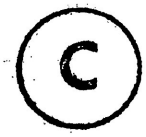
PHOTOSYNTHETIC EFFICIENCY OF FROZEN-STORED *PICEA GLAUCA* SEEDLINGS IN RELATION TO ROOT PRUNING, ROOT GROWTH, AND SHOOT MOISTURE STRESS

by



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A Thesis submitted in partial fulfillment
of the requirements of Lakehead University
for the degree of Master of Science in Forestry.

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ABSTRACT

This study examines the influence of duration of frozen storage (-2 C) and root pruning on photosynthetic efficiency, shoot moisture stress and root growth of white spruce (Picea glauca (Moench) Voss) seedlings planted in a glasshouse. Photosynthetic efficiency was measured using infra-red gas analysis, and shoot moisture stress by the pressure chamber technique. Root growth was determined using trees planted in glass-faced root boxes.

Photosynthetic efficiency of root pruned and non-pruned trees which were not frozen was significantly greater 2 and 4 weeks after planting than that of stock frozen 92 days. Rates of photosynthesis of trees which had been frozen for 50 days were inexplicably lower than other storage treatments up to four weeks after planting. After six weeks photosynthetic efficiency was high regardless of duration of storage. Shoot moisture stress of seedlings stored 92 days remained significantly greater than non-frozen stock throughout the experiment, in spite of greater root growth by those frozen 92 days. Root pruning had a detrimental influence on all aspects of seedling physiology examined: photosynthetic efficiency was lower, shoot moisture stress greater and root growth was slower than in non-root pruned seedlings. Root growth was not strongly correlated with photosynthetic efficiency.

DEDICATION

For Carol

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INTRODUCTION

Planting is an important form of forest regeneration in Ontario. In 1980, over 58 million trees were planted, of which white spruce (Picea glauca (Moench) Voss) accounted for more than 24 percent (Ontario Ministry of Natural Resources 1981).

To deal with so many trees it becomes advantageous to store stock until planting is possible. Frozen storage through winter is an especially useful tool in Ontario, where it permits planting early in the spring when nursery beds remain inaccessible (Deffenbacher and Wright 1954, Mullin 1966). Frozen storage also helps to avoid damage in the nursery beds by animals or disease (Hocking and Nyland 1971). However, nursery stock can be damaged if trees are not physiologically dormant when put into frozen storage (Hocking and Nyland 1971, Glerum 1976, Mullin and Parker 1976). Even trees which are dormant may suffer reduced survival and slower growth as a result of storage (Aldhous 1964, Hocking and Nyland 1971, Mullin and Parker 1976).

With few exceptions (cf. Stone 1967, Lavender and Wareing 1972, McCracken 1978) most published reports have not investigated the influence of storage on seedling physiology. In the study presented in this thesis, three aspects of seedling physiology, namely photosynthetic efficiency, shoot moisture stress and root growth, were observed with respect to the effects of frozen storage.

Root loss when seedlings are lifted is inevitable, and it is augmented by root pruning at grading - a standard nursery practise (Armson and Sadreika 1974). Root pruning is used to

make stock handling more efficient and to improve the ease and quality of planting, even though root pruning has in many cases adversely affected seedling physiology (Sutton 1967, Wareing et al. 1968, Brown 1969). As a result, this study was designed to investigate the effects of 0, 50 and 92 days of frozen storage (-2 C) and root pruning on the physiological condition of white spruce seedlings. The physiological condition is studied in terms of photosynthetic efficiency, shoot moisture stress and root growth.

LITERATURE REVIEW

The Effect of Cold Storage on Gas Exchange

If trees are in good condition when placed in storage, two important factors which will determine the effect of storage on photosynthesis are prolonged absence of light and exposure to low temperatures.

Lavender and Wareing (1972) examined the influence of dark storage on Douglas-fir (Pseudotsuga mensiezii (Mirb.) Franco) seedlings lifted in the fall and stored for six weeks at 2 C, either in the dark or with illumination. Mortality was 11.5 percent following storage in the dark. When storage was conducted with a daily period of illumination (nine hour photoperiod at 6000 lux provided by fluorescent and incandescent sources) mortality was reduced to about 2.5 percent. Even a light intensity of 600 lux, at which appreciable levels of photosynthesis would not be expected, seedlings suffered only five percent mortality. Lavender and Wareing considered that storage with intermittent exposure to light allowed a photo-dependent stimulus to be produced in the needles which improved post-planting survival. They hypothesized that levels of gibberellins were responsible for improved survival when storage was conducted in the light. Gibberellin levels are increased by red light through the phytochrome system even at light intensities allowing only low rates of photosynthesis (Leopold and Kriedemann 1975).

Lavender and Wareing's work can also be interpreted in

terms of photosynthetic activity after transplanting. McCracken (1978) found that radiata pine (Pinus radiata D. Don) and mugo pine (P. mugo Turra) seedlings suffered a loss of photosynthetic ability following cold storage. He hypothesized that cold storage resulted in the disorganization of the internal structure of needle chloroplasts, which resulted in lower rates of photosynthesis. McCracken did not observe chloroplast structure. However, Perry and Baldwin (1966) found that, in winter, chloroplasts become disorganized and dispersed in cells of Picea, and Neilson et al. (1972) measured the resultant decrease in photosynthesis which occurs after exposure to freezing temperatures. The reduction of photosynthesis following cold storage may in addition be caused by a disruption of chloroplast structure as the result of darkness. Etiolation is caused by the absence of light and disrupts chloroplast structure (Packer et al. 1967), which should inhibit photosynthesis. Dark storage thus places trees into conditions unfavourable for photosynthesis to take place after planting.

Another indication of the possible influence of cold storage influencing post-planting rates of photosynthesis is seen in the effects of the exposure of nursery stock to low temperatures. Pharis et al. (1972) examined the effect of periods of low temperature on photosynthesis. Three-year-old ponderosa pine (Pinus ponderosa Laws.) seedlings, grown 12 months at 23 C, were exposed for 1, 4, or 17 days to temperatures of 3 C. Photosynthesis was monitored following transfer back to 23 C conditions. The chamber provided an 8 hour photoperiod with a total light intensity of about 13,000 lux (incandescent

and fluorescent light sources). Photosynthesis was significantly affected for up to one week after cold exposure. After one day at 3 C, photosynthesis increased by between five and 15 percent in comparison to pre-treatment rates. The rates of photosynthesis of seedlings exposed to 3 C for four or 17 days were reduced by about 20 to 30 percent respectively. Thus, exposure to temperatures of 3 C for even short periods can reduce rates of photosynthesis when warmer temperatures are resumed.

Pharis et al. (1972) did not determine whether the decrease in photosynthesis following exposure to cold was due to closure of the stomates or was caused by a slowing of the rate at which photosynthesis occurred due to biochemical factors. With respect to this question, Christersson (1972) studied the effect of low temperature on the gas exchange of Norway spruce (Picea abies (L.) Karst) and Scots pine (Pinus silvestris L.) seedlings. Six-month-old seedlings were grown for 3 months in a greenhouse at about 20 C before cold acclimatization at 3 C for three months. Following acclimatization, trees were returned to 20 C. Immediately upon being returned to the warm environment, transpiration rates of acclimatized Norway spruce and Scots pine were as much as 50 percent less than those of seedlings not exposed to cold. However, the transpiration rates of spruce seedlings increased rapidly. After three to five days, cold acclimated spruce transpired at the same rates as those not exposed to cold. Pine seedlings did not experience such rapid increases in rate of transpiration, and did not achieve the rates of transpiration of warm-grown seedlings until after 12 to 14 days.

According to Christersson's results, exposure to cold may restrict gas exchange by causing stomatal closure. This may be why Pharis et al. (1972) observed reduced rates of photosynthesis. In comparison, McCracken (1978) suggested that following cold storage in the dark there is a loss of stomatal control. Possibly, stomatal physiology may be affected by dark storage in such a way that the closure mechanism is temporarily disrupted, over-riding the tendency for cold exposure to promote closing of the stomates.

The Influence of Roots on Photosynthesis

Roots can influence photosynthesis by their role in the control of moisture stress. In one instance, it was found that maximum rates of photosynthesis in Douglas fir and ponderosa pine normally occurred below 10 bars shoot moisture stress (Cleary et al. 1978). When shoot moisture stress exceeded 10 bars the rates of photosynthesis declined, until at 20 bars photosynthesis was only 40 percent of the maximum rates. Photosynthesis was significantly reduced in sitka spruce (Picea sitchensis (Bong.) Carr.) by shoot moisture stress over 18 bars (Watts and Neilson 1978).

Roots, in addition to their role in regulating photosynthesis by control of plant moisture stress, also synthesize cytokinins which are important in the biochemical regulation of photosynthesis. Cytokinins are translocated to the leaves, where they promote the activity of photosynthetic enzymes (Wareing et al. 1968, McDavid et al. 1976, Okoro and Grace 1976). Rates

of photosynthesis have been decreased by root pruning in maize (Zea mays L.) (Wareing et al. 1968) and pea (Pisum sativum L.) (McDavid et al. 1976). In both cases, root pruned seedlings did not photosynthesize at rates comparable to those with intact root systems, but, when the leaves of pruned seedlings were sprayed with cytokinin, photosynthesis increased. These results support the hypothesis that roots are able to regulate photosynthetic rates by means of cytokinin production which occurs in the root tips.

The Influence of Root Pruning on Root Activity

When nursery stock is lifted, large root systems are often reduced in size by chopping off roots that are overly long. This root pruning or trimming is done to facilitate the handling of seedlings as well as to improve the ease and quality of planting. Root pruning is also done when the trees are in the seedbeds, by running horizontal and vertical blades through the soil of the nursery beds.

Root pruning generally induces a greater proliferation of roots than would form on seedlings with intact root systems. Brown (1969) examined the influence of root pruning on the subsequent root development of one-month-old Scots pine seedlings. Thirty days after pruning he found that the average length of white lateral roots was significantly greater on seedlings whose lateral roots had been pruned to half their original length than when no roots were removed. Similarly, Owston and Seidel (1978) reported that ponderosa pine seedlings whose roots had been

pruned produced a greater dry weight of roots than did trees with intact root systems. They observed that root pruning stimulated the initiation of lateral roots. Rook (1971) found that the rates of root growth of pruned (by wrenching) and non-pruned radiata pine seedlings in their first growing season were similar - but there were large differences in root form. Trees with undisturbed root systems had long taproots while wrenched trees had a mass of fibrous roots.

Sutton (1967) and MacDuff (1979) examined the influence of root pruning on the subsequent regrowth of the root systems of white spruce seedlings. Sutton found that partial root pruning, in which either the lateral roots were pruned to within 5 cm of the tap root or in which the taproot was cut off 10 cm below the root collar, induced a greater proliferation of roots than occurred in non-pruned trees. In contrast, MacDuff found that root growth, as measured in root boxes, was greatest by white spruce seedlings which had not been pruned.

Severe root pruning can be detrimental to subsequent root development. When Sutton (1967) removed all laterals from the taproot of three-year-old white spruce seedlings, the total amount of new root tissue was significantly less than for partially- or non-pruned stock. Larson (1975) found that red oak (Quercus rubra L.) seedlings whose taproots were severed just 2.5 cm below their root collar had significantly lower root dry weight following planting, as the result of reduced numbers and lengths of new root tissue in comparison to trees pruned 7.5, 15.0 or 20.0 cm below the root collar. Brown (1969) questioned the desirability of severe root trimming Scots pine seedlings prior

to field planting, as he found that the least amount of new root formed on seedlings pruned most severely.

Evidence exists that root pruning can be harmful to seedlings by reducing subsequent root system development. However, little information is available on its effect on white spruce.

The Effect of Root Pruning on Plant Moisture Stress

The development of moisture stress in plants is controlled by the balance between water uptake by roots and water loss by transpiration. Root pruned seedlings will be unable to provide as much moisture as non-pruned seedlings after planting, because fewer old lignified and suberized roots will be present to absorb water (Kramer 1949). In addition, non-pruned seedlings resume root growth more quickly, as they possess root tips ready to elongate while pruned trees must initiate new roots before elongation can begin. It is only after root pruning has stimulated the development of large numbers of new roots that pruned seedlings will be better able to provide moisture than non-root pruned seedlings (McCracken 1978).

MATERIALS AND METHODS

Plant Materials

Thirty-three hundred two-year-old (2-0) white spruce seedlings from the Thunder Bay Forest Station were lifted by hand using a spade on November 1, 1978. All seedlings came from about fifteen metres of each of two adjacent nursery beds containing trees from the same seedlot of the Thunder Bay District of the Ontario Ministry of Natural Resources. On average a sample of 60 trees were 14.4 cm tall (S.D. = 0.4 cm)[1] and had a root collar diameter of 3.0 mm (S.D. = 0.8 mm).

Seedling Treatments

Immediately after lifting, seedlings were placed in polyethylene bags containing damp, milled sphagnum moss and placed in cool storage (4 C) at Lakehead University. On November 2nd seedlings within ± 17.5 percent of mean shoot length and root collar diameter were selected for study. Following grading, the root systems of half of the selected trees were pruned by excising portions of lateral roots more than three cm from the point of attachment to the thickest lateral root, according to the method of Sutton (1967). The oven-dry weight and proportion of the root system removed by pruning is shown in Appendix A. A total of 540 seedlings were prepared - half of which were root pruned trees. Seedlings had their roots wrapped in damp sphagnum moss and were

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[1] S.D. standard deviation

double-bagged in polyethylene within a kraft paper bag. Each of three bags contained 180 trees - 90 pruned and 90 non-pruned.

After 36 days in cool storage the seedlings of one bag were removed and planted in a mixture consisting of one part peat, one part milled sphagnum moss, one part perlite and two parts sandy loam. Thirty seedlings of each root pruning treatment were grown in root boxes and placed glass face down at an angle of 60 degrees from the vertical, and sixty seedlings of each pruning treatment were planted in 80 mm deep plastic pots. At the same time the remaining 360 seedlings in two bags were placed in frozen storage (-2 C) at the Thunder Bay Forest Station. After both 50 and 92 days, one bag of seedlings was removed from frozen storage and the seedlings allowed to thaw overnight in their bags at about 8 C before being planted. A summary of the times of observation and sample sizes for each experimental treatment is shown in Table 1.

Trees were grown on a greenhouse bench under eight fluorescent lights (Gro-lux very high output, wide spectrum tubes) which supplemented natural daylight to provide a 16 hour photoperiod with an average mid-day light intensity of 14,447 $\mu w \cdot cm^{-2} \cdot sec^{-1}$ between the photosynthetically active wavelengths of 400 to 750 $m\mu$. Spectral intensity distribution is shown in Figure 1. Spectral intensity distribution was measured using a factory calibrated Instrument Specialties Company Model SR Spectroradiometer. Temperature varied between 13 and 16 C by night and 18 and 23 C by day.

Table 1. Summary of times of observation and sample sizes of seedlings from each frozen storage and root pruning treatment combination.

	Number of Seedlings Sampled		
Days Since Planting	Photosynthesis	Shoot Moisture Stress	Root Growth
3	11	12	--
5	--	--	30
7	11	12	--
10	--	--	30
14	11	12	--
15	--	--	30
25	--	--	--
28	11	12	--
30	--	--	30
34	--	--	30
39	--	--	30
42	11	12[1]	30

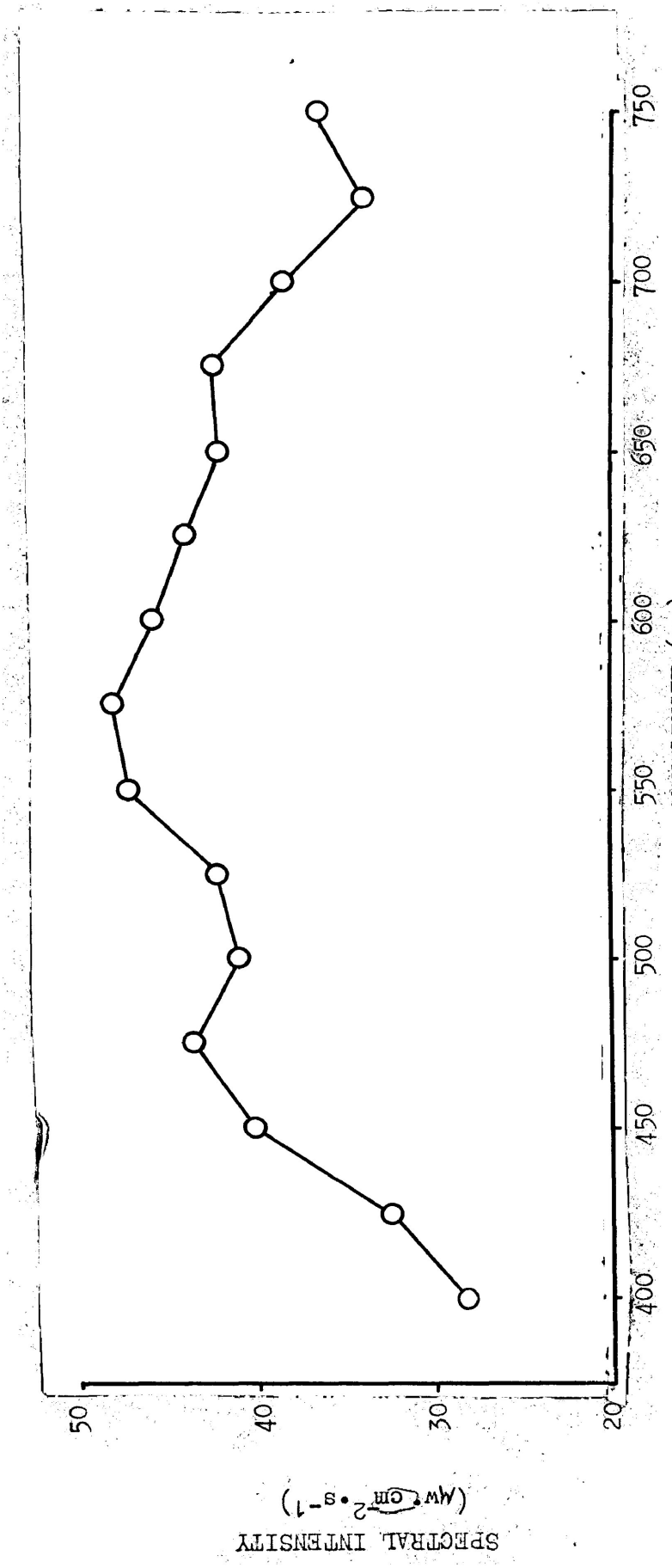

FIGURE 1. Typical mid-day spectral intensity distribution available to seedlings on a greenhouse bench with supplemental fluorescent lighting.

Techniques and Equipment Used to Measure Physiological Quality

Each seedling was grown for 42 days during which periodic measurements of photosynthesis, shoot and root moisture stress and root growth were made. In addition, bud flushing and the number of seedlings with new root growth were observed using trees planted in root boxes. Survival was assessed using trees planted in pots and in root boxes.

Photosynthesis

Measurements of photosynthesis were taken on eleven seedlings from each root pruning and storage treatment that were planted in root boxes. Seedlings used for photosynthetic measurements were replaced if they died, the new tree thenceforth being used. Trees were transported for measurement from the greenhouse in a polyethylene-lined box, to a laboratory where they were placed in a cardboard box covered with transparent polyethelene until measurement was made. Air was maintained above 50 percent relative humidity by pumping humidified air into the box. Seedlings remained in the box without supplemental lighting no longer than 90 minutes before measurement of photosynthesis, and they were returned to the greenhouse immediately thereafter.

Photosynthesis is expressed as apparent photosynthetic efficiency, which is the net amount of CO_2 absorbed by the seedling per unit needle oven dry weight per unit time. Apparent photosynthetic efficiency consists of gross photosynthesis (the amount of CO_2 absorbed) minus the amount of CO_2 evolved in the light as a result of respiration.

Photosynthetic efficiency was calculated as follows:

$$P_N = \frac{F(\Delta CO_2) \times 10^{-2}}{M}$$

where, P_N = photosynthetic efficiency (ml $CO_2 \cdot g$ needle $ODW^{-1} \cdot hour^{-1}) \times 10^{-5}$,

F = air flow rate = 28.6 litres$\cdot h^{-1}$,

ΔCO_2 = change in CO_2 content of air stream passing over seedling (percent)

and, M = foliage oven dry weight (g) of all needles at the end of the experiment.

Each seedling was prepared for measurement by fitting a split rubber stopper about the stem near the root collar. When necessary, small, one-year-old branches near the base of the stem were excised to allow fitting of the stopper. The rubber stoppers were sealed on the seedlings using mastic, after which the seedling shoot was raised through an opening into a transparent plexiglass chamber, the rubber stopper forming an airtight seal at the opening to the chamber. Photosynthesis was measured as the seedlings progressed through the stages of bud swell and stem and needle elongation - 3, 7, 14, 28 and 42 days after planting.

The equipment used for determining rates of photosynthesis had three major components: gas handling, gas conditioning and environmental control.

Gas Handling

A stream of air, continuously drawn from the outside, was pumped through the equipment used in gas conditioning and into a plant chamber, from which it passed through an infra-red gas analyser (Beckman Model 815) before being exhausted. This is

known as an open-flow gas system (Sestak et al. 1971), and has the advantage that at all points the gas contained in the system is under positive pressure, preventing ingress of air from the laboratory.

The gas handling system began where outside air was pumped through two-136 litre and a 12 litre mixing tanks before bubbling through a column of water and into another, 23 litre mixing tank. Mixing tanks reduced fluctuations in flow rate and CO_2 concentration of the incoming air. The incoming air was next split into four separate streams, each supplying a two litre, 25 cm tall, cylindrical plexiglass chamber submerged in water. As the air passed through a chamber it exchanged CO_2 with the enclosed seedling shoot. The air stream leaving each chamber passed through its own drying column of calcium sulphate after which it was either exhausted through a separate flow meter or diverted through another flow meter and through the gas analyser. Flow rates of the air streams were maintained at the same level. All connections between chambers, drying columns, and gas analyser were made using 3.2 mm (inside diameter) copper tubing.

Air Conditioning

Air conditioning was necessary to regulate the CO_2 concentration and humidity of the incoming air stream.

The concentration of CO_2 was controlled by drawing a portion of the incoming air from the first mixing tank and bubbling it through two columns of 2.5 molar potassium hydroxide (KOH). This reduced the CO_2 concentration of the air which was then recombined in the second large mixing tank with the remainder

of the incoming air, so that the concentration of CO_2 always was between 260 and 320 ppm.

Humidity of the incoming air was kept at no less than 55 percent by bubbling the air stream through a column of water. This humidifying unit was positioned between the 12 and 23 litre mixing tanks. Before entering the plant chamber humidity was monitored by passing the incoming air through a sealed flask containing a Yellow Springs Instrument Company Series 700 thermilinear thermistor probe and Model 91 dew point hygrometer probe.

Environmental Control

Temperature and light intensity within the plant chambers were controlled in order to provide a uniform environment in which to measure photosynthesis. Air temperature was maintained between 20 and 25 C by surrounding the plant chambers with water. In addition, seedling needles were kept near air temperature by circulating the air in each assimilation chamber by fan. Air temperature was measured using a shielded thermocouple placed inside the plant chamber, and also by passing the air stream from the plant chamber over a thermistor sealed in a flask.

Light was provided by six fluorescent tubes (Sylvania Gro-Lux Lifeline, wide spectrum very high output 48 inch lamps), suspended 45 cm above the seedlings. Light, passing through about 25 mm of water and the 6.4 mm thick plexiglass top of the plant chamber before reaching the tree, had a spectral intensity between 400 and 750 mμ of 2, 499 $\mu w \cdot cm^{-2} second^{-1}$ at average plant height, with the spectral distribution shown in Figure 2. Preliminary investigations had shown this to be near the light

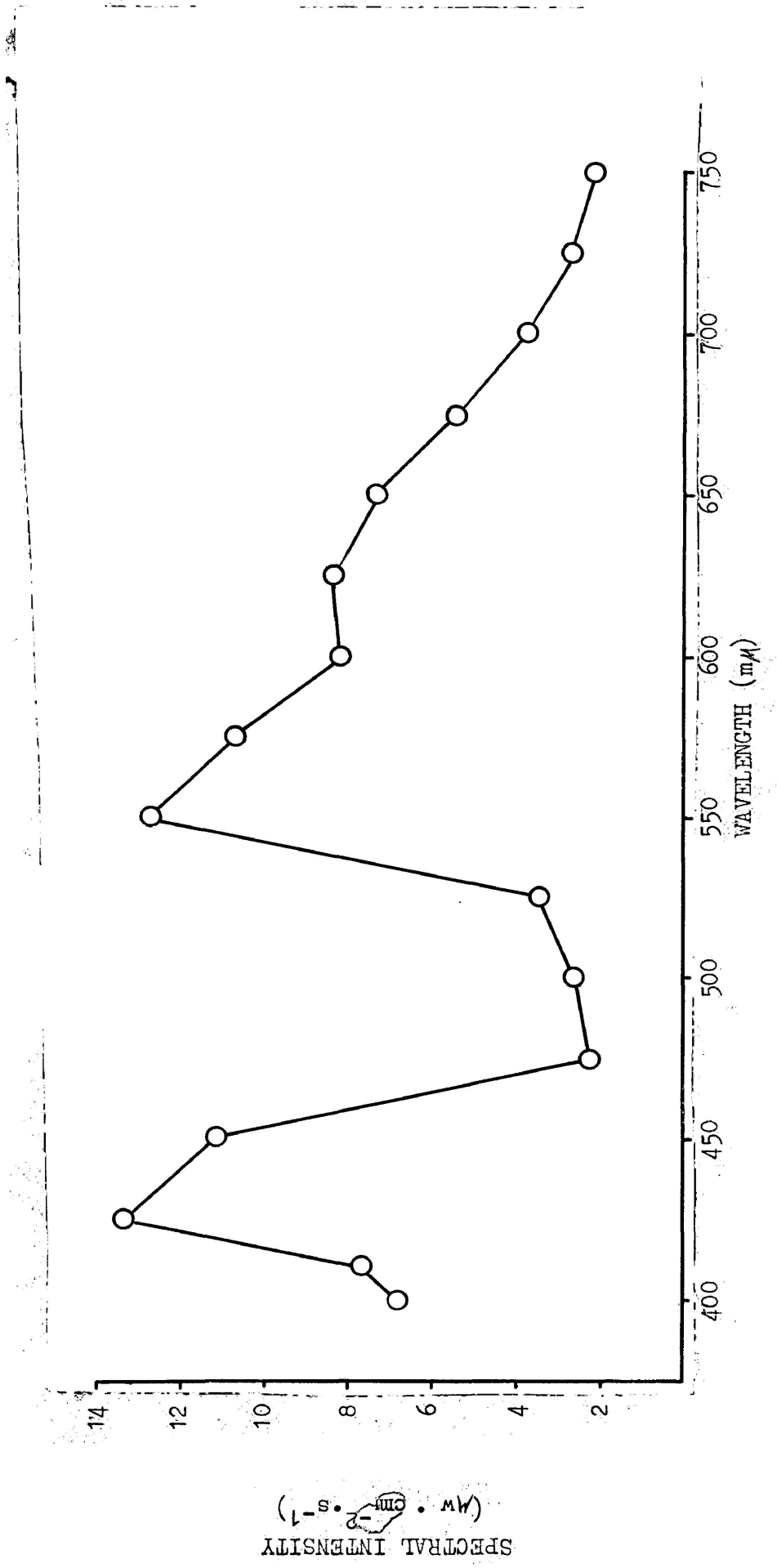

FIGURE 2. Spectral intensity distribution during photosynthetic measurements.

saturation point.

Moisture Stress

Moisture stress was determined according to the pressure chamber method of Cleary (1968) and Pierpoint (1969), except that the bark and phloem were not peeled from the cut ends before measurement. Cleary and Zaerr (1980) recommend peeling before measurement of moisture stress, because phloem exudate can obscure xylem sap at the endpoint. In this trial peeling was not necessary because little or no exudate came from the phloem. In addition, a supplementary trial failed to show any significant difference in shoot moisture stress measured with and without the phloem peeled (Appendix B). However, without peeling phloem exudate made the end point more difficult to see.

Moisture stress was determined using seedlings planted in pots. Measurements were made after bisecting seedlings at the root collar and placing first the roots and then the shoots in the pressure chamber. Measurements were in pounds per square inch and are presented in bars of moisture stress (100 pounds per square inch = 6.89 bars). Pressure was increased at about 0.34 bars $\cdot$ sec^{-1}, and was recorded at the time when a bead of moisture emerged from the xylem at the cut end.

Shoot moisture stress follows a diurnal pattern, in which stress is lowest just before dawn but rises rapidly during the morning as temperature and vapour pressure saturation deficit increase. Moisture stress reaches a daily maximum by the afternoon which is not relieved until temperature falls later in the day (Cleary 1968). All measurements were made using well watered stock between 12:00 and 4:00 p.m., the time of day when moisture

stress was expected to reach high afternoon levels.

Root Growth

Root growth was determined from measurements of seedlings grown in glass-faced root boxes. The glass-faces were not blacked-out because the root boxes were leaned glass-face down toward the bench. All new seedling root growth, visible through the glass of each root box, was traced and later measured on a plastic sheet. All roots, regardless of length or diameter, were included.

Experimental Design and Analysis of Data

Seedlings were randomized on a greenhouse bench. Root boxes were arranged randomly on one-half of the bench, while the rest of the bench contained trees planted in pots for use in determining moisture stress. Pots were arranged in four randomly placed blocks of 6 seedlings in order to evaluate the influence of bench position.

A factorial analysis of variance was performed on observations of photosynthesis and shoot moisture stress. Treatment effects were declared significant if the probability of F exceeding or being equal to the variance ratio was 5 percent or more. Factors considered were time of observation, duration of frozen storage and degree of root pruning. Variance of treatment means was homogeneous regardless of the size of the mean for both shoot moisture stress and photosynthetic efficiency. Thus, transformation of the data was unnecessary (Jeffers 1959).

There were 11 seedlings per treatment combination used in the determination of photosynthetic efficiency, each seedling

being a replicate. The shoot moisture stress of 12 seedlings was measured for each treatment combination. In the analysis of variance of shoot moisture stress only four observation times were used, for a total of 24 treatment combinations. Stock frozen 0 days was not observed on day 42, as day 28 was originally chosen as the final sampling date. However, because changes in root growth were observed after day 28, the length of the experiment was extended by two weeks. Due to the destructive nature of moisture stress measurements no trees of the non-frozen storage treatment were available at day 42. To maintain orthogonality of design, day 42 observations were not included in the analysis of variance of shoot moisture stress. Analysis of root moisture stress data was not performed because the response of root moisture stress was similar to that found for the shoot for all treatments (Appendix C and D).

Root growth data were not examined using standard analysis of variance procedures because sample sizes were disproportionate, as root growth for seedlings of different treatments began at different times. Therefore, Student-Newman-Keul's multiple range test (five percent level) was employed to test the difference between treatment means (Nie et al. 1978). This test was performed following logarithmic transformation of the data, because the variance of root growth increased proportionately with the mean. Differences in root growth due to length of storage were compared for each time of observation, separately for root pruned and non-pruned seedlings.

Student-Newman-Keul's test was also used to test the significance of differences between treatment means (five percent

level) for photosynthetic efficiency and shoot moisture stress. The highest order interactions found to be significant were analyzed.

The relationship between root growth and photosynthetic efficiency was examined by regression. Only seedlings which were photosynthesizing and had elongating roots were used in the development of the regression equation. Best fit was achieved using square root transformation of root growth and logarithmic transformation of photosynthetic efficiency.

RESULTS

Photosynthetic Efficiency

Photosynthetic efficiency responded significantly to time in storage, and this response differed according to both time since planting and root pruning treatment, as indicated by the significant interaction of these three factors (Appendix E). Photosynthetic efficiencies are shown in Appendix F.

On the third day after planting photosynthetic efficiency of root pruned and non-pruned trees stored for 0 and 92 days was significantly greater than that of seedlings stored for 50 days (Figures 3 and 4). By the seventh day this significant difference had disappeared, as the photosynthetic efficiency of trees stored 50 days had risen and that of seedlings stored 0 and 92 days had fallen. On subsequent occasions photosynthetic efficiency of each group of seedlings generally increased, but this increase was most rapid in non-frozen stock. By the fourteenth day after planting both pruned and non-pruned stock which had not been frozen had achieved significantly higher levels of photosynthetic efficiency than trees frozen 50 or 92 days.

Recovery was faster for non-root pruned trees frozen 50 and 92 days than it was for pruned seedlings. However, regardless of root pruning it was 42 days before the levels of photosynthetic efficiency of trees frozen 50 and 92 days had reached levels comparable to those of non-frozen stock.

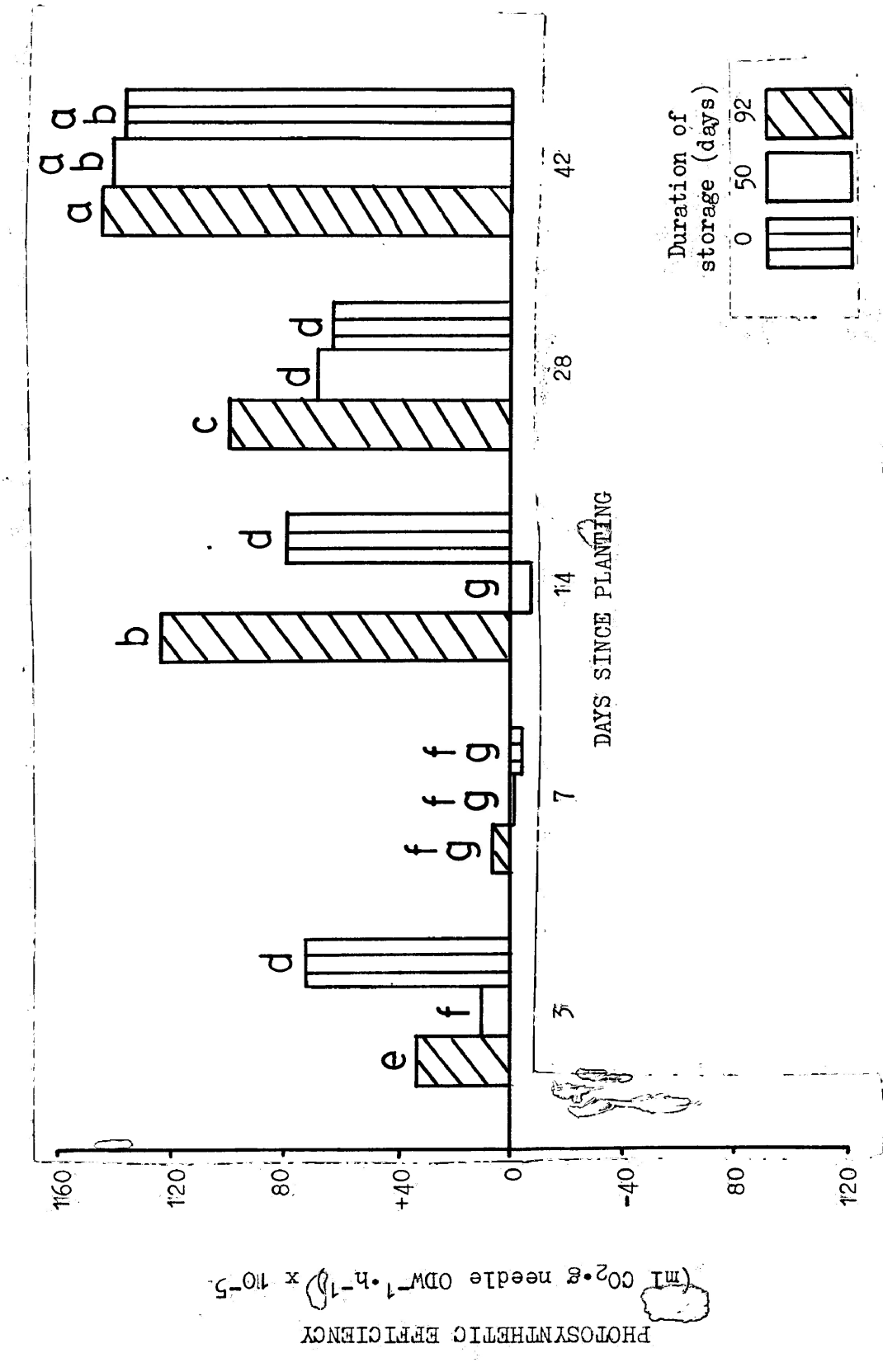

FIGURE 3. Effect of frozen storage on periodic photosynthetic efficiency of non-root pruned seedlings.[1]

[1]Bars associated by common letters do not differ significantly (5% level).

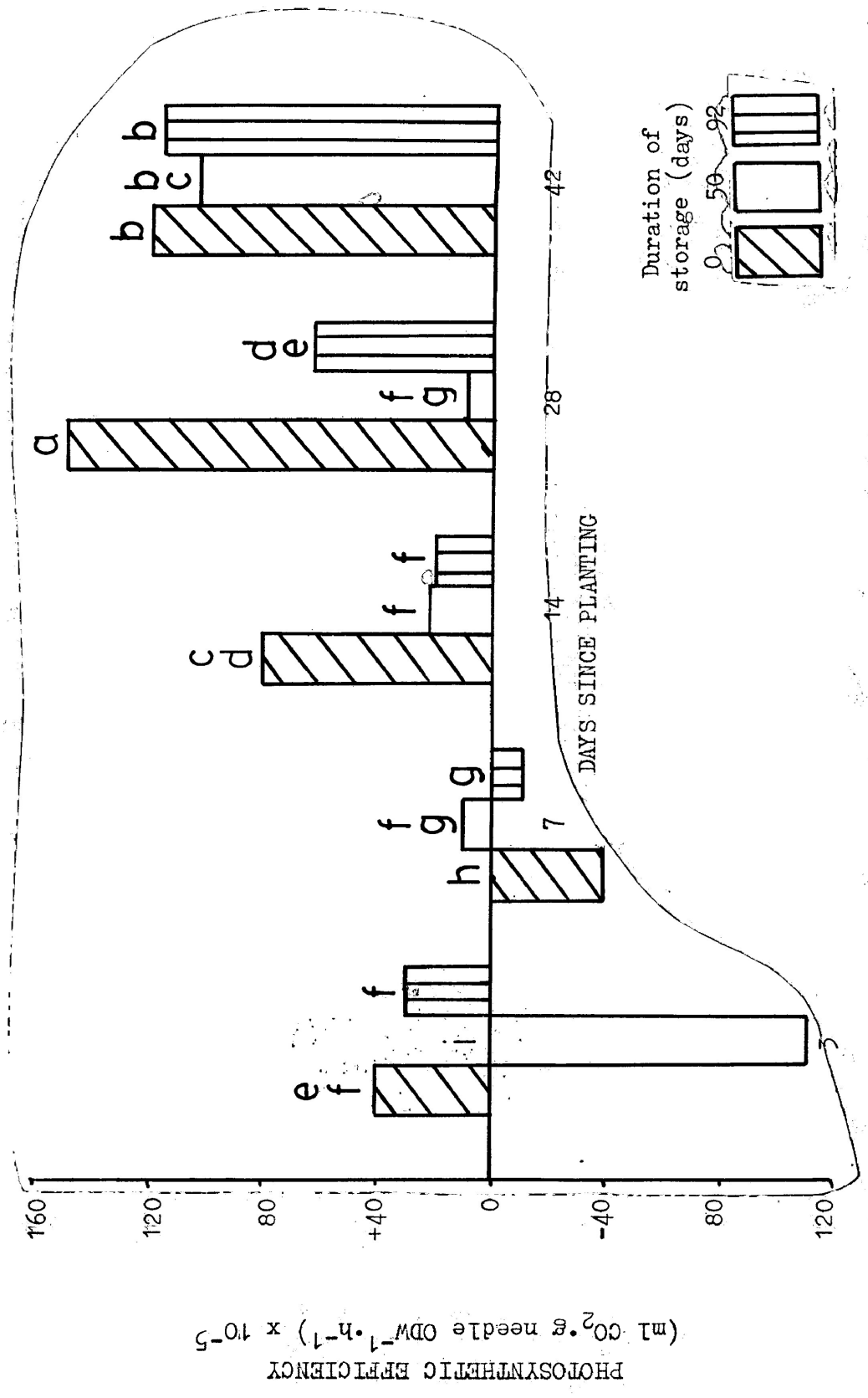

FIGURE 4. Effect of frozen storage on periodic photosynthetic efficiency of root pruned seedlings.[1]

[1]Bars associated by common letters do not differ significantly (5% level).

Shoot Moisture Stress

Shoot moisture stress decreased as time since planting increased irrespective of length of storage, and, except for day 7, the shoot moisture stress of seedlings stored for 92 days was always highest and that of non-frozen trees lowest (Figure 5). However, the significance of the differences in shoot moisture stress between storage treatments was not the same on each occasion. Three days after planting shoot moisture stress was significantly greater (5 percent level) the longer the period of storage: trees frozen 0, 50 and 92 days had shoot moisture stress of 17.9, 20.7, and 22.2 bars respectively. When measured seven and fourteen days after planting, trees frozen for 0 and 50 days did not have significantly different levels of shoot moisture stress, but trees frozen 92 days had significantly greater shoot moisture stress levels than the other groups. The difference in shoot moisture stress between seedlings frozen 0 and 92 days remained significant 28 days after planting.

The response of shoot moisture stress of pruned trees to increasing lengths of storage was different than that of non-root pruned stock (Appendix G, Figure 6). With each increase in time of frozen storage, there was a significant increase in shoot moisture stress (5 percent level) in the root pruned trees. Non-pruned seedlings had no significant differences in shoot moisture stress after frozen storage of 0 and 50 days, but following 92 days of storage shoot moisture stress increased significantly.

Differences in shoot moisture stress between blocks were significant, as a result of greater rates of air flow across the

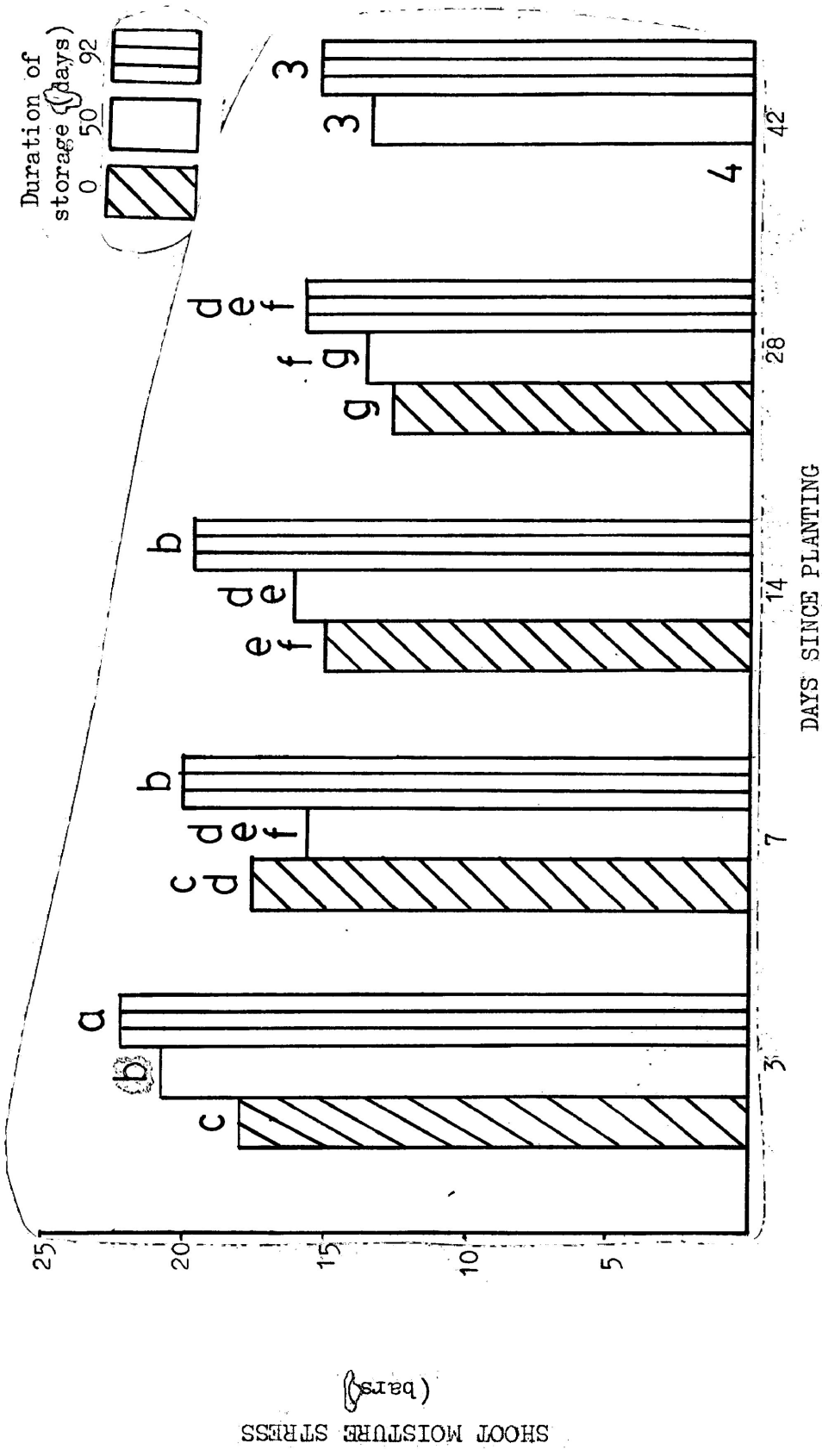

FIGURE 5. Effect of frozen storage on periodic shoot moisture stress.[1,2]

[1]Bars are means of root pruned and non-root pruned seedlings.

[2]Bars associated by common letters do not differ significantly (5% level).

[3]Trees observed 42 days after planting were not included in this analysis.

[4]Not observed.

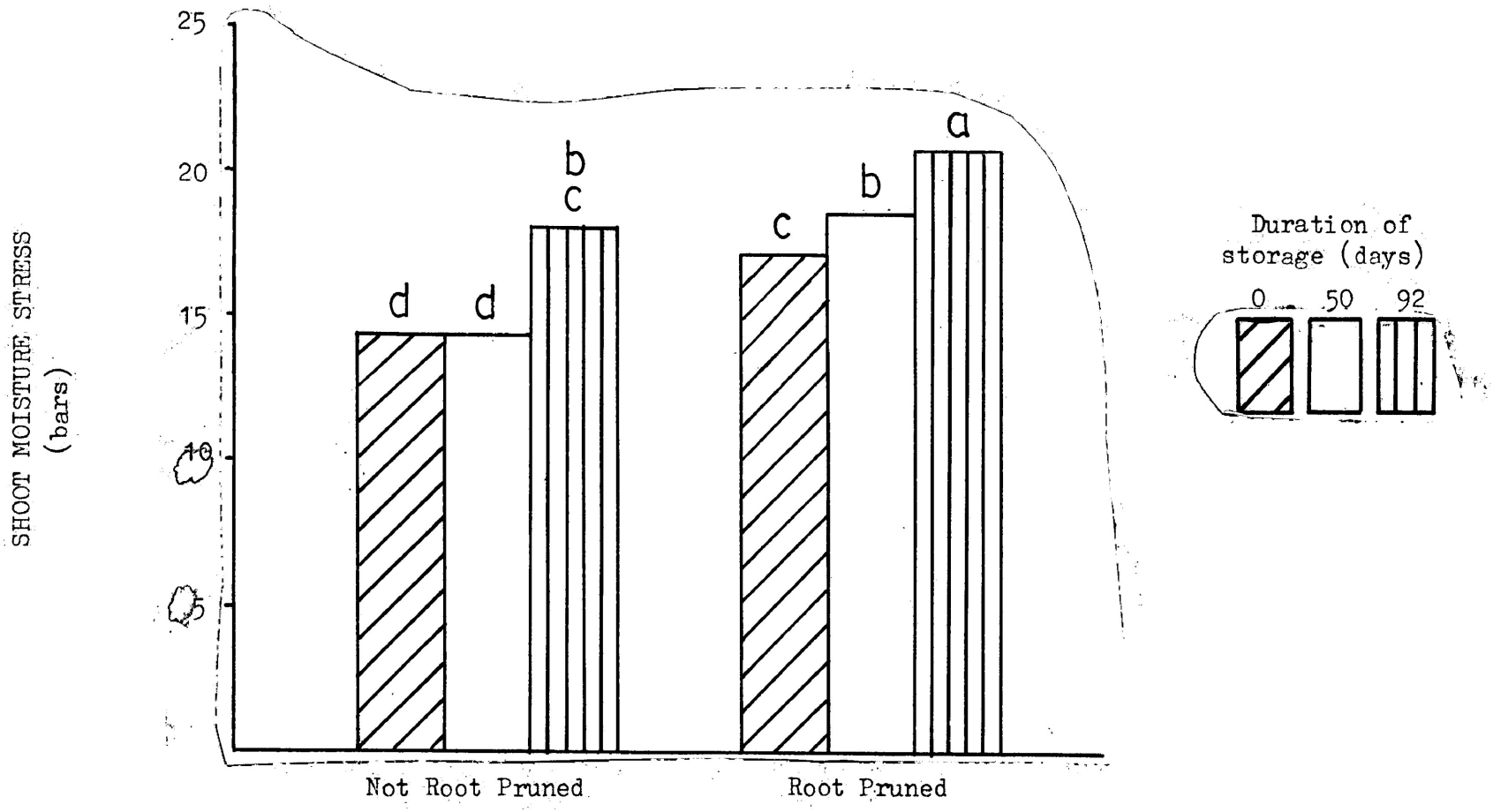

FIGURE 6. Effect of frozen storage and root pruning on shoot moisture stress.[1,2]

[1]Bars are means of trees sampled on days 3, 7, 14, and 28.

[2]Bars associated by common letters do not differ significantly (5% level).

bench where block 3 was located.

Root Growth

Root growth of non-pruned seedlings frozen 0 and 92 days began 6 to 10 days after planting, while trees frozen 50 days did not begin until between day 11 to 15 (Appendix H, Figure 7). Root growth of pruned trees began later than non-pruned, regardless of length of storage (Figure 8).

There were significant differences in root growth due to storage for pruned trees only 40 to 42 days after planting, at which time stock frozen 92 days produced 22.8 mm of new root$\cdot$ seedling$^{-1}\cdot$ day^{-1} while seedlings frozen 0 or 50 days produced 7.5 and 7.6 mm$\cdot$seedling$^{-1}\cdot$day^{-1} respectively. In comparison, non-pruned seedlings frozen 92 days produced significantly more root than other storage lengths between days 21 and 25.

Correlation of Root Growth and Photosynthetic Efficiency

A highly significant relationship ($P < 0.01$) was found to exist between root growth and photosynthetic efficiency, with r^2 equal to 7.2 percent ($n = 208$). The closest relationship was achieved using a reciprocal square root transformation of the root growth data and logarithmically transformed photosynthetic efficiency (Figure 9), in which:

$$1/(RG + 0.5)^{0.5} = 0.6957-0.1336 \ (\log 10 \ P_N)$$

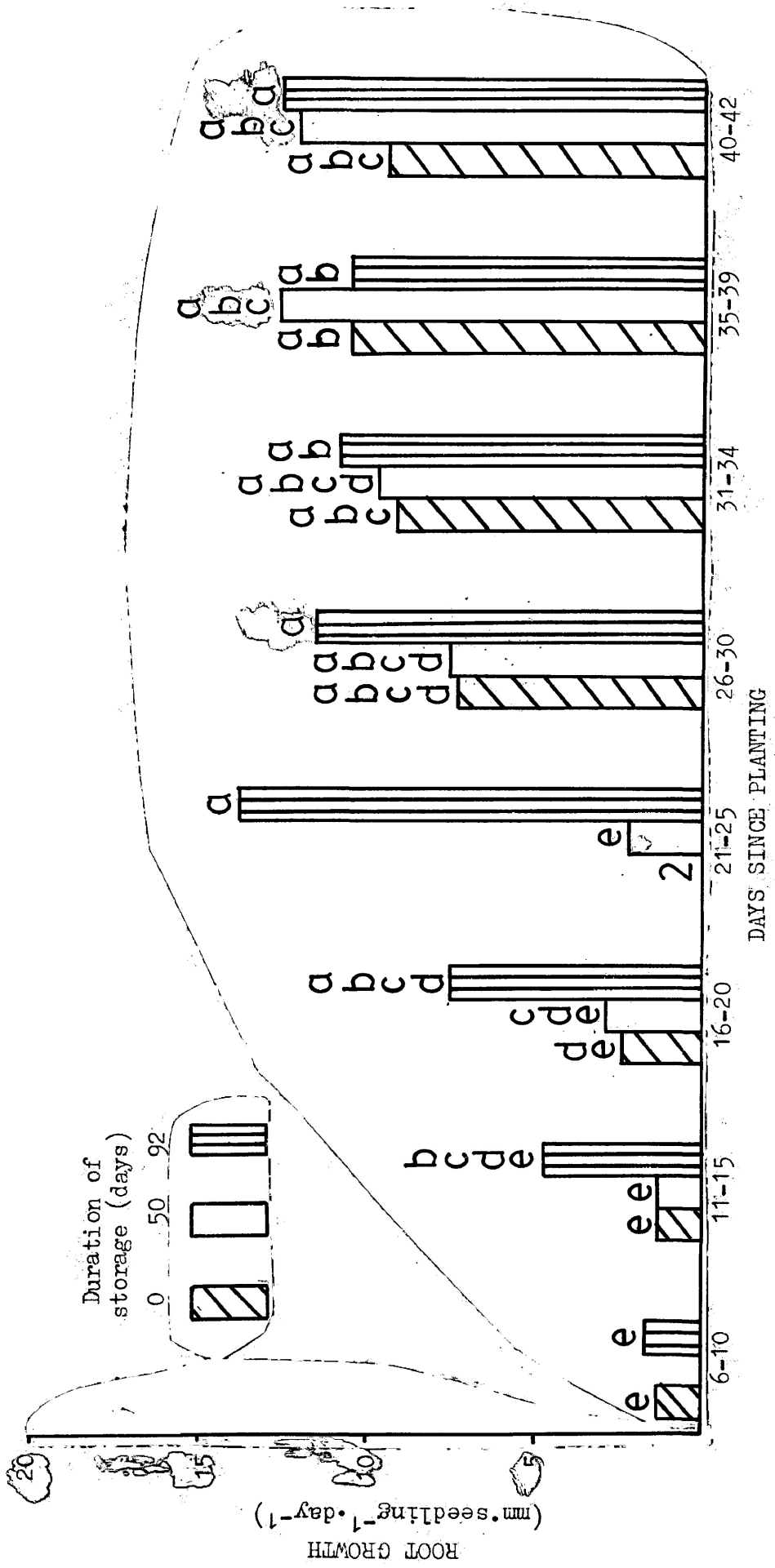

FIGURE 7. Effect of frozen storage on periodic root growth of non-root pruned seedlings.[1,2]

[1]Bars associated by common letters do not differ significantly (5% level).

[2]Not observed.

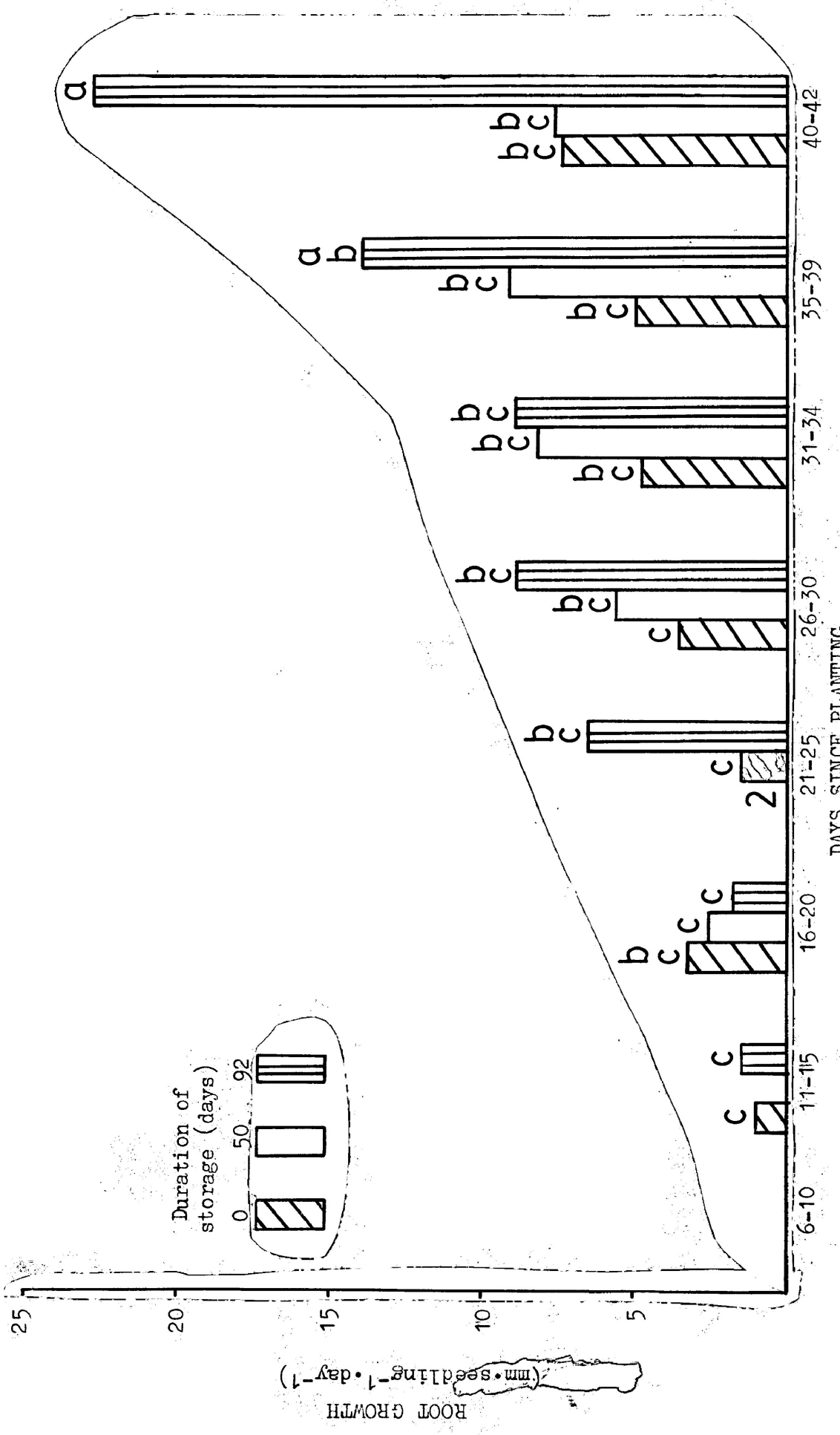

FIGURE 8. Effect of frozen storage on periodic root growth of root pruned seedlings.[1,2]

[1]Bars associated by common letters do not differ significantly (5% level).

[2]Not observed.

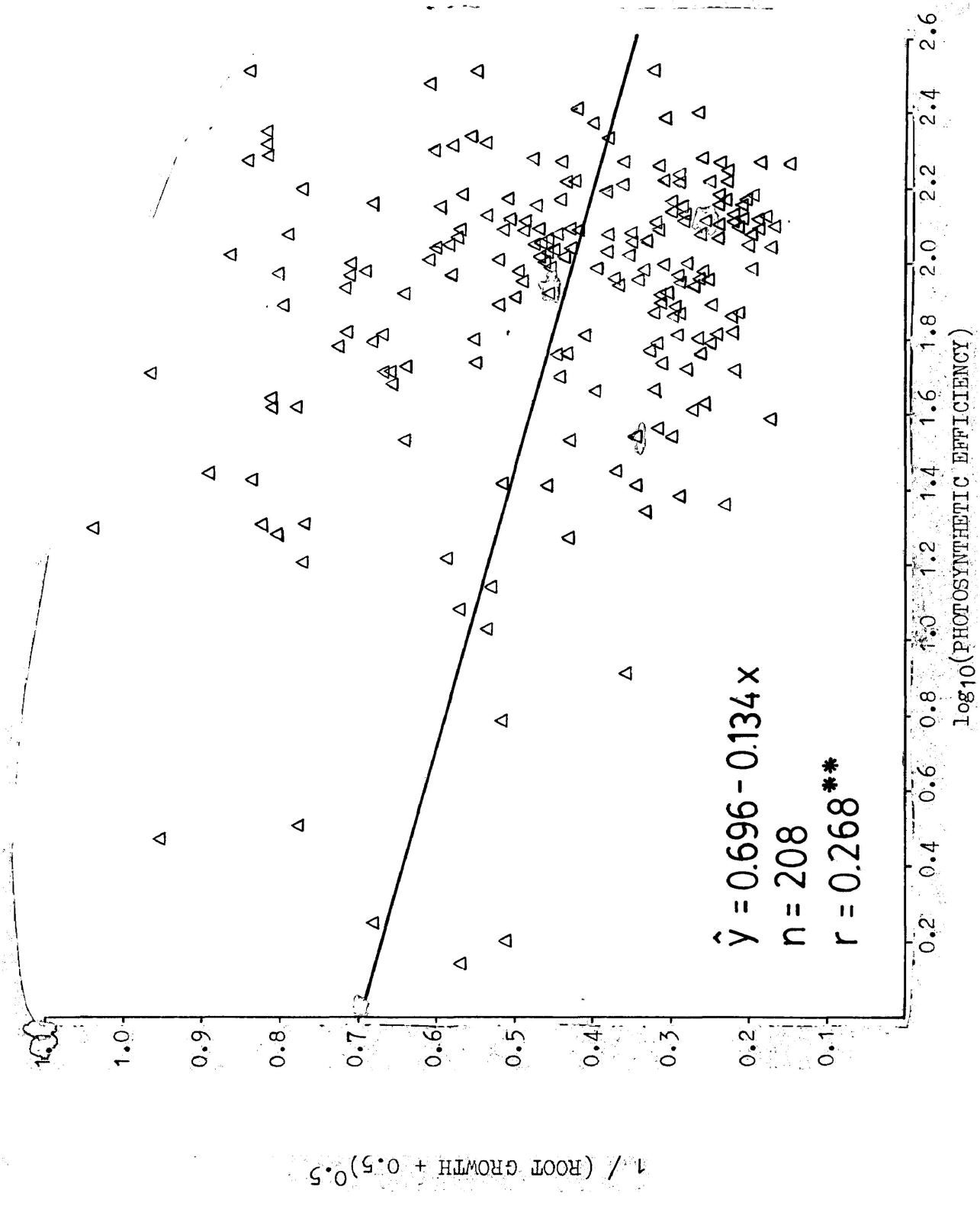

FIGURE 9. Correlation of root growth and photosynthetic efficiency.

where, RG = root growth (mm new root$\cdot$day^{-1}),

and P_N = photosynthetic efficiency
(ml $CO_2\cdot$g needle ODW$^{-1}\cdot$hour^{-1}) x 10^{-5}.

DISCUSSION

Photosynthetic efficiency was significantly affected by frozen storage. Regardless of root pruning treatment, photosynthetic efficiency of non-frozen stock was significantly greater than stock in other storage treatments 14 and 28 days after planting. In addition, trees frozen 50 days had significantly lower rates of photosynthesis than trees frozen 0 and 92 days on the 3rd and 28th days after planting.

The patterns of variation in photosynthetic efficiency and shoot moisture stress often did not correspond to changes in root growth. Stock frozen 92 days had significantly higher levels of shoot moisture stress and lower levels of photosynthetic efficiency than non-frozen trees up to four weeks after planting. There was no comparable response in root growth.

Seedlings frozen 0 and 92 days had lower levels of photosynthetic efficiency on day 7 than on the third day after planting. Photosynthetic efficiency decreased because buds began to flush during this time. Keller (1980) observed a similar trend, and attributed it to the coupling of bud break with high rates of respiration.[1] In comparison, seedlings frozen 50 days had low levels of photosynthetic efficiency throughout the first two weeks after planting for non-root pruned stock, and photosynthetic efficiency remained low for four weeks if the seedlings were root pruned. Stock frozen 50 days also inexplicably

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[1] T. Keller. Swiss Federal Institute of Forestry Research, Birmensdorf, Switzerland. Personal communication.

began root growth later than other storage treatments. Whether or not these results are anomalies or true treatment effects is a matter of speculation, but they may demonstrate the sensitivity of nursery stock to variation in handling, storage, or growing conditions.

Just three days after planting shoot moisture stress was significantly greater the longer the seedlings had been kept in frozen storage. Since shoot moisture stress was not measured as soon as the trees came from storage, it could be suggested that these differences developed during storage. Storage methods were meant to minimize moisture loss: roots were covered with damp moss and the trees double-bagged in preparation for storage. However, moisture loss by sublimation from the foliage could have occurred during storage, but this was not tested.

Significant differences in shoot moisture stress were still present 42 days after planting. At the same time, it was observed that seedlings stored 92 days had produced a greater amount of new root than the other storage treatments, which suggests that these trees should have had the greatest capacity to absorb moisture and thereby reduce shoot moisture stress. The fact that shoot moisture stress remained at high levels contrary to expectations is best explained in terms of transpiration. If cold exposure is done in the dark, as it was in this trial, stomata may be unable to close (McCracken 1978). The interaction between the effects of cold and dark on stomatal physiology is undoubtedly complex. Exposure to cold in the light will result in stomatal closure (Christersson 1972) but, if cold exposure is carried out in the dark, stomatal control will be lost (McCracken

1978). In this experiment, high levels of shoot moisture stress of frozen stock may have been caused and maintained after planting by unrestricted transpiration through stomata which, under other conditions, would be closed.

Despite stomata which apparently were open, trees frozen 50 and 92 days required more time to reach high levels of photosynthetic efficiency than non-frozen seedlings. This seeming contradiction, in which seedlings are unable to photosynthesize despite open stomata, can be explained in terms of a breakdown in chloroplast structure due to frozen storage in the dark (McCracken 1978, Perry and Baldwin 1966).

All trees in this trial were stored in the dark for 36 days at 4 C. Seedlings planted immediately after this period of cool storage had higher levels of photosynthetic efficiency than stock placed in frozen storage for 50 or 92 days. This may be accounted for in either of two ways. Firstly, the breakdown of chloroplast structure may occur gradually: the longer the period of storage, the greater the breakdown in structure. Secondly, chloroplast structure could have been disrupted by freezing rather than non-freezing temperatures, although McCracken (1978) stored trees at non-freezing temperatures and still suspected that the chloroplast had been damaged. Confirmation of these hypotheses would be valuable in planning modifications of storage conditions. In order to know how to modify storage conditions, further information about the effects of frozen storage on tree seedling physiology is necessary. For instance, is the breakdown in chloroplast structure during frozen storage temperature-dependant? If so, at what temperature will the least damage

occur? If warm temperatures help to restore chloroplast integrity, a warm pre-conditioning period before planting would be warranted. Furthermore, what is the role of darkness in the disruption of chloroplast structure? Perhaps frozen storage with exposure of the seedling shoots to light could prevent or lessen the degree of chloroplast damage.

Root growth of seedlings frozen 92 days was almost always greater than root growth of those from any other storage treatment, although the differences were seldom significant. It has been demonstrated using ponderosa pine (Krugman and Stone 1966, Stone 1967) that root activity depends upon the duration of exposure to cold. Similarly, it has been shown using Douglas-fir that chilling results in increased root growth (Lavender and Wareing 1972). Day, Stupendick and Butler (1976) hypothesized that the increase in root activity of white spruce in the fall season was due to chilling. Root activity in this experiment increased with the length of the period of cold exposure. Root growth may have been promoted further if frozen storage had continued past 92 days, although there is a point at which further exposure to cold will not result in further increases in root growth (Krugman and Stone 1966).

Root pruning was detrimental to all aspects of seedling physiology which were studied. For most storage treatments and times of observation pruning resulted in lower levels of photosynthetic efficiency and slower rates of root growth. Shoot moisture stress was in all cases greater in root pruned stock.

The adverse effect of root pruning on photosynthetic efficiency may be due to the loss of a large number of root tips,

known to be a source of cytokinin (Van Staden 1977), thus chemically reducing the capability of the needles to photosynthesize (Wareing et al. 1968). Photosynthetic efficiency may also have been reduced by high levels of shoot moisture stress attributable to root pruning. In another study (Watts and Neilson 1978), a shoot moisture stress of 18 bars resulted in significantly lower rates of photosynthesis in sitka spruce, and Cleary, Greaves, and Owston (1978) found that photosynthesis in Douglas fir and ponderosa pine gradually declined up to 40 percent as shoot moisture stress increased from 10 to 20 bars. In the trial reported here, high shoot moisture stress levels in root pruned stock may have contributed to restriction of the photosynthetic processes.

The removal of fibrous roots by root pruning in this experiment was designed to give an indication of the effects of nursery stock root trimming. My results indicate that this practice may be harmful, since root pruned stock had low levels of photosynthesis, reduced rates of root growth, and resulted in greater shoot moisture stress. Root pruned seedlings also suffered greater mortality than trees which were not root pruned (7.8 versus 0.4 percent), and fewer pruned seedlings broke bud (68.3 percent) than non-pruned stock (95.0 percent). Root pruning is used to improve stock handling and planting, and is not done to improve stock performance. In my view, if more compact root systems are desired these should be achieved while the trees are still in the nursery beds, by means of undercutting or wrenching.

The relationship between photosynthetic efficiency and

root growth was weak, even though significant. Only 7.2 percent of the variation in photosynthetic efficiency was attributable to root growth. Perhaps a causal relationship does exist. However, there is little evidence to support this hypothesis. There are at least two explanations for the weak correlation. Firstly, root growth is a poor parameter to use when comparing photosynthesis and root activity. Root growth consists of root elongation, which depends on photosynthesis (Wassink and Richardson 1951, Webb 1976), but also on root initiation, which is independent of photosynthesis in white spruce (Carlson 1976, 1977). van den Driessche (1978) similarly failed to find differences in root growth capacity which he could relate to carbohydrate reserves in white spruce, perhaps because his method of measuring root growth capacity by changes in root volume (Burdett 1979) also depended on root initiation. In this experiment the importance of root initiation in influencing root growth may be why only a small percentage of the variation in root growth could be explained by photosynthetic efficiency.

A second explanation may be that root growth depends upon reserve substances as substrates for root growth rather than current photosynthate. Reserve substances are known substrates for root elongation shortly after growth resumption in the spring (Lyr and Hoffman 1967, Ronco 1973), and could play a role as substrates for root growth at other times in white spruce. Reserve substances play an important but unspecified role in the survival of conifer seedlings after planting (Hocking and Nyland 1971, Navratil 1976). Their role could be as substrates for root growth, which would aid in explaining why photosynthetic

efficiency was not closely related to root growth in this experiment.

CONCLUSIONS

Photosynthetic efficiency of white spruce seedlings was measured during a six week period following frozen storage for 0, 50 or 92 days. Photosynthetic efficiency was examined with respect to root pruning, root growth and shoot moisture stress.

Photosynthetic efficiency of non-frozen stock was significantly greater two and four weeks after planting than it was for trees frozen 92 days, but trees frozen 50 days had inexplicably lower levels of photosynthesis for up to four weeks after planting. In the same way shoot moisture stress was in all cases significantly lower for non-frozen trees than for stock which was frozen for 92 days, in spite of greater root growth by those frozen 92 days.

Root pruning was invariably detrimental, resulting in lower levels of photosynthetic efficiency, reduced rates of root growth and higher levels of shoot moisture stress. Root pruning is a practise which should be critically reviewed.

The absence of light during storage may explain why seedlings frozen 92 days had significantly lower levels of photosynthetic efficiency and higher shoot moisture stress than non-frozen stock. Exposure to light during storage could be necessary to prevent deterioration of chloroplasts which is damaging to the photosynthetic process, and to allow stomatal

closure in response to high levels of shoot moisture stress.

Root growth was not strongly correlated with photosynthetic efficiency. This may be because root growth consisted of measurements of both root initiation and root elongation - but only root elongation depended on photosynthesis. In addition, the correlation may have been weak because root elongation relied upon stored food reserves as a substrate instead of currently produced photosynthate.

To the nursery man the results of this thesis should indicate that frozen storage and root pruning are both practices which need to be modified in order to optimize nursery stock quality. Storage conditions need to be altered to prevent breakdown in chloroplast structure, perhaps by exposure of the needles to light during storage or a pre-conditioning, warm period before planting. Root pruning has no obvious benefits in terms of nursery stock physiological condition, and should be discontinued in preference to undercutting or wrenching.

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APPENDIX

APPENDIX A. Oven-dry weight (g) of total root system and excised roots of root pruned white spruce seedlings.

	R_{PRUNED}[1]		R_{FINAL}[2]	R_{TOTAL}[3]
	g	%	g	g
Not Frozen				
$\bar{x}$	0.251	44.0	0.309	0.562
S.D.	0.093	11.7	0.079	0.122
Range	0.12-0.39	22.8-68.0	0.16-0.44	0.37-0.75
Frozen 50 Days				
$\bar{x}$	0.193	36.1	0.364	0.544
S.D.	0.066	10.1	0.110	0.143
Range	0.10-0.31	20.0-53.6	0.15-0.64	0.26-0.83
Frozen 92 Days				
$\bar{x}$	0.201	39.9	0.334	0.506
S.D.	0.082	9.5	0.105	0.163
Range	0.10-0.41	25.9-58.5	0.15-0.60	0.20-0.81

[1] R_{PRUNED} = O.D.W. of root tissue excised before planting.

[2] R_{FINAL} = O.D.W. of root systems of root pruned seedlings after 42 days of growth.

[3] R_{TOTAL} = total root O.D.W. = $R_{PRUNED} + R_{FINAL}$

[4] Percent $R_{PRUNED} = (R_{PRUNED}/R_{TOTAL}) \times 100$

APPENDIX B. Paired comparison of shoot moisture stress measured with and without peeled phloem.

Pair No.[1]	Not Peeled	Peeled	X_i D (X_1-X_2)	d Deviation $(X_i - 0.705)$	d^2
1	8.5	8.0	0.5	1.205	1.452
2	6.0	9.5	-3.5	-2.795	7.812
3	4.5	8.0	-3.5	-2.795	7.812
4	5.0	4.5	0.5	1.205	1.452
5	4.0	5.0	-1.0	-0.295	0.087
6	6.0	7.0	-1.0	-0.295	0.087
7	7.5	9.5	-2.0	-1.295	1.677
8	7.5	10.0	-2.5	-1.795	3.222
9	7.5	7.0	0.5	1.205	1.452
10	11.5	9.0	2.5	3.205	10.272
11	12.0	10.0	2.0	2.705	7.317
12	12.0	9.0	3.0	3.705	13.727
13	7.0	6.0	1.0	1.705	2.907
14	7.0	8.0	-1.0	-0.295	0.087
15	7.0	7.0	0.0	0.705	0.497
16	7.0	10.0	-3.0	-2.295	5.267
17	8.5	7.0	1.5	2.205	4.862
18	7.5	8.5	-1.0	-0.295	0.087
19	16.0	19.0	-3.0	-2.295	5.267
20	5.5	8.0	-2.5	-1.795	3.222
21	5.0	6.0	-1.0	-0.295	0.087
22	7.5	9.5	-2.0	-1.295	1.677
Total	170.0	185.5	-15.50	0.0100	80.3290
$\bar{x}$	7.727	8.432	-0.705	0.0005	3.6513
S.D.	2.869	2.855			

s^2 $\frac{80.3290}{21}$ 3.8252

$s_{\bar{x}}$ 0.4170

t $\frac{-0.705}{0.4170}$ -1.6907

0.10 P (t 1.6907) 0.05

[1] Pairs are lateral branches matched for length from individual 3-0 white spruce seedlings.

APPENDIX C. Periodic root moisture stress (bars) of white spruce seedlings frozen for 0, 50 and 92 days.

(i) Non-root pruned stock.

Length of Storage (Days)	Days Since Planting	Block I	II	III	IV	Average
0	3	12.8[a]	14.3	14.3	12.7	13.5
	7	8.4	14.7	14.9	15.1	13.3
	14	8.6	10.5	9.8	13.1	10.5
	28	7.3	7.3	9.9	8.5	8.3
	42		NOT MEASURED			
	Average	9.3	11.7	12.2	12.4	11.4
50	3	12.2	12.5	15.0	12.5	13.1
	7	13.4	14.0	15.1	13.8	14.1
	14	7.7	9.9	13.7	14.0	11.3
	28	8.0	8.2	7.9	8.6	8.2
	42	11.4	9.0	9.7	9.9	10.0
	Average	10.5	10.7	12.3	11.8	11.3
92	3	18.1	16.3	17.6	19.6	17.9
	7	15.6	13.0	16.2	13.1	14.5
	14	15.8	14.2	17.0	17.5	16.1
	28	8.7	9.2	10.5	10.4	9.7
	42	9.2	10.1	10.1	10.8	10.1
	Average	13.5	12.6	14.3	14.3	13.7
GRAND AVERAGE		11.1	11.7	12.9	12.8	12.1

[a]Each value is the mean of three observations

APPENDIX C.

(ii) Root-pruned stock.

Length of Storage (Days)	Days Since Planting	Block I	II	III	IV	Average
0	3	16.4[a]	16.7	19.1	16.9	17.3
	7	21.0	17.7	16.9	19.3	18.7
	14	15.0	12.3	19.7	16.3	15.8
	28	10.0	12.0	11.2	13.9	11.8
	42		NOT MEASURED			
	Average	15.6	14.7	16.7	16.6	15.9
50	3	20.3	21.8	21.7	21.0	21.2
	7	13.6	20.0	15.6	16.8	16.5
	14	15.5	17.0	20.3	19.1	18.0
	28	17.5	12.0	19.0	17.8	16.6
	42	12.2	9.7	11.3	11.9	11.3
	Average	15.8	16.1	17.6	17.3	16.7
92	3	20.8	22.2	23.2	24.2	22.6
	7	21.7	20.8	22.2	21.7	21.6
	14	20.0	22.8	16.8	17.9	19.4
	28	9.7	15.7	11.2	13.5	12.5
	42	10.3	15.9	11.9	11.1	12.3
	Average	16.5	19.5	17.1	17.7	17.7
GRAND AVERAGE		16.0	16.8	17.1	17.2	16.8

[a] Each value is the mean of three observations

APPENDIX D. Periodic shoot moisture stress (bars) of white spruce seedlings frozen for 0, 50 and 92 days.

(i) Non-Root Pruned Stock.

Length of Storage (Days)	Days Since Planting	Block I	II	III	IV	Average
0	3	15.6[a]	19.3	16.6	15.2	16.7
	7	13.3	17.7	17.3	16.1	16.1
	14	13.5	14.0	14.1	12.9	13.6
	28	11.6	8.8	12.6	9.8	10.7
	42		NOT MEASURED			
	Average	13.5	15.0	15.2	13.5	14.3
50	3	17.1	18.6	19.9	17.6	18.3
	7	13.4	14.0	15.0	13.8	14.1
	14	10.3	14.1	17.1	16.1	14.4
	28	10.3	10.0	10.8	9.9	10.3
	42	14.2	11.8	13.2	13.8	13.3
	Average	13.1	13.7	15.2	14.2	14.1
92	3	19.2	21.5	21.0	21.8	20.9
	7	17.6	17.7	18.3	17.8	17.9
	14	18.6	18.1	18.1	19.5	18.6
	28	13.7	13.9	16.2	15.4	14.8
	42	14.0	15.2	13.8	15.4	14.6
	Average	16.6	17.3	17.5	18.0	17.4
GRAND AVERAGE		14.4	15.3	16.0	15.2	15.2

[a] Each value is the mean of three observations

APPENDIX D.

(ii) Root-Pruned Stock

Length of Storage (Days)	Days Since Planting	Block I	II	III	IV	Average
0	3	18.7[a]	18.1	21.1	18.2	19.0
	7	19.5	18.4	19.2	18.5	18.9
	14	15.7	13.3	19.5	16.8	16.3
	28	12.9	16.3	13.2	15.6	14.5
	42		NOT MEASURED			
	Average	16.7	16.5	18.3	17.3	17.2
50	3	21.6	24.0	24.1	22.3	23.0
	7	15.4	20.0	16.7	16.3	17.1
	14	14.0	17.5	20.4	18.1	17.5
	28	18.7	12.6	19.4	17.8	17.1
	42	13.6	13.7	14.6	13.2	13.8
	Average	16.7	17.6	19.0	17.5	17.7
92	3	21.8	23.0	24.3	25.0	23.5
	7	23.2	21.6	22.3	21.1	22.1
	14	21.1	21.4	19.8	20.2	20.6
	28	16.9	17.9	15.0	16.5	16.6
	42	14.0	19.4	15.5	14.5	15.9
	Average	19.4	20.7	19.4	19.5	19.7
GRAND AVERAGE		17.6	18.3	18.9	18.1	18.2

[a] Each value is the mean of three observations

APPENDIX E. Analysis of variance of photosynthetic efficiency of frozen stored and root pruned white spruce seedlings measured following transplanting.

Source of Variation	df	Mean Square	Variance Ratio	Level of Significance[1]
Replications	10	5764.085	1.204	N.S.
Treatments	29	43258.270	9.039	***
Storage (S)	2	73494.810	15.357	***
Time Observed (T)	4	186979.760	39.069	***
Root Pruning (R)	1	53042.180	11.083	***
S x T	8	21532.250	4.499	***
S x R	2	4497.000	0.940	N.S.
T x R	4	4591.750	0.959	N.S.
S x T x R	8	13365.120	2.793	***
Error	191	4785.910		
Total	329			

[1] N.S. = not significant, * = 0.05, ** = 0.01, *** = 0.005

APPENDIX F. Photosynthetic efficiency (mg $CO_2 \cdot g$ needle $ODW^{-1} \cdot hour^{-1}$) of white spruce seedlings frozen for 0, 50 and 92 days.

(i) Three days after planting

	Length of Frozen Storage (days)					
	0		50		92	
	Not Pruned	Pruned	Not Pruned	Pruned	Not Pruned	Pruned
	-39.0	-96.1	-31.9	-85.3	24.7	-92.7
	59.8	18.3	49.6	-136.3	52.0	9.0
	12.4	127.2	-9.9	-116.0	42.7	42.7
	-51.0	139.8	-10.9	-144.7	11.0	129.0
	30.0	-52.5	-8.1	-197.3	111.3	11.6
	-10.6	129.7	16.4	-51.2	45.3	-3.2
	47.7	85.2	12.0	-201.7	136.4	36.8
	76.1	115.1	32.0	-43.9	124.2	112.9
	143.3	163.4	61.9	-39.9	83.1	98.3
	73.6	0.0	42.7	-30.3	111.7	-24.4
	-23.2	-188.7	-33.3	-181.5	52.8	4.6
$\bar{x}$	33.23	40.13	10.95	-111.65	72.29	29.51

(ii) Seven days after planting.

	Length of Frozen Storage (Days)					
	0		50		92	
	Not Pruned	Pruned	Not Pruned	Pruned	Not Pruned	Pruned
	23.7	75.0	6.1	-3.3	-37.3	0.0
	8.4	-106.5	-36.5	-13.6	0.0	-25.6
	10.2	-37.0	-46.1	29.6	-20.9	-24.0
	0.0	35.1	0.0	67.9	-5.5	34.1
	34.8	-42.0	13.2	-1.6	13.8	-34.2
	44.2	-206.9	5.8	-2.1	12.8	0.0
	2.9	-37.1	42.1	2.2	2.4	13.4
	74.0	-128.1	26.1	-7.1	-12.1	-72.0
	-44.6	26.6	-53.1	41.8	-3.5	-10.9
	-147.8	75.9	12.5	29.1	6.1	0.0
	57.1	-90.0	38.1	-20.4	0.0	-5.4
$\bar{x}$	5.72	-39.55	-0.36	10.68	-4.02	-11.33

(iii) Fourteen days after planting.

	Length of Frozen Storage (Days)					
	0		50		92	
	Not Pruned	Pruned	Not Pruned	Pruned	Not Pruned	Pruned
	135.5	53.9	6.3	22.3	43.6	-115.4
	96.8	-46.7	8.8	27.9	65.2	13.3
	96.4	99.8	-40.3	39.7	86.9	123.6
	100.1	34.3	24.6	-67.6	92.3	-3.9
	94.7	166.7	0.0	79.4	116.2	43.1
	369.7	138.9	21.9	51.9	19.3	48.0
	76.8	122.7	-33.5	-18.4	38.7	10.2
	81.8	102.0	4.6	28.3	47.7	31.2
	105.6	80.9	24.3	39.5	124.5	12.6
	90.4	67.0	-46.7	94.8	108.3	7.9
	120.1	70.0	-45.6	-47.0	117.8	51.2
$\bar{x}$	124.35	80.86	-6.87	22.80	78.23	20.1

(iv) Twenty eight days after planting

	Length of Frozen Storage (Days)					
	0		50		92	
	Not Pruned	Pruned	Not Pruned	Pruned	Not Pruned	Pruned
	216.8	328.1	84.1	-5.3	27.6	97.1
	-108.5	105.1	53.4	-35.3	146.6	87.9
	52.9	220.2	94.2	-21.0	34.4	78.9
	90.4	-123.5	64.6	32.8	38.6	0.0
	156.5	253.0	10.0	222.8	126.4	135.9
	188.3	103.4	65.8	-20.1	106.5	20.4
	200.1	44.3	-19.6	4.1	75.3	48.8
	162.0	360.0	20.8	13.7	78.9	50.6
	-41.9	7.5	122.0	-82.4	53.1	80.7
	3.4	107.9	92.7	-62.1	133.2	25.4
	183.1	233.4	156.5	55.0	42.5	66.4
$\bar{x}$	100.28	149.04	67.68	9.29	78.46	62.92

(v) Forty two days after planting

	Length of Frozen Storage (Days)					
	0		50		92	
	Not Pruned	Pruned	Not Pruned	Pruned	Not Pruned	Pruned
	211.3	296.4	126.4	124.1	21.8	48.0
	187.1	-45.5	136.2	100.6	55.8	73.6
	89.1	192.0	104.9	126.7	133.7	135.9
	129.1	-11.5	128.1	112.8	132.0	199.6
	188.3	262.6	163.8	141.0	193.4	70.4
	228.9	182.5	129.5	145.4	164.7	53.7
	87.5	-17.0	144.2	126.3	156.4	166.0
	232.4	382.9	197.2	36.7	93.0	64.6
	97.2	-66.6	147.9	67.5	197.1	151.7
	50.4	194.6	117.8	64.8	190.3	151.5
	104.5	-53.3	156.9	84.1	172.5	146.6
$\bar{x}$	145.98	119.74	141.17	102.73	137.34	114.69

APPENDIX G. Analysis of variance of shoot moisture stress of frozen stored and root pruned white spruce seedlings measured following transplanting.

Source of Variation	df	Mean Square	Variance Ratio	Level of Significance[1]
Blocks	3	10.167	4.452	**
Treatments	23	45.585	19.960	***
Storage (S)	2	115.708	50.665	***
Time Observed (T)	3	154.322	67.572	***
Root Pruning (R)	1	260.542	114.083	***
S x T	6	8.630	3.779	***
S x R	2	6.815	2.984	***
T x R	6	2.235	0.979	N.S.
S x T x R	6	3.568	1.562	N.S.
Error	69	2.284		
Total	95			

[1] N.S. = not significant, * = 0.05, ** = 0.01, *** = 0.005

APPENDIX H. Root growth (mm new root·day^{-1}) of white spruce seedlings.

(i) Non-frozen, non-root pruned.

Time of Observation (Days)								
0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	0.80	0.87	1.91	[b]	8.07	4.18	5.53	3.36
---	1.73	1.45	1.34		5.83	7.03	4.88	4.54
---	1.65	2.50	2.67		4.90	4.90	6.62	10.51
---	---	1.52	2.16		4.44	5.08	5.21	5.69
---	---	1.10	5.01		16.37	12.08	11.28	3.53
---	---	0.82	0.49		4.91	8.58	5.33	4.71
---	---	1.48	1.79		3.79	3.70	0.47	---
---	---	1.43	2.96		12.33	8.23	8.72	10.62
---	---	1.50	5.04		16.36	15.60	17.49	6.47
---	---	0.57	0.83		4.21	28.68	44.66	22.62
---	---	---	---		11.37	20.05	19.38	16.71
---	---	---	---		1.23	3.28	2.47	3.94
---	---	---	---		3.11	2.88	5.38	4.59
---	---	---	---		4.54	4.08	10.38	4.53
---	---	---	---		1.59	1.73	5.33	6.22
---	---	---	---		4.84	7.83	6.78	2.98
---	---	---	---		3.66	2.38	3.94	4.99
---	---	---	---		30.44	40.45	21.01	9.65
---	---	---	---		3.29	4.95	1.65	4.66
---	---	---	---		0.91	4.70	6.47	7.36
---	---	---	---		9.60	11.28	9.86	8.53
---	---	---	---		21.26	17.90	32.24	53.76
---	---	---	---		3.29	9.75	10.59	5.18
---	---	---	---		1.40	5.48	14.33	10.81
---	---	---	---		9.17	16.20	8.24	3.65
---	---	---	---		4.73	4.73	8.74	11.16
---	---	---	---		1.04	3.73	8.04	10.87
---	---	---	---		---	1.00	9.13	12.71
---	---	---	---		---	5.80	---	---

[a] No root growth observed.

[b] No observations made.

APPENDIX H.

(ii) Non-frozen, root pruned.

Time of Observation (Days)								
0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	---	1.02	3.20	[b]	7.50	11.63	3.35	10.83
---	---	---	---		2.20	9.28	10.27	13.91
---	---	---	---		2.49	8.13	11.59	9.15
---	---	---	---		0.36	0.58	2.01	9.64
---	---	---	---		2.44	5.78	13.45	7.12
---	---	---	---		8.73	7.45	4.82	1.87
---	---	---	---		0.89	4.00	4.93	9.87
---	---	---	---		---	1.13	2.61	8.79
---	---	---	---		---	1.25	1.12	3.09
---	---	---	---		---	5.23	2.84	6.75
---	---	---	---		---	2.95	8.48	12.69
---	---	---	---		---	2.38	4.56	5.47
---	---	---	---		---	2.85	3.99	1.41
---	---	---	---		---	---	6.18	24.56
---	---	---	---		---	---	1.67	3.52
---	---	---	---		---	---	2.53	4.47
---	---	---	---		---	---	2.35	1.20
---	---	---	---		---	---	1.75	0.39

[a] No root growth observed.

[b] No observations made.

APPENDIX H.

(iii) Frozen 50 days, non-root pruned

Time of Observation (Days)								
0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	---	2.80	2.40	1.08	13.72	8.15	0.72	---
---	---	0.64	3.06	4.60	21.80	11.80	6.48	6.26
---	---	1.54	1.76	2.86	31.34	75.60	127.75	88.29
---	---	1.40	4.60	2.80	10.98	8.98	3.02	---
---	---	0.60	0.40	0.30	7.60	3.48	4.12	4.86
---	---	1.06	---	1.40	8.08	2.60	6.36	7.17
---	---	0.78	3.40	3.68	7.48	---	---	---
---	---	1.92	2.54	1.00	11.58	2.73	---	---
---	---	---	2.02	0.80	11.28	15.25	14.72	17.71
---	---	---	4.78	5.40	7.00	4.58	7.24	9.11
---	---	---	2.76	1.68	2.60	13.50	18.18	13.54
---	---	---	3.46	0.60	1.40	0.93	---	3.83
---	---	---	---	3.24	---	3.48	5.20	12.80
---	---	---	---	1.00	13.96	---	6.44	16.51
---	---	---	---	---	1.88	0.90	3.10	5.51
---	---	---	---	---	3.40	4.65	10.88	9.97
---	---	---	---	---	7.82	3.23	3.18	---
---	---	---	---	---	5.00	8.08	7.82	4.86
---	---	---	---	---	6.80	9.23	9.38	7.40
---	---	---	---	---	3.58	4.78	5.90	1.51
---	---	---	---	---	2.08	---	1.86	1.46
---	---	---	---	---	1.58	2.13	5.54	8.34
---	---	---	---	---	4.64	---	5.92	3.11
---	---	---	---	---	3.78	17.28	18.80	7.14
---	---	---	---	---	0.96	1.65	---	---
---	---	---	---	---	2.40	7.23	8.80	3.00
---	---	---	---	---	---	1.58	2.20	---
---	---	---	---	---	---	20.63	28.20	31.71
---	---	---	---	---	---	---	4.18	3.97
---	---	---	---	---	---	---	---	---

[a] No root growth observed.

APPENDIX H.

(iv) Frozen 50 days, root pruned.

Time of observation (Days)								
0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	---	---	2.58	2.10	18.20	23.43	17.86	20.06
---	---	---	2.54	0.86	7.08	4.25	1.08	---
---	---	---	2.40	0.80	11.00	19.40	12.58	17.43
---	---	---	---	1.88	8.80	14.68	13.56	4.14
---	---	---	---	---	2.80	2.75	2.40	---
---	---	---	---	---	0.92	---	1.00	1.11
---	---	---	---	---	2.78	2.93	13.44	15.26
---	---	---	---	---	3.00	7.98	10.72	7.74
---	---	---	---	---	1.00	8.00	8.44	8.86
---	---	---	---	---	1.38	3.25	7.24	5.60
---	---	---	---	---	2.76	2.25	3.86	4.57
---	---	---	---	---	6.98	7.00	11.98	6.06
---	---	---	---	---	10.42	18.48	21.60	4.26
---	---	---	---	---	1.92	---	---	---
---	---	---	---	---	---	4.40	3.70	14.09
---	---	---	---	---	---	0.88	9.60	4.54
---	---	---	---	---	---	3.90	5.80	4.26
---	---	---	---	---	---	---	9.92	1.54
---	---	---	---	---	---	---	---	2.63

[a] No root growth observed.

APPENDIX H.

(v) Frozen 92 days, non-root pruned.

Time of Observation (Days)

0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	1.90	2.78	6.80	10.78	12.96	12.53	17.54	20.00
---	3.02	8.86	8.58	12.18	2.66	4.28	7.68	20.37
---	1.38	14.06	22.22	24.40	17.18	1.08	1.38	5.90
---	2.16	3.92	10.78	25.40	26.20	27.58	29.40	20.00
---	2.22	6.86	6.46	14.08	11.60	12.60	9.58	9.20
---	1.30	4.98	9.44	22.84	20.90	12.00	7.48	23.00
---	0.72	0.60	10.80	33.34	2.32	14.05	6.74	8.00
---	1.40	6.64	12.04	14.94	6.60	3.23	6.00	6.80
---	2.58	3.10	1.94	2.82	---	1.93	---	1.47
---	1.16	2.90	12.40	20.20	5.78	9.23	7.82	12.23
---	---	6.92	13.38	18.78	11.10	1.75	---	10.33
---	---	2.10	3.10	8.38	7.40	5.73	---	4.90
---	---	1.44	2.52	10.40	13.02	7.60	10.92	10.10
---	---	1.00	2.62	24.36	20.66	15.98	11.00	17.60
---	---	23.00	20.10	17.98	14.70	5.23	9.30	---
---	---	1.40	0.50	2.40	6.62	---	4.60	7.03
---	---	1.00	1.78	3.28	1.58	3.95	4.42	5.17
---	---	7.92	14.58	33.88	10.72	15.50	11.30	13.97
---	---	5.14	10.06	24.74	24.06	10.78	9.98	14.03
---	---	1.06	2.42	5.00	4.00	5.23	3.96	7.03
---	---	4.40	4.82	13.80	22.26	25.60	36.18	27.40
---	---	0.76	1.16	1.80	1.72	---	1.18	1.00
---	---	3.64	3.72	12.18	27.00	17.50	18.38	16.53
---	---	---	1.62	3.36	5.86	4.13	5.64	7.03
---	---	---	9.20	13.90	12.40	11.25	8.42	16.27
---	---	---	4.30	8.58	20.98	39.25	26.76	40.93
---	---	---	---	4.32	4.30	---	5.34	1.80
---	---	---	---	5.00	6.86	8.00	11.58	8.50
---	---	---	---	7.82	13.04	14.38	11.58	20.33
---	---	---	---	---	2.50	2.25	1.16	9.20

[a] No root growth observed.

APPENDIX H.

(vi) Frozen 92 days, root-pruned.

Time of Observation (Days)								
0-5	6-10	11-15	16-20	21-25	26-30	31-34	35-39	40-42
---[a]	---	1.40	2.46	7.82	14.44	20.75	29.84	33.33
---	---	1.44	2.58	6.36	4.34	6.85	10.08	14.33
---	---	0.52	0.78	0.44	---	---	---	---
---	---	2.60	0.88	0.40	---	---	---	---
---	---	---	1.78	17.22	16.20	14.35	19.88	22.17
---	---	---	4.00	24.16	30.06	21.75	35.12	81.63
---	---	---	2.76	4.66	8.40	8.08	18.96	13.67
---	---	---	1.02	6.82	14.34	8.63	12.86	11.17
---	---	---	1.00	0.80	3.02	2.75	3.64	5.67
---	---	---	0.38	2.38	5.50	10.63	9.00	9.67
---	---	---	---	12.20	20.10	11.45	16.28	24.30
---	---	---	---	6.22	4.12	4.00	3.42	4.70
---	---	---	---	3.02	1.78	2.25	7.42	3.60
---	---	---	---	4.20	3.40	2.98	12.22	19.67
---	---	---	---	1.18	0.42	---	---	---
---	---	---	---	---	4.80	9.23	9.48	11.00
---	---	---	---	---	1.20	1.73	2.28	2.63
---	---	---	---	---	---	---	18.12	34.10
---	---	---	---	---	---	---	---	22.50
---	---	---	---	---	---	---	---	73.00

[a] No root growth observed.