The Effects of Elevated Post-Storage Temperatures on the Physiology and Survival of White Spruce Seedlings'

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Abstract.--The objectives of this study were to determine the effects of elevated temperature and exposure time on the physiology and survival of post-cold stored white spruce *(Picea glauca* (Moench) Voss.) seedlings, and whether such effects could be detected quickly physiologically prior to planting. Foliage and root temperatures lagged behind ambient temperatures after transfer from storage (-2°C), to thawing (5°C), and from thawing to heat treatments (10, 20, 30, 40°C). Although no visible seedling damage was apparent after 12 h at 40°C, damage was 32% after 24 h. Seedling mortality was 489 after 48 h, and reached 100'6 after 72 h. At 30, 20 and 10°C no seedling mortality was observed for 24, 72 and 96 h respectively. Root Growth Capacity was poor in treated seedlings showing poor survival after planting. Seedling mortality of over 10% from elevated temperatures may be detected in about 24 h from specific conductivity of tissue leachates. This test, however, does not predict significant non-lethal heat stress tissue damage of seedlings prior to planting. Exposure of seedlings in boxes to temperatures above 10°C is not recommended.

INTRODUCTION

Survival of conifer seedlings after planting depends on their physiological state prior to storage, at the time of planting, and upon planting site conditions (Ritchie 1984; Duryea 1985). After lifting seedlings may be culled, counted, stored, loaded in and out of transporters, temporarily stored in the field and finally planted (Trewin 1978). During these operations seedlings may be exposed to a range of stresses including drying or freezing, mechanical damage to shoots and roots through impact (Trewin 1978; Tabbush 1986), and heating (DeYoe et al. 1986).

Exposure of conifer seedlings to heat may have a number of effects on seedling physiology and subsequent survival including stress metabolism (Haard 1983), excessive expenditure of food reserves (Mattsson 1986; Ritchie 1984; Puttonen 1980, 1986), and loss of cold hardiness (Levitt 1980). Stress due to heat increases with temperature and duration of exposure (Levitt 1980).

Although extreme heat results in rapid, extensive tissue death, the effects of more moderate heat stress on seedling vigour are not always visible and are usually only manifested after the stock has been planted (Kauppi 1984). Determining seedlings vigour status using potting trials often takes too long for the nurseryperson to make a decision about stock quality before shipment to the planting site. However, some physiological attributes may be detected which reveal relationships between stock quality and subsequent survival after planting (Wakely 1948; Zaerr 1985).

This study reports: i) the amount of heat stress which can be applied to white spruce seedlings after cold storage without a decrease in survival or vigour, ii) and whether the results of heat stress can be detected by physiological tests prior to planting. Results indicate the tolerance of the test seedlot to heat stress during thawing, pre-shipment storage, transportation, or storage at the planting site.

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METHODS OF INVESTIGATION

General

White spruce 1-0, PB313 styroblock containerized seedlings (seedlot Sw, 8504, 87G09005), destined for spring planting in northeastern British Columbia, were grown under operational conditions at a nursery near Vancouver, British Columbia in 1987. Seedlings were lifted in late November 1987 and cold stored (-2°C) at the Ministry of Forests, Research Laboratory, Glyn Road, Victoria, B.C.

Waxed cardboard boxes (36x14 in.) were lined with a wax paper bag and seedlings were packed, 500 to a box in bundles of 20 with plugs wrapped in plastic. Boxes were then sealed with tape so that they could be reopened to remove samples.

In the spring of 1988 physiological measurements were made during two time intervals because of the short sampling period and available growth chamber space. Each period included an 8-day thaw at 5°C followed by up to 96 h heat. The first sample period began on April 11, 1988 and the second on April 26.

Samples were taken of frozen seedlings, and seedlings which had been thawed for 8 days. Following the thaw period seedlings were placed into the randomly assigned temperature treatments, 5, 10 and 40°C during the first experimental period and 20 and 30°C during the second. Seedlings were sampled and measured after 12, 24, 48, 72, 96 h.

The temperature inside boxes was recorded constantly throughout the experiment with thermistors monitored by a Campbell CR10 datalogger. Each box contained three temperature probes measuring foliage, and root temperatures of inner and outermost seedling bundles.

Each temperature treatment was represented by only one growth chamber because of limited equipment availability.

Specific Conductivity of Leachates

The amount of electrolyte leakage from tissues is a relative measure of the degree of cell membrane damage caused by exposure of seedlings to stress (i.e. low or high temperatures). The method used here is modified from van den Driessche (1976) and Burr et al. (1986).

Measurements were made after 24, 48, 72 and 96 h. Fifteen seedlings were divided at random into three replicates of five seedlings. The middle 8 cm section of stem of each seedling was cut into 15, 0.5 cm stem segments. Three segments were randomly selected from each of the five seedlings and placed in a covered test tube.

Needle segments 1 cm long, cut at both ends, were taken from one side of the stem of each seedling. All needles removed from one seedling were mixed and subsamples of needles from each of five trees in each replicate combined to give an approximate final weight of 0.7 g.

During preparation the stem and needle segments were kept in small plastic petri dishes on moist filter paper before transferring them to test tubes. Deionised water was added to the tissue segments in a ratio of 10:1 by weight. The specific conductance of the leachates were determined with a Radiometer CDM83 Conductivity meter after 24 h in a water bath at 25°C.

Root Growth Capacity

Root Growth Capacity (RGC) tests were conducted on 16 seedlings removed; i) directly from cold storage ii) at time zero of the treatment period (end of thaw) and, iii) after 48 and 96 h. For the 20 and 30°C heat treatments tests were also conducted after 24 h. Test conditions were carried out according to B.C. Ministry of Forests standards for white spruce, (400 9 $mol^{-2}.s^{-1}$, 30°C day/25°C night, 75%RH and a 16 h light period. Pots, containing a peat/vermiculite mixture (pH 5.7) were watered to field capacity at time zero and after 5 days. After a test period of 7 days the numbers of roots >1 cm produced during the test were counted and the Index of Root Growth calculated (IRG) (Burdett 1979).

Survival of Heat Treated Seedlings

Immediately after treatment seedlings were planted on site at the Glyn road Research Laboratory. Seedlings were planted in a completely randomized design. No water or fertilizer was applied.

Seedlings were evaluated for mortality and damage two months after planting. A seedling was considered dead if needle damage extended over the whole shoot. Damage (between 10 and 90%) to each seedling was scored as a percentage of the total plot sample (50 seedlings) if the seedling was not dead.

RESULTS AND DISCUSSION

Temperature inside boxes did not immediately reach target levels (Figs. 1 and 2). This lag time, may account for the surprising tolerance of this seedlot at the highest heat treatment. The 40°C treatment temperature was not reached until almost 20 h after treatment started, but was about 36°C within 6 h (Fig. 1) and over 30°C within 3 h. There can also be a differential heating rate up to 8 h between outer and inner bundles in an box (compare Fig. 1 to Fig. 2).

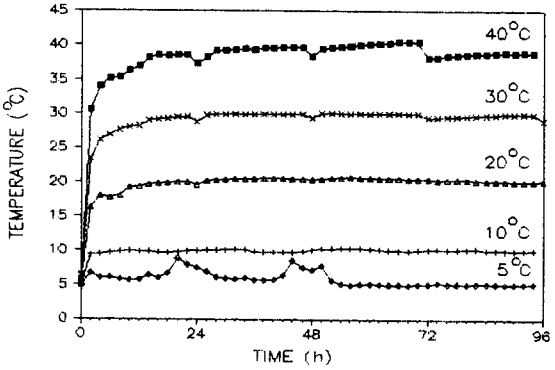

Figure 1. Foliage temperatures (°C) inside boxes during both experimental treatment periods, from April 18-22 (40, 10, and 5°C) and May 02-06 (20 and 30°C).

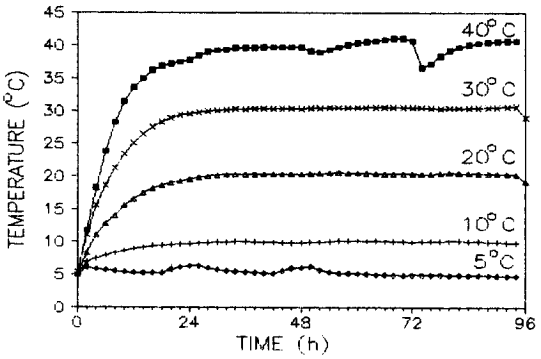

Figure 2. Root temperatures (°C) inside boxes during both experimental periods, from April 18-22 (40, 10 and 5°C) and May 02-06 (20 and 30°C). A bundle from the centre of the box was monitored.

Survival

Table 1 shows the percentage of damage (D) and mortality (M) of treated seedlings 2 months after planting on a moist site. Mortality was <4% and there was no damage to live seedlings which were planted after removal from cold storage (2°C), and after thawing at 5°C for 8 days. After 96 h at 5, 10 and 20°C mortality was <10% and damage was zero.

Mortality and damage increased with length of treatment at 30 and 40°C. At 30°C mortality was <10% up to 48 h but increased to 18 and 409 at 72 and 96 h. At 40°C visible damage was observed at 24 h and mortality was 489 by 48 h and 100% by 72 h.

Root Growth Capacity

Figure 3 shows that the Index of Root Growth (IRG) was acceptable by operational standards over the thawing period and there was no significant

Table I. Percentage of field mortality (M) and damage (D) to white spruce seedlings resulting from heating for up to 96 h. Dashes indicate damage or mortality was <4%.

TEMP. (°C)		DURATION OF TREATMENT (H)											
		0		12		24		48		72		96	
		M	D	M	D	M	D	M	D	M	D	M	D
THAW	5	-	-	-	-	-	-	-	-	-	-	-	-
HEAT	5	-	-	-	-	-	-	-	-	-	-	6	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	8	-	6	-
	30	-	-	-	-	6	16	4	-	18	16	40	20
	40	-	-	-	-	4	32	48	4	100	--	100	--

change over the treatment period at 5, 10 and 20°C. At the start of the heat treatments IRG was about 3.5, after 48 h at the 30 and 40°C treatments IRG had decreased to 2.5 and to 0.2 respectively. Only a slight decrease in IRG was noted in seedlings which received 20°C for 96 h, but IRG decreased to 0.5 at 30°C and was zero at 40°C.

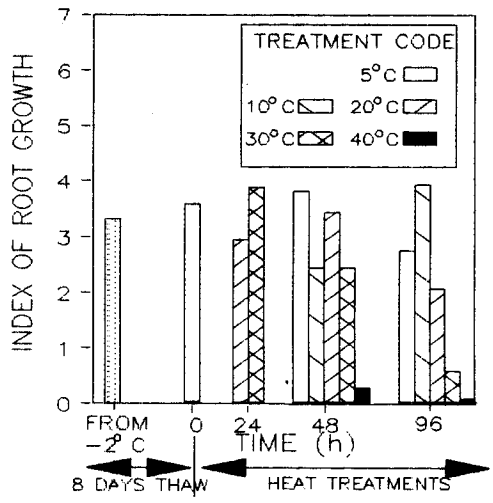

Figure 3. Index of Root Growth (IRG) of white spruce seedlings after treatment at five storage temperatures (5, 10, 20, 30 and 40°C) for up to 96 h.

Figures 4, 5 and 6 show roots on seedlings at 10°C and 30°C for 96 h and 40°C for 48 h. Seedlings in the latter two temperature treatments had visible shoot damage compared with those which were held at 10°C for 96 h. A comparison of Table I and Figure 3 indicates that IRG data seem to reflect quite well the field survival results.

Figure 4. Root development after an RGC test of seedlings previously treated to a 10°C storage temperature for 96 h. There was no visible damage to stems or needles. Buds were flushing.

Figure 5. Root development after an RGC test of seedlings previously treated to a 30°C storage temperature for 96 h. Considerable discolouration of stems and needles was noted in some seedlings. Buds were not flushing.

Figure 6. Root development after an RGC test of seedlings previously treated to a 40°C storage temperature for 48 h. Discolouration in many stems and needles was severe. Buds were not flushing.

Specific Conductivity

Specific conductivity of leachates from stem segments increased with length of treatment and temperature (Fig. 7). At 40°C specific conductivity increased after 48 h indicating cell damage (Fig. 7). At 20 and 30°C cell damage occurred after 72 h treatment. (Actual temperatures inside boxes are shown in Figures 1 and 2).

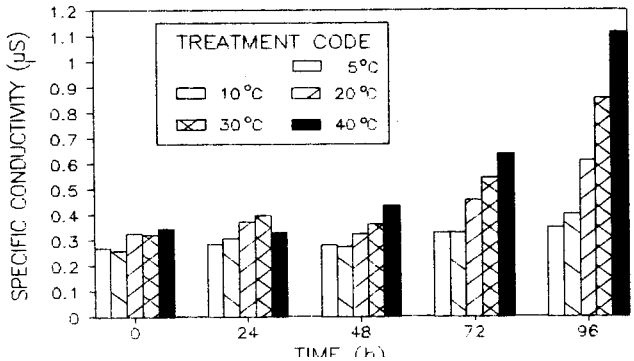

Figure 7. Specific conductivity (uS) of leachates from stem segments of white spruce after holding intact seedlings in five treatment temperatures up to 96 h. Segments were soaked in 10 times their fresh wt. (g) of deionised water for 24 h.

Specific conductivity of leachates from needle segments (Fig. 8) increased in 30 and 40°C treatments indicating tissue damage after 72 h. At 20°C an effect, although slight, was also noted after 72 h. However, this did not increase' further after 96 h. At 5 and 10°C no change occurred up to 96 h.

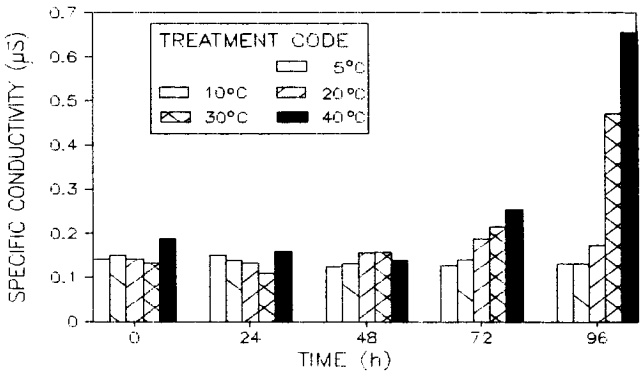

Figure 8. Specific conductivity WS) of leachates from white spruce needle segments after holding intact seedlings in five treatment temperatures for up to 96 h. Segments were leached into 10 times their fresh wt.(g) of deionised water for 24 h.

Measurement of leachate conductivity from tissues may be useful to assess stock quality quickly (24 h) after damage from excessive heating. Conductivity has been used to assess other types of physiological stress e.g. frost damage (Colombo et *al.* 1984) for some time. However, the test at present seems to lack sensitivity. Below 48 h heat treatments did not result in a detectable increase in leachate conductivity (Figs. 7 and 8) despite significant visible seedling damage after outplanting (see 30°C after 24 h, Table 1). Results indicate mortality of greater than 10% is detectable. However, the amount of leachate detected increases with time within a specific treatment temperature and continues to increase even after mortality has reached 100% (c.f. Table 1 and Figures 7 and 8 at 40°C at 72 and 96 h). Measurement of leachates from stem segments appears to be a more sensitive indicator of heat damage than from needle segments.

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LITERATURE CITED

Burdett, A.N. 1979. New methods for measuring root growth capacity: Their value in assessing lodgepole pine stock quality. Can. J. For. Res. 9:63-67.

Burr, K.E., R.W. Tinus, S.J. Wallner, and R.M. King. 1986. Comparison of four cold hardiness tests on three western conifers. *In:* Proceedings of the Combined Western Forest Nursery Association Meeting, Tumwater, Wash., August 12-15. p. 87-95.

Colombo, S.J., D.P. Webb and C.O. Glerum. 1984. Frost Hardiness testing: An operational monitoring manual for use with extended greenhouse culture. Ontario Ministry of Natural Resources. Forest Research Report No. 110.

DeYoe, D., H.R. Holbo, and K. Waddell. 1986. Seedling protection from heat stress between lifting and planting. WJAF 1: 124-126.

Duryea, M.L. 1985. Evaluating seedling Quality: Importance to reforestation. *In:* Evaluating Seedling Quality: Principles, procedures, and predictive abilities of major tests. M.L. Duryea. ed. Proceedings of the Workshop held October 16-18 1984. Forest Research Laboratory, Oregon State University, Corvallis.

Haard, N.F. 1983. Stress Metabolites. *In:* Postharvest physiology and crop preservation. M.Lieberman, ed., Plenum Publishing Co., New York.

Kauppi, P. 1984. Stress, strain and injury: Scots pine transplants from lifting to acclimation on the planting sites. Acta. For. Fenn. 185: 5-50.

Levitt, J. 1980. Responses of plants to environmental stress. I: chilling, freezing and high temperatures. Academic Press, New York.

Mattsson, A. 1986. Planting site storage: effects on survival and growth of overwinter-stored Scots pine *(Pinus* sylvestris*)* containerized seedlings. Can. J. For. Res. 16: 84-89.

Puttonen, P. 1980. Effect of temporary storage temperature on carbohydrate levels in Scots pine seedlings and planting success. Proceedings of the IUFRO meeting working group s. 1.05-04 Frieburg i.Br. p.

Puttonen, P. 1986. Carbohydrate reserves in *Pinus* sylvestris seedlings as an attribute of seedling vigour. Scand. J. For. Res. 1: 181193.

Ritchie, G.A. 1986. Relationships among bud dormancy status, cold hardiness, and stress resistance in 2+0 Douglas fir. New Forests. 1: 29-42.

Ritchie, G.A. 1984. Assessing seedling quality. In: Forest Nursery Manual: Production of bareroot seedlings. M.L. Duryea and T.D. Landis, eds. Martinus Nijhoff/Dr. W. Junk Publishers. The Hague, for Forest Research Laboratory., Oregon State University, Corvallis.

Tabbush, P.M. 1986. Rough handling, soil temperature, and root development in outplanted Sitka spruce and Douglas fir. Can. J. For. Res. 16: 1385-1388.

Trewin, A. 1978. Pine seedlings-handle with care. What's New in Forest research. 67: 3 p.

van den Driessche, R. 1976. Prediction of cold hardiness in Douglas fir seedlings by index of injury and conductivity methods. Can. J. For. Res. 6: 511-515.

Wakely, P.C. 1948. Physiological grades of southern pine nursery stock. *In:* Proceedings of the Society of American Foresters, 1948. p. 311322.

Zaerr, J.B. 1985. The role of biochemical measurements in evaluating vigour. *In:* Evaluating Seedling Quality: Principles, procedures, and predictive abilities of major tests. M.L. Duryea ed. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis.