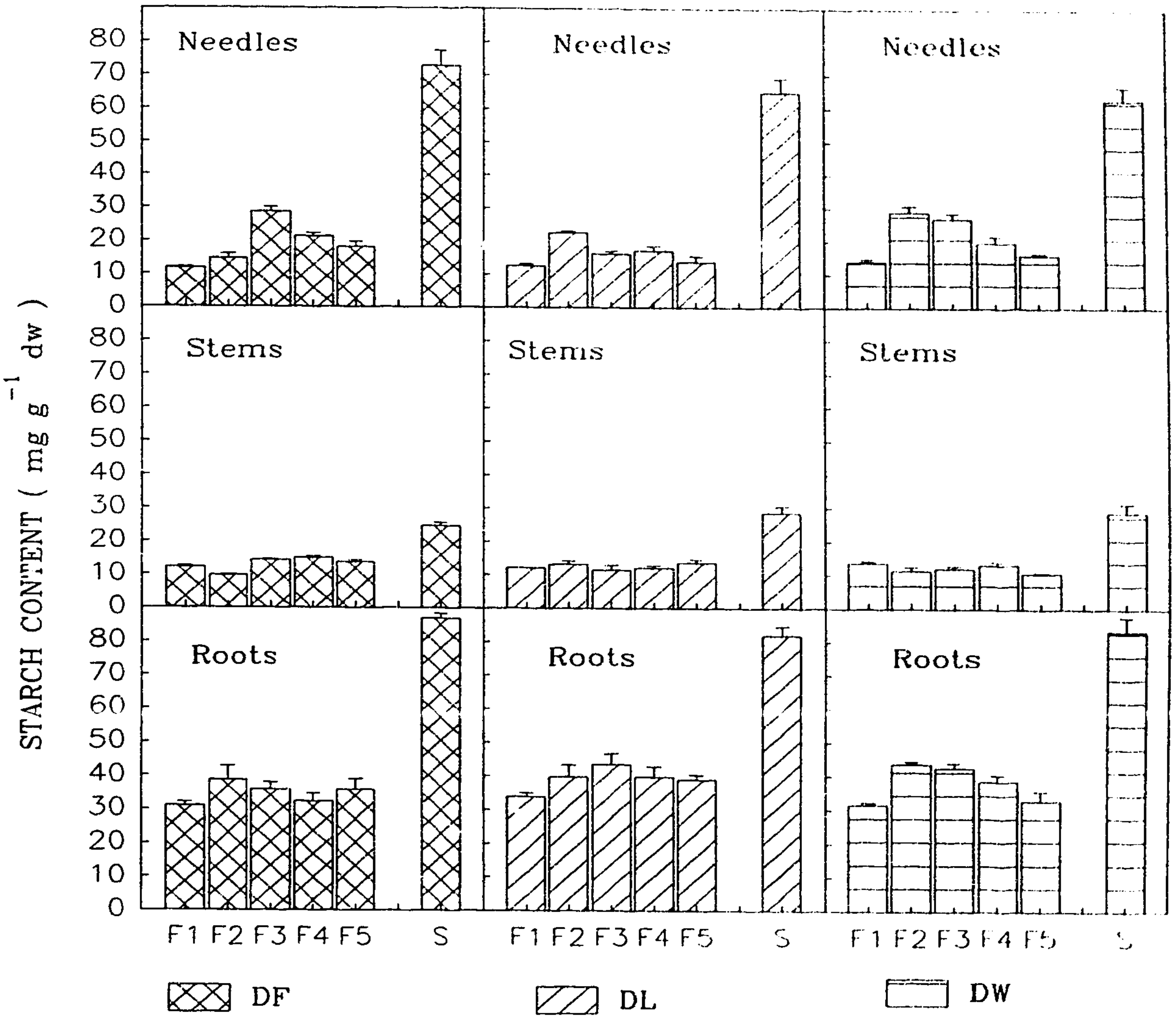

Figure 2-3. Starch content of spring-lifted (S) and fall-lifted white spruce seedlings before (F1=Oct.), during (F2=Nov., F3=Jan., F4=Mar.) and after (F5=May) cold storage. Means (n=5) and standard errors are shown.

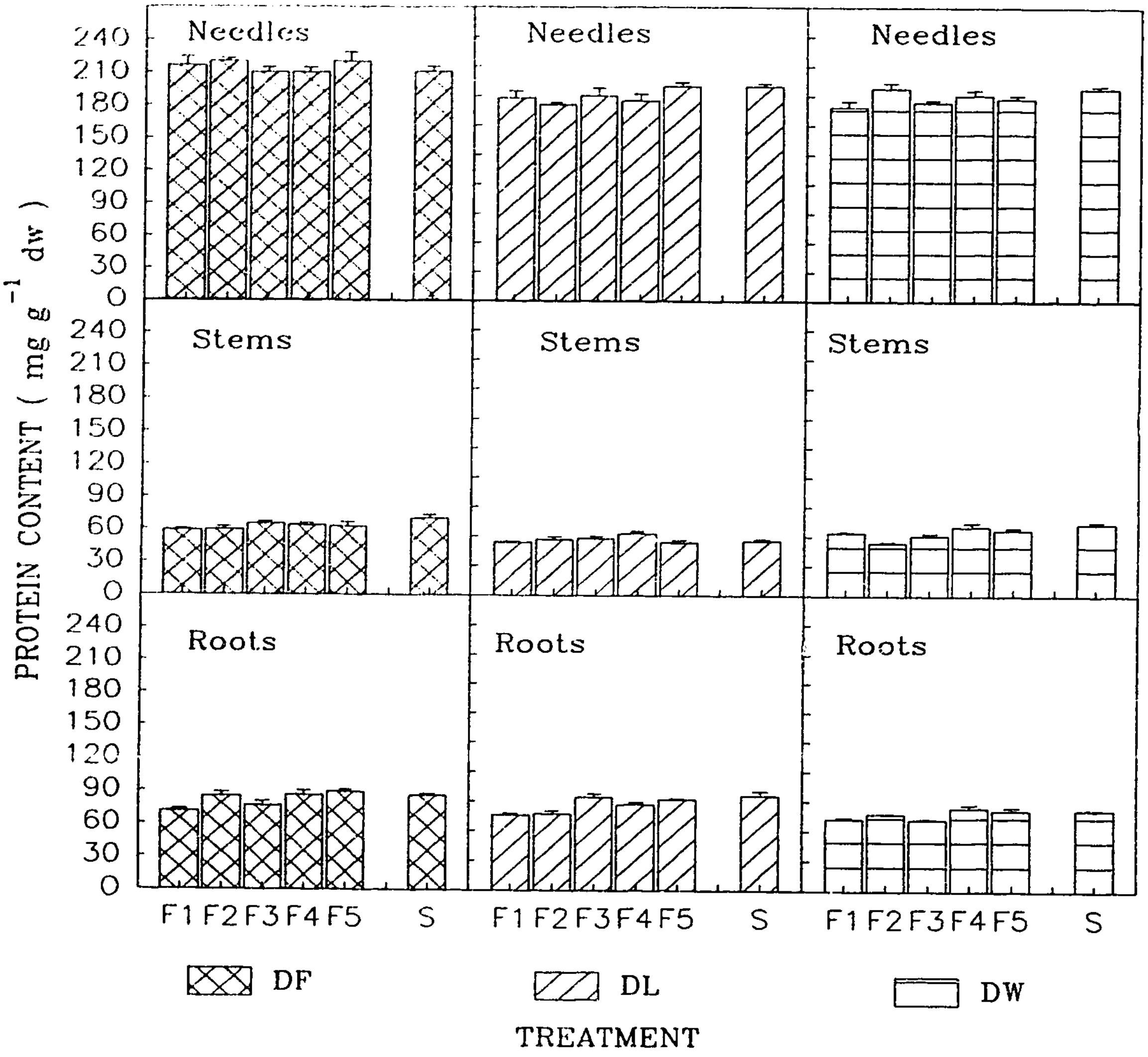

Figure 2–4. Protein content of spring-lifted (S) and fall-lifted white spruce seedlings before (F1=Oct.), during (F2=Nov., F3=Jan., F4=Mar.) and after (F5=May) cold storage. Means (n=5) and standard errors are shown.

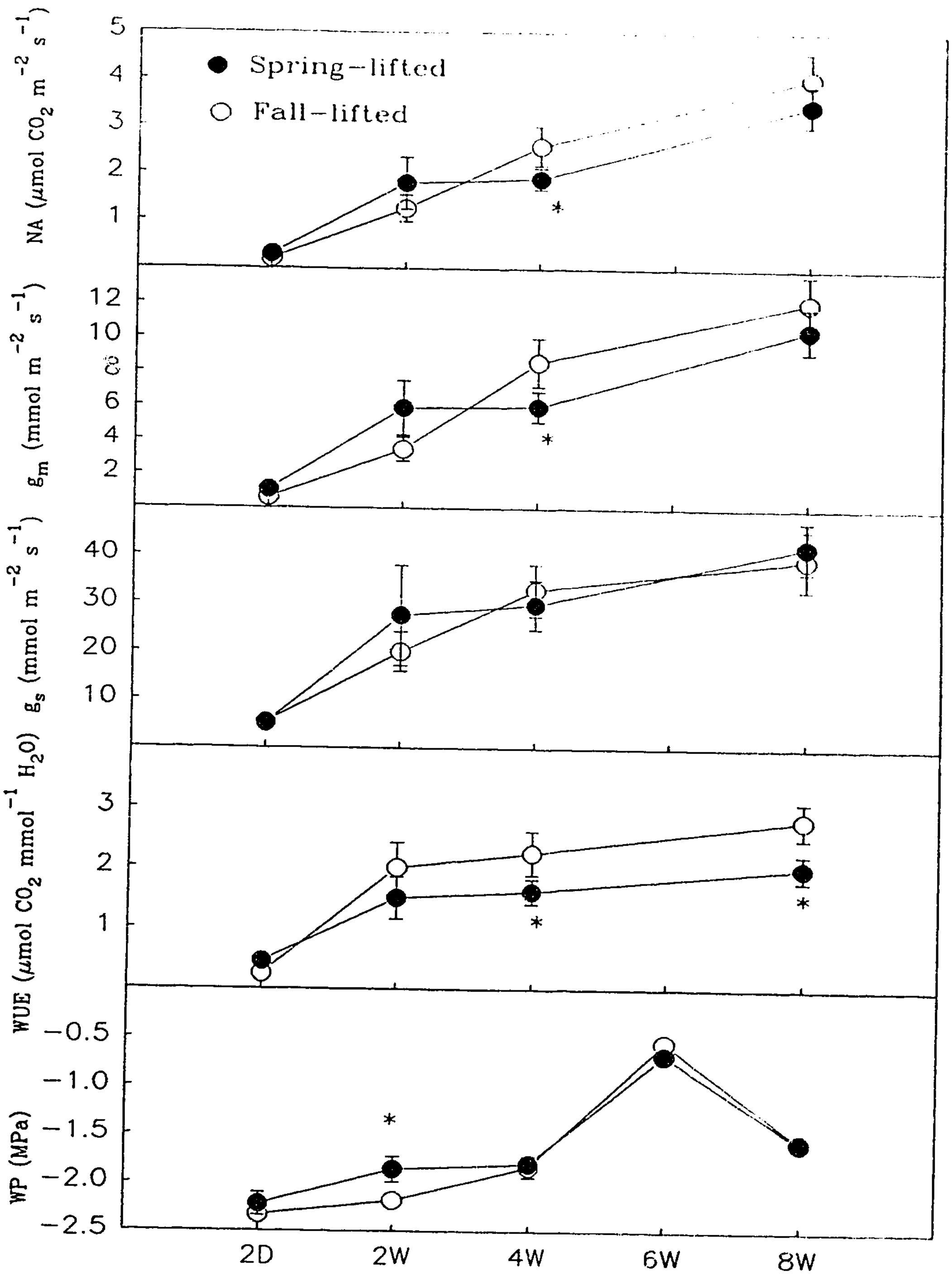

Figure 2-5. Gas exchange and water potential values in white spruce seedlings two days (2D), two weeks (2W), four weeks (4W), six weeks (6W), and eight weeks (8W) after planting in Calling Lake (DL). *. Significant difference ($p<0.05$). Means ($n=16$) and SE are shown.

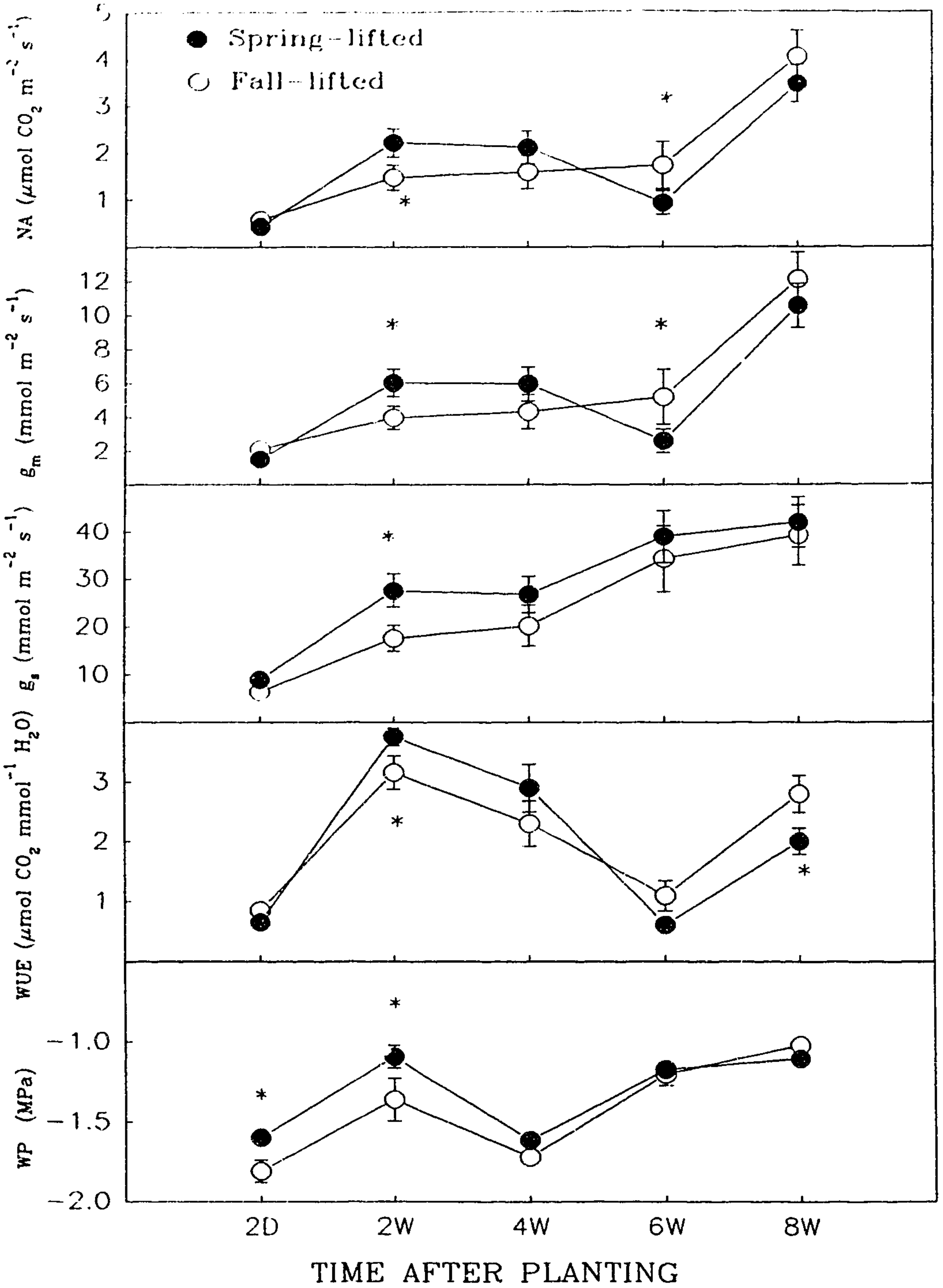

Figure 2-6. Gas exchange and water potential values in white spruce seedlings two days (2D), two weeks (2W), four weeks (4W), six weeks (6W), and eight weeks (8W) after planting in Fort Assiniboine (DW). * Significant difference ($p<0.05$). Means ($n=16$) and SE are shown.

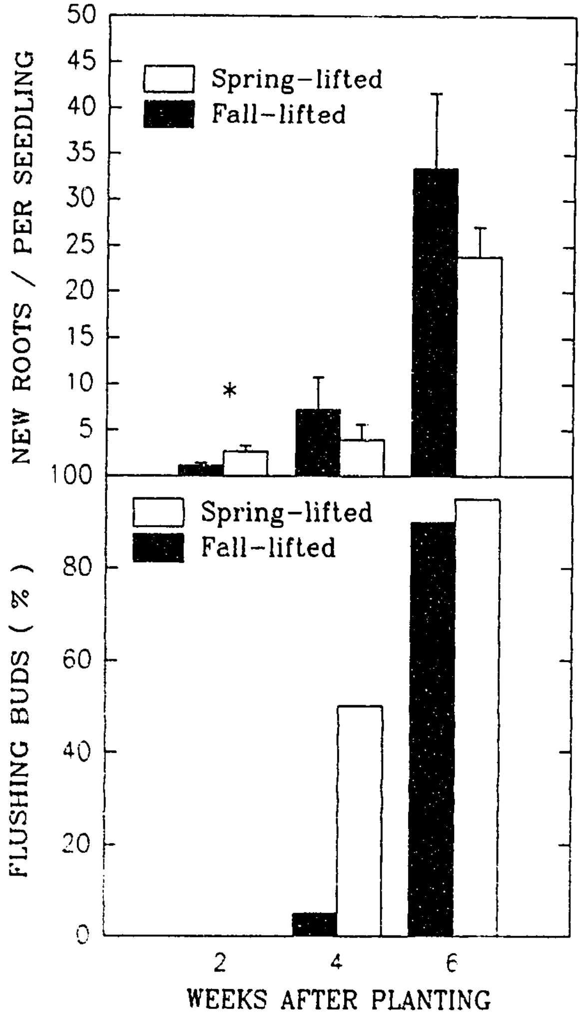

Figure 2-7. Means (n=16) of new root number and bud flushing two weeks, four weeks and six weeks after planting in Calling Lake (DL seedlot).

*. Statistically significant difference ($p<0.05$).

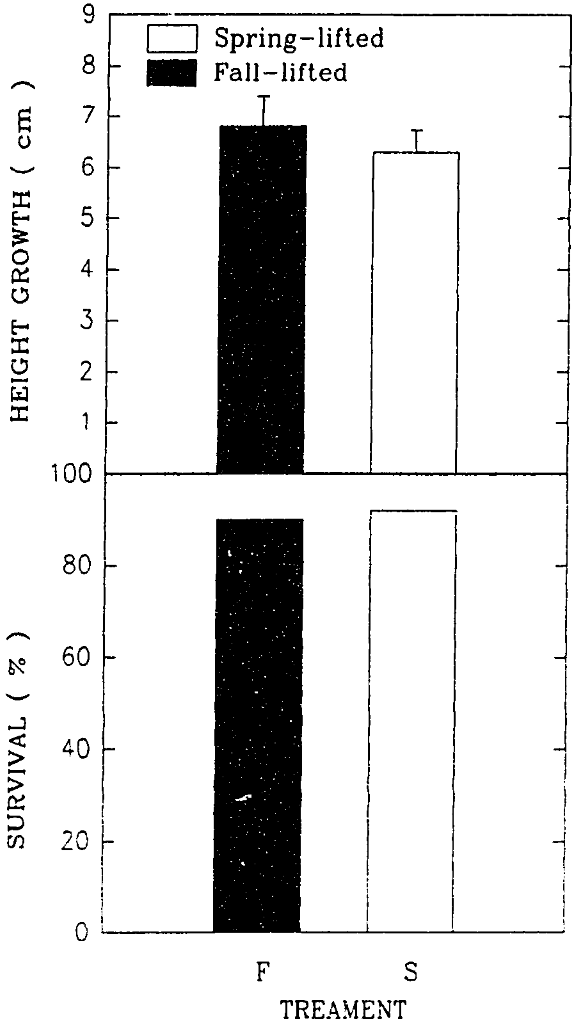

Figure 2-8. Mean height growth (n=40) and survival (%) of white spruce seedlings after one year growth in Calling Lake (DL seedlot). S=Spring-lifted, F=Fall-lifted.

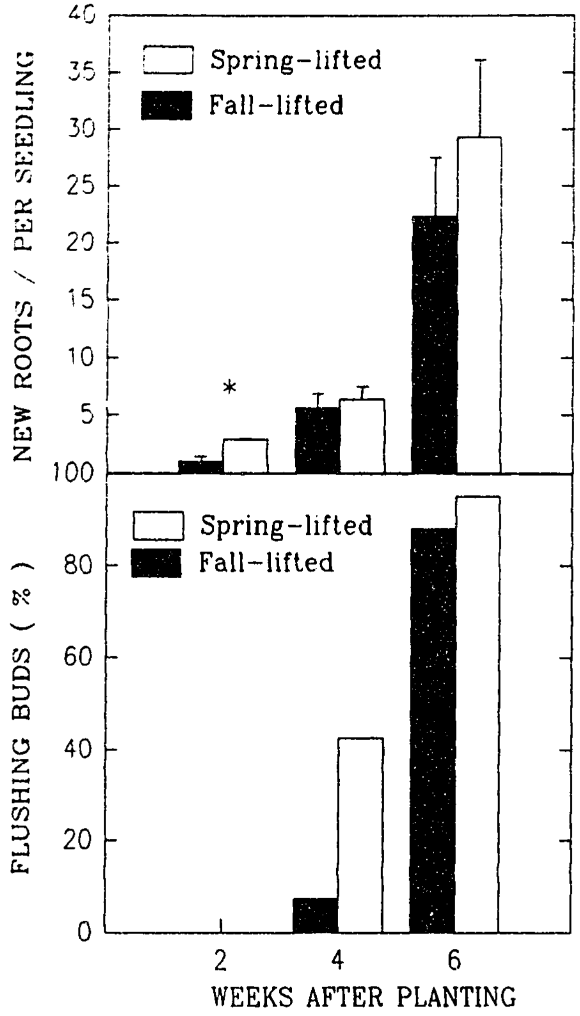

Figure 2-9. Means (n=16) of new root number and bud flushing two weeks, four weeks and six weeks after planting in Fort Assiniboine (DW). * Significant difference (p<0.05).

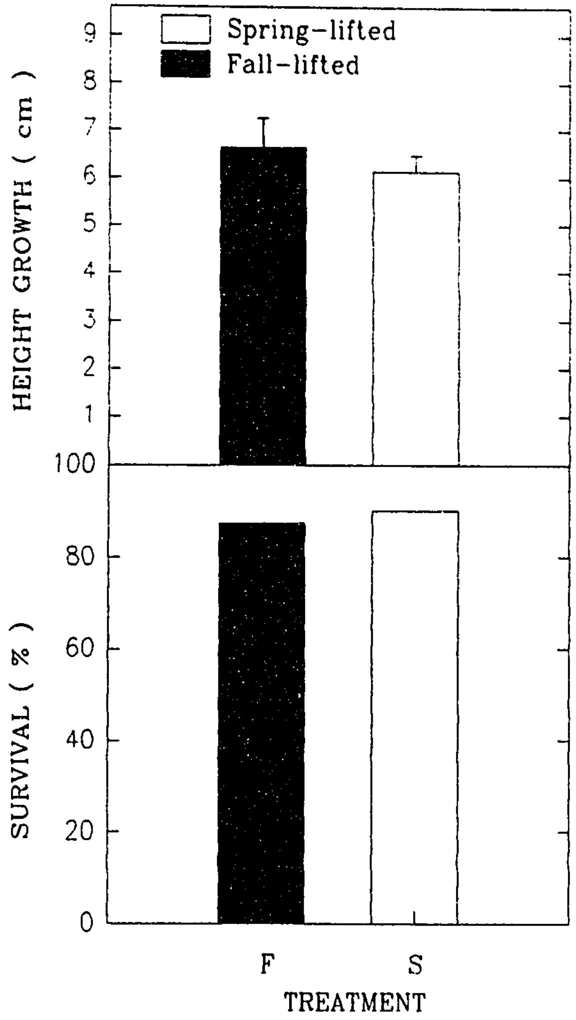

Figure 2-10. Mean height growth (n=40) and survival (%) of white spruce seedlings after one year growth in Fort Assiniboine (DW). S=Spring-lifted; F=Fall-lifted.

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# Chapter Three

## Water Relations and Gas Exchange of White Spruce Seedlings in Different Lifting Time and Water Stress Treatments

### 3.1 Introduction:

Water stress is one of the most important environmental factors affecting survival, growth and distribution of plants. Water stress develops often in newly planted conifer seedlings because of the poor contact between roots and soil and the absence of new absorbing roots (Sands 1984, Burdett 1990). This stress can result in limited initial growth of outplanted seedlings and higher mortality (Kozlowski and Davies 1975; Burdett 1990). Therefore, the successful establishment of newly planted seedlings depends largely on their drought resistance (Blake and Sutton 1987).

Water stress can influence plants in many different ways. The lower rate, or sometimes complete cessation, of photosynthesis is a common response of plants to water stress (Zwiazek and Blake 1989a; Kozlowski et al. 1991). The ability of newly planted seedlings to maintain photosynthesis under drought conditions is related to their water status (Kubiske and Abrams 1992), the treatments they experienced before stress (Etter and Carson 1973; Seiler and Johnson 1988) and the speed of expanding new roots (Burdett 1990). Current photosynthesis as well as reserve energy are important for the growth of new roots in newly planted seedlings (Johnson-Flanagan and Owens 1985; Philipson 1988; van den Driessche 1987). In turn, the rapid growth of new roots can help the seedlings overcome water stress, resulting in improved photosynthesis (Grossnickle and Reid 1984; Burdett 1990).

It has been shown that the reserve energy of seedlings can be reduced by cold storage (McCracken 1979; Ritchie 1982; Chapter Two) and that cold-stored seedlings show a slow recovery of photosynthetic capacity after planting (McCracken 1978; Chapter Two). However, these studies either did not attempt to or failed to relate the survival and growth of cold-stored seedlings in the field to their reduced photosynthesis and energy reserves. It is plausible that high photosynthetic rates and energy reserves are more important when seedlings are under severe stress.

The present study was conducted to test the hypothesis that cold-stored white spruce (_Picea glauca_ (Moench) Voss) seedlings which have lower rates of photosynthesis and lower energy reserves will not be able to survive as well under water stress conditions as spring-lifted seedlings. It is also hypothesized that the lower rates of photosynthesis in cold-stored seedlings are due to the deleterious effect of cold storage on seedling water status.

## 3.2 Materials and Methods

Two experiments were conducted to test the hypotheses separately in January and May of 1992.

### 3.2.1 Experiment in January

1.5 + 1.5 white spruce seedlings from a Whitecourt (54° 30', 115°05') seedlot (Seedlot number DW 62-22-5-83) were grown at Pine Ridge Nursery, Smoky Lake, Alberta according to the standard cultural practices in the nursery. In October 1991, some seedlings were lifted and placed in cold storage at -2°C to -5°C for overwinter. Another group of seedlings was left in the nursery beds for the winter. On January 14, 1992, about 300 seedlings from nursery beds were lifted. These seedlings, together with another 200 seedlings from cold storage, were shipped to the University of Alberta where they were thawed for one week in a dark growth chamber at 5°C. Fall- and January-lifted seedlings (27 from each lifting time) with similar shoot length and good root system were selected for the following experiment.

On January 22, nine seedlings from each lifting time were grown in three plastic containers (4 litres in volume) with aerated half-strength Hoagland's solution (Zwiazek and Blake 1989a) in a growth chamber under a constant temperature of 20°C (18-h photoperiod; light intensity of 450 $\mu$mol $m^{-2}$ $s^{-1}$). There were six seedlings, three replicates from each lifting time, in every container. Seedlings were exposed to -1.1 MPa or -2.7 MPa final osmotic stress and control (half-strength Hoagland's solution). Osmotic stress was induced by a stepwise increase in the concentration of PEG 3350 in the nutrient culture solution. PEG was increased from 0 (control), 100 and 100 g.$L^{-1}$ for the first 3 days, then an additional 0, 50 and 100 g.$L^{-1}$ for 3 days and another addition of 0, 50 and 100 g.$L^{-1}$ for 3 days. Nine days after planting, photosynthesis was recorded using a portable infrared gas analyzer system (Chapter Two) and the water relations parameters of shoots were measured using the pressure-volume technique with a pressure chamber (Tyree and Hammel 1972; Roberts et al. 1981).

The pressure-volume measurements were conducted on 7-8 cm segments of terminal shoots excised from seedlings. The cut ends of the shoots were placed in sealed beakers with distilled water and rehydrated overnight in the dark at 4°C. The xylem pressure potential ($\psi_x$) and weight loss from the cut shoots were recorded in simultaneous measurements from the fully rehydrated shoots after collecting xylem sap expressed by applying pressure in the pressure chamber. The resulting data were plotted as $1/\psi_x$ vs. cumulative volume of xylem sap expressed to obtain the pressure-volume curve. Osmotic potentials at full turgor ($\psi_o$) and at zero turgor ($\psi_{TLP}$) and symplastic volume ($V_o$) were determined from the graph. Turgor potentials (P) were estimated as the difference between water and osmotic potentials. The bulk modulus of elasticity ($\epsilon$) of cells was calculated as the change in P per unit change in V:

$$\varepsilon = \frac{dP}{dV/V_o} \qquad (3.1)$$

where dP represents the change in bulk turgor potential and dV is the change in water volume associated with the change in turgor (Roberts et at. 1981). $V_o$ is symplastic volume. The maximum bulk modulus of elasticity ($\epsilon_m$) was calculated by extrapolation to full saturation.

The experiment was conducted as a split-plot design. Because the measurements could not be carried out in a short period of time due to the large sample size, blocks were used to eliminate errors incurred by the time of measurement. There were three blocks in the experiment which represented three planting times, January 22, 24, and 26.

All data were analyzed by using analysis of variance (ANOVA) with the aid of the SAS computer package. A significant level of $p \leq 0.05$ was used in all cases. The model for the analysis was as follows:

$$Y = \mu + B_i + W_j + BW_{ij} + L_k + WL_{jk} + BL_{ik} + BWL_{ijk} + \varepsilon_l \qquad (3.2)$$

where

$\mu$ = population mean;
$B_i$ = blocks: i=1,2,3;
Wj = water stress levels: j=1,2,3;
$BW_{ij}$ = the errors to test W;
$L_k$ = the lifting times: k=1,2;
$WL_{jk}$ = the interaction between water W and L;
$BL_{ik}$ + $BWL_{ijk}$ = the error to test L;
$\epsilon_l$ = random error: l=1,2,3.

### 3.2.2 Experiment in May

In late April of 1992, seedlings from the nursery beds were lifted before bud break and placed in cold storage at 2°C. In early May, these seedlings and fall-lifted seedlings were shipped to the University of Alberta and kept in a growth champer at 5°C until May 20 when the seedlings were planted for a greenhouse experiment.

Before planting, water status parameters were measured in 9 seedlings of each stock type using the pressure-volume techniques described previously, and then 280 seedlings were planted in 140 7-inch pots containing Metro-Mix growing medium. Each pot had two seedlings, one fall-lifted and one spring-lifted. Immediately after planting, the seedlings were placed in a greenhouse and well watered. From the 140 pots, 20 pots were randomly selected and divided into two groups of 10. Seedlings of the first group which were used as a control were watered every two days. Seedlings in the second group were not watered for 20 days to induce a gradual water stress and then released from the water stress by watering them every two days for a six day period. During the treatments, photosynthesis was measured in either two day or three day intervals.

Photosynthesis was measured as described earlier (Chapter Two) and all measurements were conducted between 2:00 and 5:00 pm. The gas exchange was carried out on laterals using the previous year's foliage. To diminish the errors in determining leaf areas due to the different growing speeds of newly flushed buds, the flushing buds on the laterals were removed whenever observed.

From the remaining 120 pots, 90 pots were randomly selected to undèrgo a gradual water stress for 20 days followed by a stress release for 6 days as described above. On the same days as photosynthesis measurements, shoot water potentials were measured in 9 out of the 90 pots using a pressure chamber. For the measurement before releasing seedlings from water stress, the excised shoots were collected for further pressure-volume analysis after the water potential measurements. The seedlings in the last 30 pots were subjected to severe water stress by watering them only once after being planting until June 26, 1992 (27 days after planting). Thereafter, the seedlings were watered every second day until the fall of 1992 when their mortality was recorded.

The water relations data for fall- and spring-lifted seedlings before planting and after subjected to gradual water stress for 20 days were tested for statistical difference using analysis of variance (ANOVA). A significant level of $p \leq 0.05$ was used. The model for the data analysis is as follows:

$$Y=\mu+T_i+L_j+TL_{ij}+e \qquad (3.3)$$

where

$\mu$=population mean;

T=treatment (before and after planting): i=1,2;

L=lifting time: j=1,2;

$TL_{ij}$=the interaction between T and L;

$\epsilon$ = random error.

## 3.3 Results

### 3.3.1 Experiment in January

Osmotic potential at full turgor ($\psi_o$,) and at turgor loss point ($\psi_{TLP}$), symplastic volume ($V_o$) and net assimilation (NA) were not significantly different between January-lifted and fall-lifted seedlings in the control and the osmotic stress treatments (Table 3-1). However, $\psi_o$, $V_o$ and NA, but not $\psi_{TLP}$, were decreased significantly by the osmotic stress treatments. There was no significant interaction between water stress and lifting time for any parameter measured.

### 3.3.2 Experiment in May

Analysis of variance showed that $\psi_o$ and $\psi_{TLP}$ increased, and $V_o$ and $\epsilon_m$ declined significantly ($p \leq 0.05$) after planting with the gradually increasing water stress for 20 days in the greenhouse (Table 3-3). There was a significant interaction between lifting time and water stress for $\psi_{TLP}$, which showed that the $\psi_{TLP}$ of cold-stored seedlings increased significantly after the gradual water stress while the $\psi_{TLP}$ of spring-lifted seedlings did not change. Except for $\psi_{TLP}$, other parameters of water relations did not show significant differences between fall- and spring-lifted seedlings and there was no interaction between lifting date and water stress (Table 3-3).

In all 10 measurements in well watered pots, spring-lifted seedlings had higher NA, $g_m$, $g_s$, and WUE values than fall-lifted seedlings in well watered pots (Fig. 3-1). In the seedlings subjected to gradual water stress, parameters of gas exchange of both fall- and spring-lifted seedlings declined with the decrease in seedling water potential, and then increased after rewatering (Fig. 3-2). Generally, NA, gm, gs, and WUE of spring-lifted seedlings were higher than those of fall-lifted seedlings in the first 16 days after planting and the [illegible] days following stress relief. However, no significant differences were found in these parameters in the last measurement before releasing from stress (20 days after planting). At that time, NA, $g_m$ and WUE reached almost zero at the water potentials of about -1.7 MPa for spring-lifted seedlings and -1.8 MPa for cold-stored seedlings. The values of $g_s$ were still about 50 (mmol $m^{-2}$ $s^{-1}$) and water potentials were still higher than $\psi_{TLP}$ (Fig. 3-2 and Table 3-3). Generally, no differences were found in water potentials between fall- and spring-lifted seedlings. Higher water potentials in spring-lifted seedlings were observed in the last measurement before and first measurement after release (Fig. 3-2).

NA was positively and linearly related to gm and this was independent of lifting dates and water stress treatments (Fig. 3-3). The average coefficient of determination for NA regressed on $g_m$ was 0.99. Figure 3-4 shows that NA and $g_s$ appeared to be more closely related at low $g_s$ than at high $g_s$. The average coefficient between NA and $g_s$ was 0.46.

The observation of mortality in seedlings for 27 day water stress revealed that 6 out of 30 fall-lifted and cold-stored seedlings died at the end of the experiment. No mortality was observed among spring-lifted seedlings (Fig 3-5).

## 3.4 Discussion

Most conifers in the temperate zone can tolerate environmental stresses well in January when they are in deep dormancy (Burr 1990). Seedlings lifted in January showed a better storage capacity and higher root growth potential than those lifted in the fall and spring (Ritchie et al. 1985). At the present study, water relations parameters of fall-and spring-lifted seedlings were similar when the seedlings were subjected to water stress. This may indicate that two months of cold storage did not decrease the ability of seedlings to resist water stress.

The values of maximum bulk modulus of elasticity ($\epsilon m$) were similar in spring- and fall-stored seedlings, while fall-lifted seedings had a lower $\psi_o$ and $\psi_{TLP}$ values before being planted in a greenhouse in May. It has been assumed that plants with the higher cell elasticity (lower values in bulk modulus $\epsilon$) and the greatest ability to maintain positive turgor would have the best survival and growth potential (Tyree 1976; Tyree and Karamanos 1981; Hinckley et al. 1983;

Robichaux 1984). When elasticity is higher, cell volume must undergo a relatively large decrease before turgor reaches zero compared to a cell of identical size but with lower elasticity (Ritchie and Shula 1984). As $\epsilon$ values will change with the turgor potential and the values of $\epsilon$ at high turgor potential more nearly reflect the elastic properties of the plant cell walls, maximum elasticity $\epsilon_m$ is recommended for comparing elasticity of tissues (Tyree 1976). Osmotic potentials are also important in turgor maintenance. Seedlings with higher osmotic potential at turgor loss point ($\psi_{TLP}$) will loss their turgor sooner than those with lower osmotic potential under water stress (Abrams 1988; Choi 1992). Therefore, fall-lifted seedlings should be more resistant to water stress than spring-lifted seedlings before planting in May because they had higher $\psi_o$ and $\psi_{TLP}$. However, fall-lifted seedlings can not maintain their drought resistance in a gradual water stress by decreasing the $\psi_{TLP}$ quickly (Table 3-3).

Osmotic potential at full turgor ($\psi_o$) and symplastic volume ($V_o$) declined significantly under PEG-induced water stress in the January experiment. The decline in osmotic potentials under water stress has been related to the accumulation of soluble carbohydrates, amino acids, organic acids and inorganic ions in cells (Ford and Wilson 1981; Clarke and Durley 1981; Jacomini et al. 1988; Zwiazek and Blake 1989b; Zwiazek 1991). A reduction in cell volume under water stress has been observed in many species as an adaption to water stress (Cutler et al. 1977; Newton et al. 1989). This reduction in cell volume which permits a lower cellular osmotic potential and an increased capacity for turgor maintenance may help plants resist water stress. In the May experiment, similar results which show a decline in cell volume were observed in both fall- and spring-lifted seedlings when subjected to gradual water stress in the May experiment. However, unlike the results in the January experiment, $\psi_o$ and $\psi_{TLP}$ increased significantly after 20 days of gradual water stress in both fall- and spring-lifted seedlings. It is possible that the lack of osmotic adjustment in the May experiment during the gradual water stress could be due to dilution within the seedlings as the shoots were elongating (Colombo 1987) or a decrease of the reserved soluble carbohydrate (Meyer and Splittstoesser 1971; Etter and Carlson 1973) and other solutes (Meyer and Splittstoesser 1971; Yamashita 1984) to support the rapid growth of new roots and shoots. It is also speculated that, instead of the decline in osmotic potential, the reduction of cell volume is a more universal response of plants to water stress and that the responses of plants to water stress vary with the differences in stress patterns.

Fall- and January-lifted seedlings had similar net assimilation (NA) in the January experiment whether they were subjected to osmotic stress or not. This may indicate that the effects of cold storage on seedling photosynthetic capacity are similar to natural conditions in the first two months after lifting or that two months of cold storage is not long enough to affect photosynthesis. However, when the seedlings were planted in the greenhouse in May, after 7 months of cold storage, the fall-lifted, cold-stored seedlings had lower NA compared to spring-lifted seedlings in both well irrigated and moderately water-stressed conditions. The slower recovery of photosynthesis after cold storage has also been observed in other species (McCracken 1978). In an experiment with Pinus sylvestris seedlings, Mattsson and Troeng (1986) observed faster recovery of photosynthesis in seedlings stored at 2°C than at 4°C. Therefore, the speed of the recovery of photosynthesis after cold storage depends on both the period of storage and temperature in cold storage. The slower recovery of photosynthesis in the seedlings which experienced prolonged cold storage may be because the photosynthetic apparatus of the chloroplasts in these seedlings kept in the continuous darkness and low temperature requires a longer time to acclimate after exposure to light (McCracken 1978).

Water balance may be another important factor related to the recovery of photosynthesis (Troeng 1991). Plants with higher resistance to water stress can maintain a positive NA at lower water potentials (Kubiske and Abrams 1992) and recover photosynthesis faster when released from water stress (Kriedemann and Downton 1981). It appears that spring-lifted seedlings have higher resistance to water stress because they showed higher photosynthetic rates after they were subjected to water stress and subsequently released from stress. However, it is difficult to attribute the higher photosynthesis in spring-lifted seedlings to water status since the measured parameters reflecting water status were generally similar between fall- and spring-lifted seedlings or better in fall-lifted seedlings before exposure to a gradual water stress.

Photosynthetic capacity can also be affected by strong carbon sinks, such as developing foliage, fruits, and reproductive structures (Jarvis and Leverenz 1983). If the carbon sinks are suddenly removed (Gifford and Evans 1981) or reduced by starch accumulation (Thorne and Koller 1974; Acock and Allen 1985), photosynthesis is hindered to some degree. Cold-stored and spring-lifted seedlings were in the same stage of development when planted in the greenhouse and cold-stored seedlings would have had a larger carbon sink because of lower tissue starch content (Chapter Two). Therefore, it is unlikely that lower photosynthetic rates of cold-stored seedlings are related to the stage of development or strength of the carbon sink.

It is widely accepted that water stress can reduce photosynthesis (Kriedemann and Downton 1981; Kozlowski et al. 1991). A decline in NA of both fall- and spring-lifted seedlings with the decrease in shoot water potentials was also observed in the present study. Photosynthesis stopped in both fall- and spring-lifted seedlings long before their turgor loss point (Fig. 3-2 and Table 3-3). It is generally agreed that the degree and speed of the reduction in photosynthesis under water stress are closely related to genetic characteristics of plants (Boyer 1970; Kubiske and Abrams 1992), patterns of the stress (Jones et al. 1981) and environmental history (Seiler and Johnson 1988; Zwiazek and Blake 1989a). However, whether the decrease in photosynthesis is due to stomatal closure which limits the diffusion of $CO_2$ into the cell or to mesophyll resistance which includes the diffusion of $CO_2$ across the cell wall, membrane, and the cytoplasm to the carboxylation sites, and various biochemical and photochemical reactions (Bradford and Hsiao 1982; Edwards and Walker 1983), is still debated (Levitt 1981; Kozlowski et al. 1991). More recent reports indicate that mesophyll, as opposed to stomatal, conductance to $CO_2$ and its responses to moisture stress (Melzack et al. 1985; Teskey et al. 1986; Guehl et al. 1991; Maier and Teskey 1992; Kubiske and Abrams 1992), and to other environmental conditions (Teskey et al. 1986) are primarily responsible for the changes in photosynthesis. The strong linear relationship (average $R^2=0.99$) between NA and $g_m$ in all treatments in the May experiment provides additional evidence to support the above findings. Although a general trend of decline in NA with the decline in $g_s$ was also observed (Fig. 3-4), the NA shows no close linear relationship with $g_s$ (average $R^2$=0.46) and this is especially true at high stomatal conductance. It is clear that photosynthesis tends to be relatively independent of stomatal conductance at high $g_s$, and stomatal control over photosynthesis may become evident only at low $g_s$.

The cold-stored white spruce seedlings with lower stomatal conductance have been assumed to tolerate water stress better because of the reduced rate of water loss (Blake 1983). The lower $g_s$ of cold-stored white spruce seedlings was also observed in the present study, but this lower $g_s$ did not help them improve their survival under water stress. In contrast, the higher mortality of cold-stored seedlings, when subjected to severe water stress, demonstrated that they were less resistant to water stress. There is no doubt that water loss can be alleviated by stomatal closure. However, the ability of seedlings to survive stress will also depend on their ability to maintain turgor and on the availability of reserve materials needed for continuous respiration when stomata close and photosynthesis ceases under severe water stress. The significant interaction which showed an increase in $\psi_{TLP}$ of cold-stored seedlings after a gradual stress for 20 days (Table 3-3) implies that cold-stored seedlings have an inferior ability for osmotic adjustment. Although starvation for energy due to photosynthesis shutting down in water stress can seldom kill a normaly growing tree directly (Levitt 1981), it might induce death in cold-stored seedlings because their reserve energy, mainly stored starch (Chapter Two) and current photosynthesis are already low. Higher starch content in spring-lifted seedlings may help these seedlings survive water stress by producing more soluble sugars to decrease the osmotic potential (Jones et al. 1981; Table 3-3), providing more energy to maintain respiration (Marshall 1985) and occupying space to reduce cell shrinkage (Levitt 1981).

It was also suggested in other studies that higher carbohydrate levels are very important in helping seedlings grow and survive under water (Ronco 1973; Marshall 1985;), ozone (Andersen et al. 1991) and other environmental stresses (Webb 1981; Friend et al. 1992). On the other hand, there are reports in which the concentration of carbohydrates can not be related to the root growth potential (Ritchie 1982; Cannell et al. 1990), shoot growth or survival (Omi and Rose 1990; Chapter Two). The function of carbohydrates may not always be obvious if their concentration is above a certain threshold level (Puttonen 1986) and the function of carbohydrates is probably heavily dependent on environmental conditions (Gordon and Larson 1970).

The results of this study indicate that lower photosynthetic rates of fall-lifted seedlings are not directly related to their water status. Spring-lifted seedlings can survive gradual water stress better than fall-lifted seedlings possibly due to their better ability to adjust osmotically and sufficient energy supply.

TABLE 3-1. ANOVA summary for the osmotic potential at full turgor ($\psi_o$), osmotic potential at turgor loss point ($\psi_{TLP}$), symplastic volume ($V_o$), and net assimilation (NA) of white spruce seedlings subjected to water stress treatment in January, 1992.

| | | $\psi_o$ | | $\psi_{TLP}$ | | $V_o$ | | NA | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | P | MS | P | MS | P | MS | P |
| Water Stress (W) | 2 | 6.93 | <0.05 | 19.64 | 0.06 | 10.41 | <0.01 | 9.13 | <0.001 |
| Lifting Time (L) | 1 | 9.45 | 0.208 | 0.14 | 0.899 | 0.01 | 0.280 | 0.73 | 0.164 |
| W x L | 2 | 6.16 | 0.350 | 18.49 | 0.09 | 0.002 | 0.773 | 0.95 | 0.063 |

TABLE 3-2. Water relations and photosynthesis in white spruce seedlings subjected to water stress in the experiment in January, 1992.

| Treatments | | $\psi_o$ | $\psi_{TLP}$ | $V_o$ | NA |
|---|---|---|---|---|---|
| W1 | F | -1.74 (0.04) | -2.57 (0.06) | 0.59 (0.05) | 3.79 (0.13) |
| | J | -1.70 (0.02) | -2.41 (0.04) | 0.54 (0.07) | 4.01 (0.22) |
| W2 | F | -1.62 (0.10) | -2.36 (0.17) | 0.21 (0.04) | 0.04 (0.01) |
| | J | -1.61 (0.09) | -2.51 (0.12) | 0.21 (0.02) | 0.13 (0.03) |
| W3 | F | -1.98 (0.10) | -2.71 (0.10) | 0.31 (0.04) | 0.07 (0.01) |
| | J | -1.72 (0.14) | -2.46 (0.10) | 0.28 (0.04) | 0.08 (0.04) |

Note: Mean values (n=9) are given with standard errors in parenthesis. W1, control; W2, -1.1 MPa; W3, -2.7 MPa; F, fall-lifted; J, Janaury-lifted; $\psi_o$, osmotic potential at full turgor (MPa); $\psi_{TLP}$ osmotic potential at turgor loss point (MPa); $V_o$ symplastic volume; NA, net assimilation ($\mu$mol $CO_2 \cdot m^{-2} \cdot s^{-1}$).

TABLE 3-3. Parameters on water relations of white spruce seedlings in May, 1992 before (T1), and after (T2) a gradually induced water stress in greenhouse for 20 days.

| Treatments | | $\psi_o$ | $\psi_{TLP}$ | $V_o$ | $\epsilon_m$ |
|---|---|---|---|---|---|
| T1 | F | -1.92 (0.57) | -2.91 (0.37) | 1.95 (0.03) | 26.87 (2.11) |
| | S | -1.88 (0.39) | -2.70 (0.49) | 2.26 (0.19) | 24.84 (1.97) |
| T2 | F | -1.63 (0.74) | -2.57 (0.56) | 1.10 (0.49) | 15.47 (2.57) |
| | S | -1.70 (0.47) | -2.74 (0.47) | 1.22 (0.55) | 16.04 (1.71) |

ANOVA SUMMARY (P values)

| | $\psi_o$ | $\psi_{TLP}$ | $V_o$ | $\epsilon_m$ |
|---|---|---|---|---|
| Treatment | 0.0009 | 0.0048 | 0.0001 | 0.0001 |
| Lifting Time | 0.5060 | 0.4414 | 0.1762 | 0.2317 |
| T x L | 0.1294 | 0.0028 | 0.5467 | 0.4212 |

Note: Mean values (n=9) are given with standard errors in parenthesis. F, fall-lifted; S, spring-lifted; $\epsilon_m$, maximum bulk modulus of elasticity; $\psi_o$, osmotic potential at full turgor (MPa); $\psi_{TLP}$, osmotic potential at turgor loss point (MPa); $V_o$, symplastic volume.

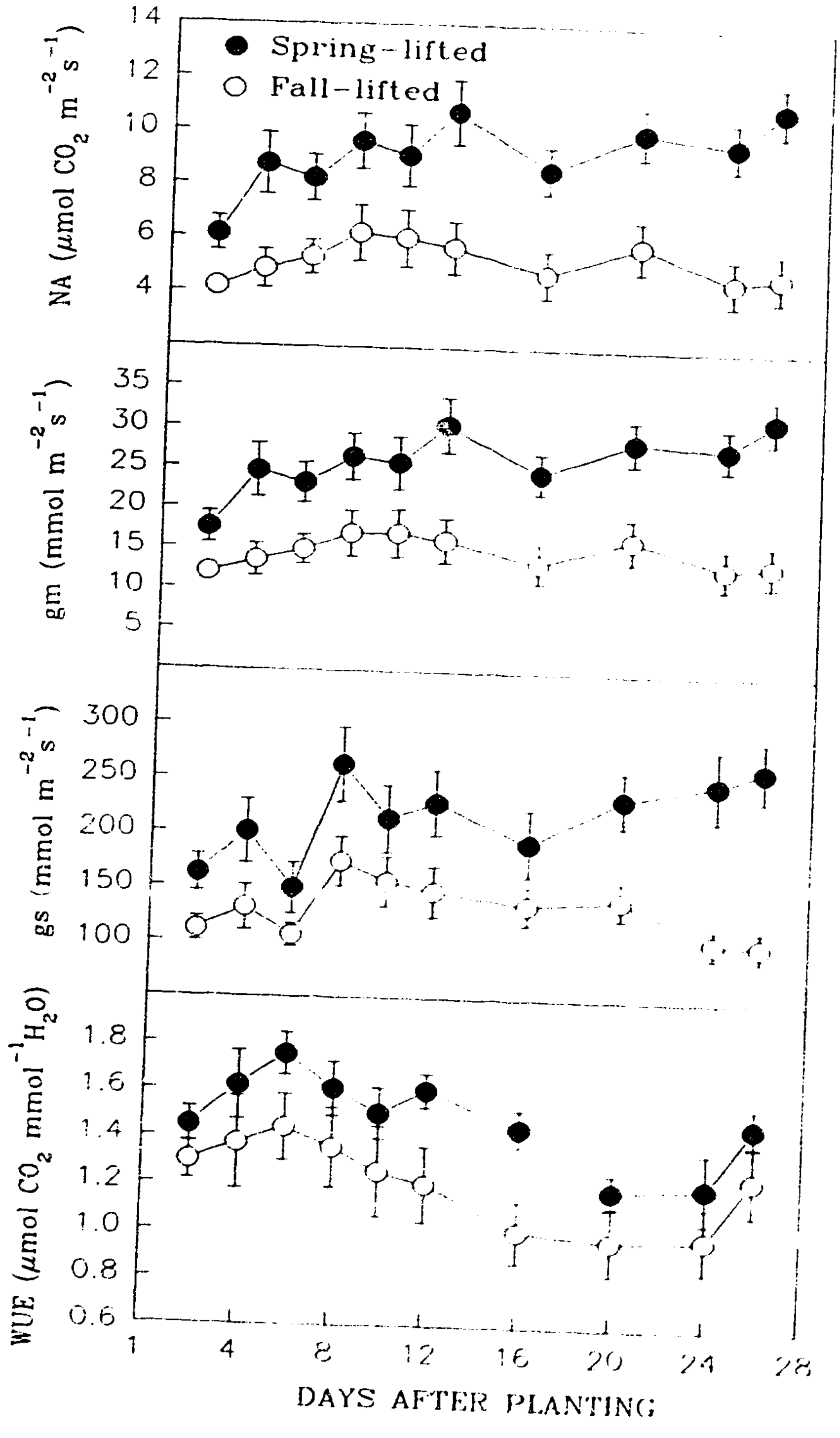


Figure 3-1. Gas exchange values of white spruce seedlings in well-watered pots in May, 1992.

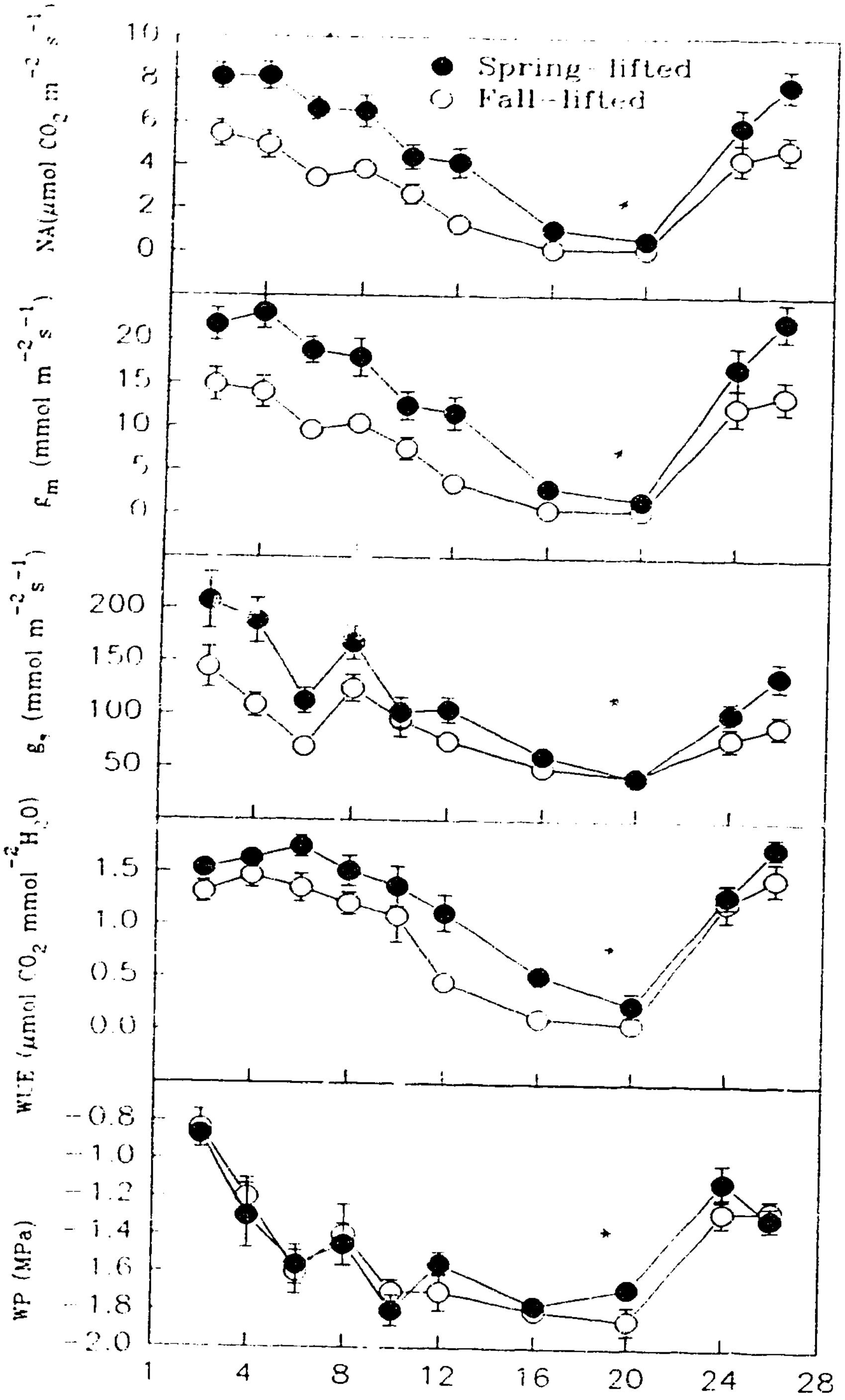


Figure 3-2. Gas exchange values of white spruce seedlings subjected to gradual water stress for 20 days and released from water stress for 6 days.
* Watered after the measurement.

Figure 3-3. The relationship of NA with $g_m$ of white spruce seedlings in (A) pots which were not watered after planting for 20 days and then released for 6 days, and in (B) pots which were well watered after planting.

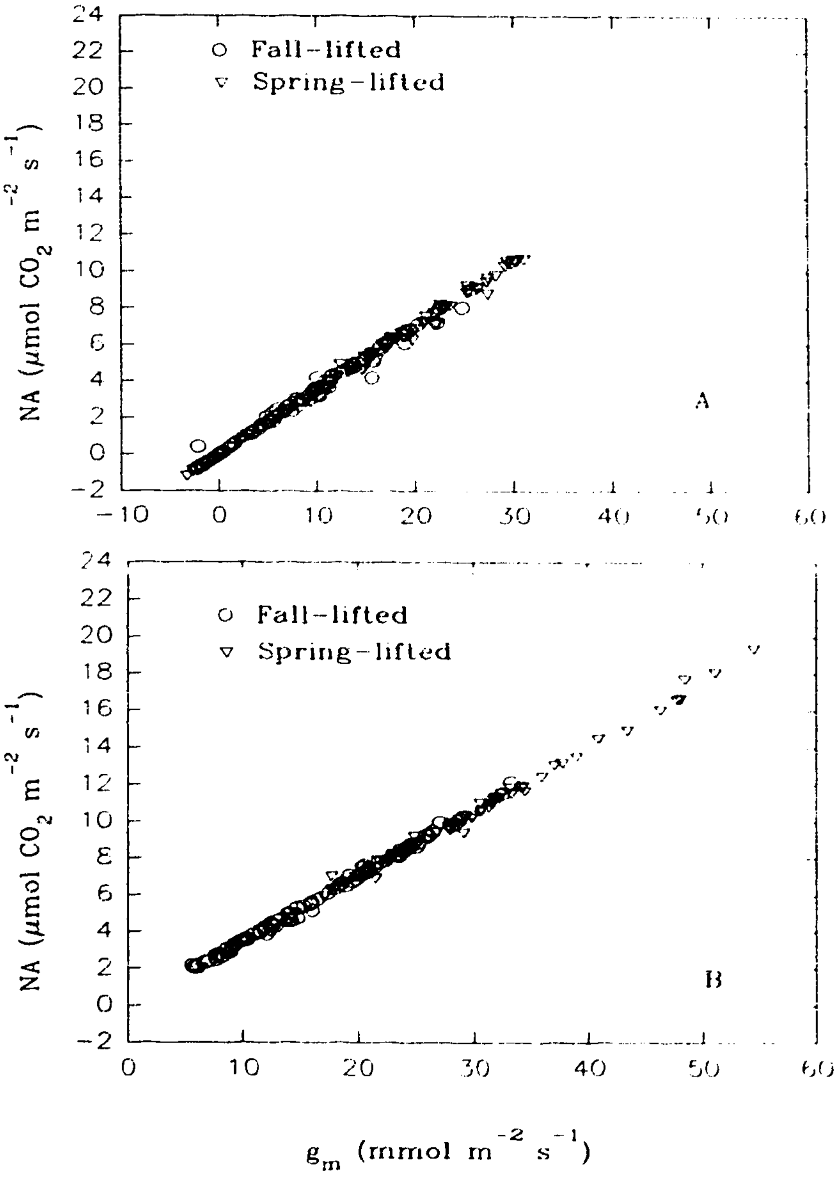
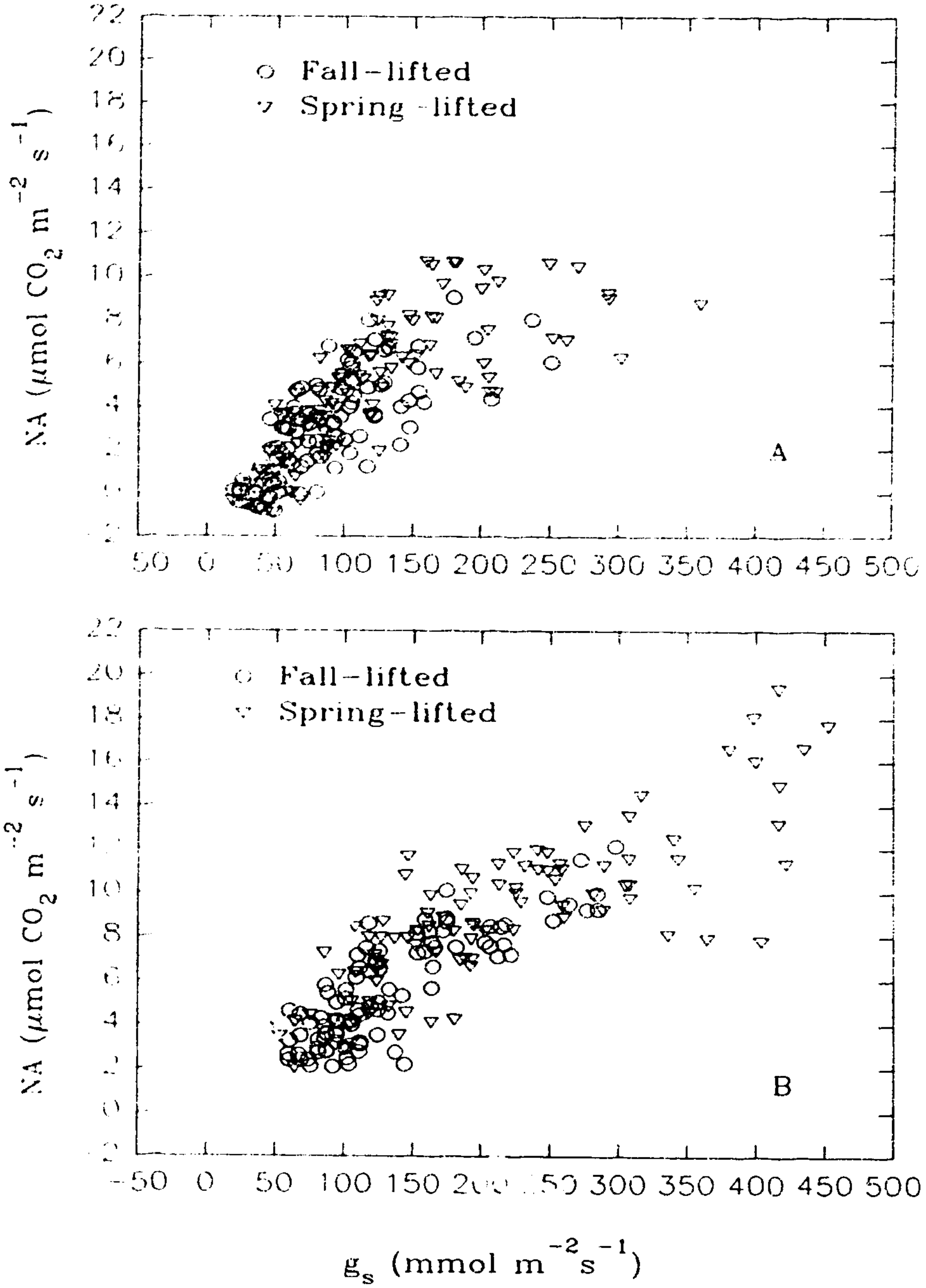


Figure 3.4. The relationship of NA with $g_s$ of white spruce seedlings in (A) pots which were not watered after planting for 20 days and then released for 6 days, and in (B) pots which were well watered after planting.



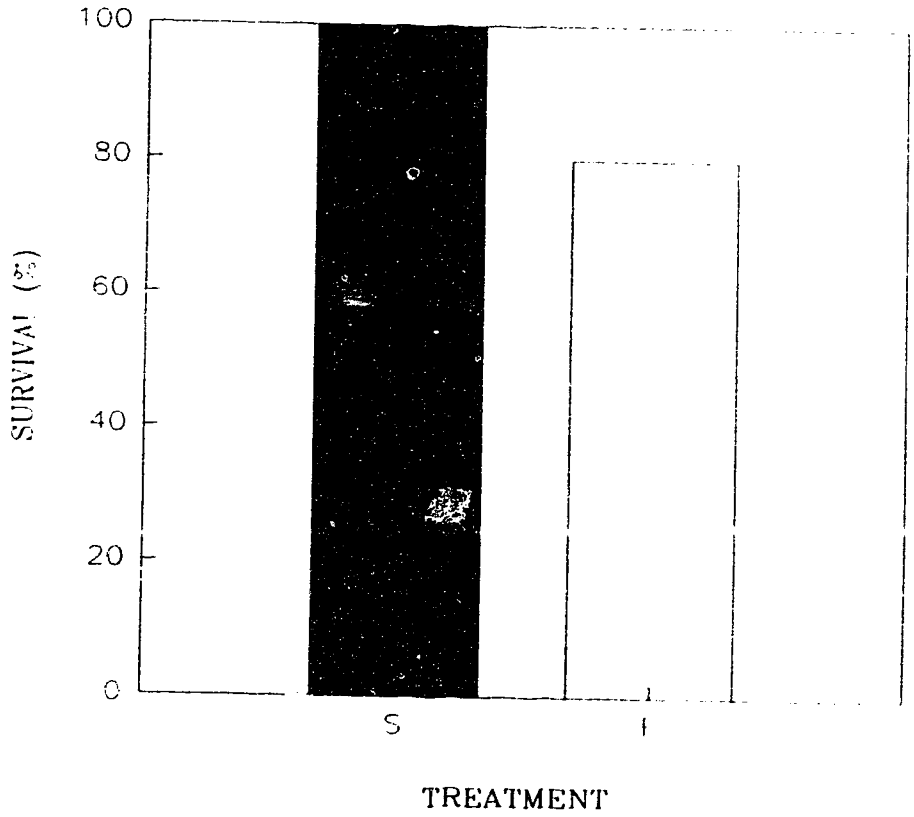


Figure 3-5 Survival of spring-lifted (S) and fall-lifted (F) white spruce seedlings in the pots which were subjected to a gradual water stress for 27 days in a greenhouse in May, 1992.

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# Chapter Four

## General Discussion

Reforestation over extensive areas has necessitated the use of cold-storage which allows the forester to extend the period of field planting (Hoching and Nyland 1971). There are variable impacts of prolonged cold storage (7-8 months in Alberta) on seedling quality (Hoching and Nyland 1971; Ritchie 1987). This study focused on the effects of prolonged cold storage on seedling physiology, and compared fall- and spring-lifted seedlings with respect to field performance and resistance to water stress.

Compared with fall-lifted seedlings, spring-lifted seedlings had higher total non-structural carbohydrates (TNC) before outplanting, and after outplanting they showed higher initial photosynthesis, faster bud flushing and growth of new roots. Spring- and fall-lifted seedlings had similar survival and shoot growth in the field during first growing season, but spring-lifted seedlings did survive better under severe water stress.

TNC decreased during cold storage. This decrease is mainly due to the decrease of soluble sugars, suggesting that soluble sugars are the major form of energy consumed by white spruce seedlings in cold storage to maintain respiration requirements. No differences in soluble sugar contents were found between fall- and spring-lifted seedlings. Starch may have accumulated during winter or early spring, which resulted in two-fold higher starch content in spring-lifted seedlings. The high starch content is the reason for high TNC in spring-lifted seedlings before outplanting. Protein content of seedlings had little change during cold storage and no significant difference in protein contents was found between fall- and spring-lifted seedlings before outplanting. The responses of seedling sugar, starch and protein contents to prolonged cold storage were similar in all 3 examined seedlots.

Photosynthesis, bud flushing and root growth of outplanted seedlings were inhibited by prolonged cold storage. However, these problems and lower TNC did not affect shoot growth and survival of outplanted cold-stored seedings if the environmental conditions for their growth are not too severe. As fall-lifted seedlings require a longer time to adjust to the new environmental conditions, it is expected that severe environmental conditions would affect more the survival and growth of fall-lifted seedlings than those of spring-lifted seedlings.

When subjected to water stress, seedlings stored in cold storage for two months had similar photosynthetic rates and water status to seedlings lifted in January. After prolonged cold storage, however, seedlings showed retarded ability to recover photosynthesis. However, the difference in photosynthesis between fall- and spring-lifted seedlings does not appear to be related to their water status. Although fall-lifted seedlings did not have altered water status immediately after cold storage, their ability to acclimate to water stress is poorer (chapter three). This might be one of the reasons for the higher mortality in fall-lifted seedlings during severe water stress. Another reason for the high mortality might be the lack of sufficient energy supply due to both lower reserve energy and lower net assimilation after planting.

Based on the results of the present study, the following recommendations may be made.

1. Seedlings can be successfully stored for 7 months at -2°C, and both fall- and spring-lifted seedlings can be used in reforestation on some forest sites in Alberta. However, it would be preferable to supply fall-lifted seedlings to the sites where soil thaws early because they need longer time to acclimate themselves to a new environment.

2. Spring-lifted seedlings have a high ability to tolerate

gradual water stress. They should be considered for planting on drier sites. However, the lifting time in spring should be carefully controlled because planting active seedlings may result in high mortality (Ritchie and Dunlap 1980).

3. More research is needed to address the possible effects of cultural practices before cold storage, and the handling procedure during and after cold storage on the outplanting survival of cold-stored seedlings.

4. Modifying seedlings to induce higher carbohydrate content through normal cultural practices in the nursery, such as fertilization and irrigation, might be desirable but difficult. It has been reported that different cultural regimes do not influence seedling carbohydrate concentration (Taylor and Van Den Ende 1970; Ericsson 1979; Nguyen et al. 1990; Gholz and Cropper 1991). Decreasing the temperature in cold storage, which can reduce the respiration of seedlings, could be a way to curtail carbohydrate loss during cold storage. However, too low a temperature may be harmful to seedlings, especially to the roots that are not protected by soil. Therefore, the methods which shorten the time it takes cold-stored seedlings to adapt to new environmental conditions are essential for enhancing the quality of fall-lifted seedlings.

There are still some questions which have not been answered by this study. The ability of plants to carry on protein synthesis under water stress is possibly related to their drought resistance (Bewley 1981). Although the total protein content is not affected by cold storage significantly, we do not know the capability of seedlings in the two stock types for protein synthesis under water stress. Further research in this area may allow us to understand better the reasons for higher mortality of cold-stored seedlings subjected to severe water stress. The responses of seedlings to water stress vary with the stress patterns (Jones et al 1981). The results observed from the present study using a gradual water stress may not necessarily reflect a unique response of fall- and spring-lifted seedlings. There is a need for more information concerning differences between fall- and spring-lifted seedlings with respect to planting shock which is often manifest as rapid and severe water stress.

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