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Heat damage in boxed white spruce (*Picea glauca* [Moench.] Voss) seedlings: Its pre-planting detection and effect on field performance

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Application. Results of this study show that boxed white spruce seedlings, removed from cold storage (−2 °C) and thawed (+5 °C), can tolerate warm (i.e. ≤ 20 °C) temperatures for up to 4 days without affecting field growth performance. However, exposure to temperatures above 5 °C for any duration during transport and field storage, is not recommended. Exposure to temperatures greater than 30 °C for longer than 48 h results in severe physiological deterioration of the seedlings and must be avoided. Physiological damage due to heat stress may be detected using electrolyte leakage or root growth potential tests prior to planting of seedlings. These tests could be used to decide whether to plant white spruce seedlings that may have been heat stressed after removal from cold storage.

Abstract. This study investigated the effects of holding 1+0 PSB313a white spruce (*Picea glauca* (Moench.) Voss) seedlings in storage boxes at air temperatures of 5, 10, 20, 30 and 40 °C for 12, 24, 48, 72 and 96 h before planting. The ability to detect physiological damage to seedlings as a result of such treatment, before planting, was also examined. After one growing season, no needle damage or mortality >8% was found for temperature treatments up to 20 °C for 4 days. At 30 °C and above, seedling damage and mortality increased, while bud flush, shoot height, stem diameter and shoot dry weight decreased with increasing temperature and duration of treatment. Seedling mortality in the field was 100% after the 40 °C treatment exposure for 72 h or longer. Pre-planting needle electrolyte leakage was indicative of visible needle damage 14 days after planting, whereas stem electrolyte leakage and root growth potential were more closely related to end of season plantation mortality. Despite the lack of damage observed at 20 °C or below, preplanting exposure of white spruce seedlings to temperatures above 5 °C, during transportation and field storage, is not recommended.

Introduction

Successful regeneration of conifer seedlings is dependent upon their physiological state at the time of planting, and upon prevailing site conditions (Ritchie 1985; Burdett 1987). Generally, seedlings are subjected to a number of potentially vigor-reducing operations between lifting and planting. In British Columbia, for example, after winter-lifting, seedlings are usually counted, placed into wax-lined cardboard boxes, cold-stored, loaded in and out of vehicles, thawed and sometimes stored in the field before finally being planted (Trewin 1978). During storage and transportation before planting the trees may be exposed to excessive heating (DeYoe et al. 1986) as well as a range of other stresses (Trewin 1978; Tabbush 1986; Mattsson 1986).

Of the studies reporting on heat stress, most deal with the effect of temperatures over 40 °C for relatively short periods, either uncovered (Kauppi 1984; Seidel 1986), or in plastic bags (DeYoe et al. 1986; McCreary and Duryea 1987; Koppenaal and Colombo 1988), immersed directly in heated water (Colombo and Timmer 1992), or only use needle sections (Burr et al. 1993). Some studies have investigated the effects of short-term temperature exposure in the range of 10–40 °C (McCreary and Duryea 1987; Koppenaal et al. 1991), but none in which boxed stock was exposed immediately after removal from cold storage. Seedling exposure to stresses before planting may present a problem, from a forest plantation management perspective, in that visible symptoms of seedling damage usually do not develop until some weeks after planting (Kauppi 1984). The time taken for seedlings to show injury after a heat treatment depends on the degree of stress suffered by the exposed tissues. Direct injury generally results from brief exposures, and is characterized by rapid cell membrane disruption and biochemical decomposition during, or immediately after, heating (Levitt 1980). Direct injury has been reported to occur above 50 °C (53–69 °C in white spruce and other conifers (Lorenz 1939, as cited in Levitt 1980, p. 377). Colombo and Timmer (1992) reported >70% direct damage to black spruce seedlings after immersion in water at 46 °C for $\geq$60 min. The membrane thermal break-point (i.e. direct killing) for these seedlings appears to be 10 minutes at 52 °C (see also Ruter (1993) using different varieties of holly). Indirect heat injury is observed after exposure to lower temperatures than those causing direct injury but is not apparent for many days or longer (Treshow 1970). The main physiological impact of indirect heat injury is through the progressive (i.e. time-dependent) impairment of major biological processes such as photosynthesis and respiration. Other damaging abnormalities such as the accumulation of toxic substances, formation of biochemical lesions, and protein proteolysis also are known to contribute to indirect heat injury (Levitt 1980). For example, no direct damage was incurred by black spruce seedlings after 40 °C for 180 min, but 40% damage due to indirect injury occurred over a 3 week period (Colombo and Timmer 1992).

Relatively few reports are available on preplanting tests which can detect sublethal heat stress in conifer seedlings and thus aid nursery managers or field foresters in deciding whether to accept or reject stock that may have been stressed and therefore be of questionable quality. McCreary and Duryea (1987) report using root growth potential, (seedling) vigor and plant moisture stress to evaluate Douglas-fir seedlings exposed to 32 °C. Colombo and Timmer (1992) also report detection of direct heat injury by visual assessment and indirect injury by dry weight differences in black spruce. Electrolyte leakage has been used in a number of studies to assess heat damage and

thermotolerance in plants. Blum and Ebercon (1981) used this method to study membrane stability of different wheat cultivars under various conditions of moisture and heat stress. They found maximal response separation of the cultivars at 44 °C. Tal and Shannon (1983) used electrolyte leakage to assess the thermostability of different tomato cultivar protoplasts after exposure to 50 °C for various amounts of time. They attribute the differences they found to the degree of saturation of fatty acids in the phospholipids of the cell membranes. Ruter (1993) used the method to show the membrane thermostability in holly was exceeded at >50 °C for 30 mins. This was attributed to loss of cell compartmentalization as a result of irreversible plasma membrane damage. The method has also been used to study recovery from sub-lethal heat stress in dogwood (Shirazi and Fuchigami 1993).

Our objectives were to determine 1) the amount of heat stress that white spruce seedlings, in storage boxes, can tolerate without affecting subsequent survival or growth potential, and 2) whether changes in seedling vigor due to heat stress can be detected by physiologically based tests before planting.

Materials and methods

A 1+0 white spruce (*Picea glauca* [Moench.] Voss) seedlot (58°48′ N and 122°46′ W, 525 m elevation) from Prophet River, Fort Nelson, British Columbia was grown in BC/CFS styroblocks (PSB 313a) (Beaver Plastics Ltd, Edmonton, Alta.) at a commercial nursery near Vancouver, British Columbia (49°28′ N). Forty-eight hundred seedlings from 50 styroblocks, sampled from a total crop of 5500 styroblocks, were lifted during the last week of November, one day before the lifting of the whole commercial crop. Seedlings were packed into 12 waxed boxes (61×30×41 cm) each containing 16 bundles of 25 seedlings. Each bundle represented a random sample from all 50 styroblocks. According to B.C. Ministry of Forests operational practices, boxes were lined with polythene-coated paper bags and the roots of bundles were wrapped in clear plastic. All boxes were cold stored at −2 °C for between 21 and 23 weeks.

Treatments

Treatments consisted of exposing whole seedlings, packed in boxes, to seven days thawing at +5 °C followed immediately by up to four days (96 h) at either, 5, 10, 20, 30 or 40 °C. Therefore all seedlings received the same thaw period but each subsequent heat treatment was applied, cumulatively, for 0, 12, 24, 48, 72, and 96 h. Each temperature was applied to one storage box of seedlings in one Conviron E15 growth chamber. Seedlings were packed in the same way in which they had been cold stored. Thermocouples installed in

the growth chamber, and inside the boxes at the needle and root zones, continuously monitored (hourly summaries) temperature using a Campbell CR 21 data logger. Box air temperature equilibrated with exterior (environmental chamber) temperature after 2 h at 10 °C, and after 14 h at 20, 30 and 40 °C. In comparison, seedling root plug temperatures required 26 h to reach 20 and 30 °C, and 28 h to reach 40 °C.

A treatment was terminated by withdrawing a sample of seedlings from the box and returning them to +5 °C. Treated seedlings were either tested or planted within 4 hours of sampling. Because of limited growth chamber space, and to allow time for sampling, testing and planting, treatments were applied over two testing periods. The first ran from April 11 through April 22 and the second from April 25 to May 06. Treatment temperatures were randomly assigned to one of the two test periods. The first test period included the 5, 10 and 40 °C treatments, and the second the 20 and 30 °C treatments.

Seedling planting and measurements of field performance

Treated seedlings were planted into a farm-field site at the Glyn Road Research Station, Victoria, B.C. (southern tip of Vancouver Island) during the periods April 11–22 and April 25 to May 06. A sample of 50 seedlings per treatment combination was planted in two plots of 25. All plots were completely randomized within an area of 12×17 m. No fertilizer, or water other than natural precipitation, were applied throughout the growing period. The test site was kept clear by hand weeding. Soil moisture tension was estimated gravimetrically from planting to August at nine randomly chosen plots. Soil water potential at 10 cm depth never fell below −0.05 MPa during planting, and remained greater than −0.3 MPa during shoot extension (mid-May to early-July). During July and August, at the end of shoot extension, the soil dried out and soil water potential decreased to −1.35 MPa. During the two planting periods mean soil temperatures were 10 °C in the morning and 13 °C in the afternoon. Soil temperature did not increase until after planting when average morning and afternoon soil temperatures for the period mid-May to June were 15 °C and 17 °C respectively.

Seedling performance was measured as mortality and visible needle damage to surviving seedlings over the growing season, and also shoot extension, stem diameter (at the root collar) and shoot, root and total plant dry weight, of surviving seedlings, assessed at seedling harvest (last week of October). Shoot extension was derived from initial and final seedling height measurements. Morality at harvest was defined as the ratio of dead trees to total number of trees in a plot, expressed as a percentage. Visible needle damage was estimated as the proportion of dead (brown or red) to healthy needles on each seedling. Each seedling was assigned to one of 20 categories between 0 and

100% (0–5, 6–10, to 96–100%). Estimates of needle damage were made 14, 28 and 63 days after planting. The presence of non-flushed terminal buds was recorded 14 and 28 days after planting. At harvest all trees were excavated taking care not to damage the roots. Roots and shoots of surviving seedlings were cleaned, bagged separately, and dried at 80 °C for 48 h to determine dry weights.

Physiological testing

Electrolyte leakage, a well established method of assessing freezing stress (Flint et al. 1967; Colombo et al. 1984; Burr et al. 1990; Sutinen et al. 1992) was used as an indicator of heat stress based on the assumption that a relationship exists between cellular membrane damage resulting from heat stress, and electrolyte efflux (Blum and Ebercon 1981; Tal and Shannon 1983; Ruter 1993; Burr et al. 1993). Efflux of electrolytes due to membrane damage was expressed as a fraction of the total electrolytes in the tissue, termed the fractional release of electrolytes (FRE). Electrical conductivity was measured using a Radiometer CDM83 conductivity meter.

Electrolyte leakage was measured for temperature treatments of 5, 10, 20, 30, and 40 °C for either 0, 24, 48, 72 or 96 h. The 12 h time duration treatment was not assessed because of time constraints. Fifteen seedlings were sampled from each treatment combination. The sample was divided into 3 groups of 5 seedlings to provide 3 replicates. A 7.5 cm section of stem, mid-way between the root collar and apex, was clipped from each seedling. Needles were excised and the stems cut into 0.5 cm sections. Three 0.5 cm sections of stem were taken from each of 5 seedlings and combined to make one replicate of 15 segments weighing approximately 0.7 g. A variable number of needle segments, cut at both ends, and approximately 1 cm long, were taken from each of the 5 seedlings sampled for stem segments and combined, resulting in approximately 0.7 g of tissue. During preparation the stem and needle segments were kept on moist filter paper in covered petri dishes at +5 °C. Tissue segments were subsequently transferred to glass tubes and 10 times the sample fresh weight of deionized water added. The samples were incubated at 25 °C for 24 h, after which electrical conductivity (EC_i) of the bathing medium was measured. Control and treated samples were than heat killed for 30 min. in a water bath at 90 °C, and the electrical conductivity (EC_k) measured again after a further 24 h incubation. The fractional release of electrolytes (FRE) was expressed as EC_i/EC_k.

Root growth potential

Root growth potential (RGP) was assessed for seedlings from the 0, 48 and 96 h temperature treatments. Testing was carried out according to B.C. Ministry of Forests standards (16 trees potted in 4, one litre pots containing peat moss at pH 5.7) (Binder et al. 1990). A Conviron E15 environmental growth chamber provided a photoperiod of 16 h at 400 μmol m^{-2} s^{-1} and 8 h dark with a synchronized thermoperiod of 25 °C (light) and 20 °C (dark). The number of new roots >1 cm produced during a 7 day test period was counted and indexed according to the Burdett (1979) scale (i.e., 0 = no new roots, 1 = any new roots < 1.0 cm in length, 2 = 1–3 new roots > 1.0 cm, 3 = 4–10 new roots > 1.0 cm, 4 = 11–30 new roots > 1.0 cm, 5 = 31–100 new roots > 1.0 cm).

Statistical analysis and experimental design

Space constraints did not allow for replication of experimental units (growth chambers) therefore all replication in this study must be considered pseudo-replication. However, chamber and box temperature were both continuously monitored. When planted, the treated seedlings were divided into two plots of 25 seedlings and arranged in a completely randomized design. Growth data collected at harvest was subjected to a one way classification ANOVA using Proc GLM of the Statistical Analysis System (SAS) with initial seedling height as a covariate. Two treatment combinations were excluded (shown in table below as asterisks) because of complete mortality leaving 28 combinations (27 degrees of freedom). Selected contrasts were performed based on a proposed response surface of weighted orthogonal contrasts shown below.

		Time (h)					
		0	**12**	**24**	**48**	**72**	**96**
Temp (°C)	**5**	9	9	9	9	9	9
	10	9	9	9	9	9	9
	20	9	9	9	9	9	−12
	30	9	9	9	−12	−33	−54
	40	9	9	−33	−54	*	*

The expected response surface was tested and found significant for all the growth measures except root dry weight where the model was not significant (p = 0.1303) at the 5% level. Visible needle damage data was subjected to an arcsine square root transformation. However, means of the transformed data did not change the r^2 of the regression against electrolyte leakage or the distribution of the residuals. Since the transformation did not change the interpretation for these data the untransformed results are presented. The means of corresponding treatment combinations for the dependent variables

Table 1. Mortality (%) of white spruce seedlings at time of harvest in November. Seedlings in sealed boxes stored for 6 months at −2 °C, and thawed for 7 days at 5 °C, were then exposed before planting to the temperatures and times shown below. Treated seedlings were planted in 2 plots of 25 seedlings each during the last week in April for treatments, 5, 10 and 40 °C and the first week in May for treatments 20 and 30 °C.

	Duration of exposure (h)					
	0	12	24	48	72	96
TEMPERATURE (°C)						
5	0	4	2	2	2	6
10	2	2	4	4	4	2
20	6	2	2	4	8	6
30	2	4	2	6	26	40
40	4	2	8	56	100	100

mortality and needle damage 14 days after planting with the independent variables FRE from stems and needles were subjected to simple linear least squares regression. Mortality at harvest against stem FRE was also subjected to regression analysis using a logistic model of the form $y = a/1 + \exp^{c+bx}$. As the FRE procedure did not allow for a 12 h treatment duration the total number of treatment combinations for the regression was 25. A natural log function was used in the regression of mortality and RGP. Limited growth chamber space only allowed for a total of 12 treatment combinations for the analysis.

Results and discussion

Mortality, and visible needle damage to surviving seedlings over one field season

Mortality was less than 8% for thawed seedlings exposed for up to 96 h to pre-planting treatment temperatures of 5, 10 and 20 °C, or up to 48 h at 30 °C and up to 24 h at 40 °C (Table 1). Seedling mortality only exceeded 20% after 72 h exposure at 30 °C and after 48 h at 40 °C. All seedlings exposed to 40 °C for 72 h or longer were dead 63 days after planting. Seedlings alive after one field season showed visible damage to needles only in the 30 °C and 40 °C temperature treatments (Fig. 1). Most damage to needles developed between 14 and 63 days after planting, and further needle damage recorded at harvest (about 6 months) was expressed by 63 days. Development of needle damage took the longest in the 40 °C/48 h treatment combination and was quickest in the most severe treatment (40 °C/96 h). Visible needle damage to

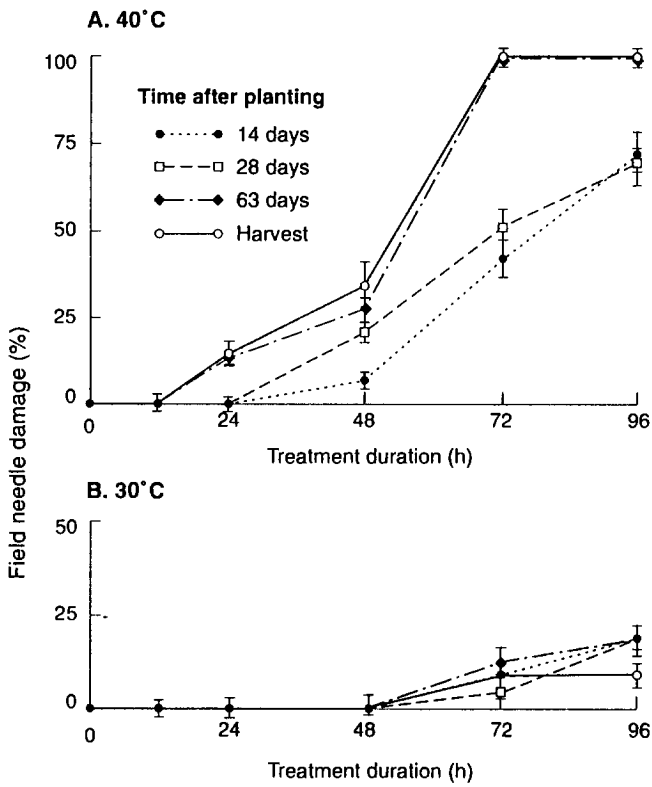

Fig. 1. Visible needle damage (%) to surviving white spruce seedlings 14, 28 and 63 days after planting, and at harvest in late October, resulting from preplanting temperature treatments of 40 and 30 °C for up to 96 h. Damage was less than 2% at 5, 10 and 20 °C and so these results are not shown. Error bars ± 1 SE, n = 20 to 50.

surviving seedlings was less than 20% for any treatment duration at 30 °C. By harvest, needle damage actually appeared to decrease in the 72 h and 96 h treatment durations at 30 °C because recovered seedlings had grown new needles. Also, dead needles which had fallen off the seedlings could not be accounted for in the assessment. Temperature, as well as its duration, clearly affected the timing and extent of visible damage after heating (Fig. 1). This inverse relationship between the damaging high temperature and the treatment duration has been shown for plant cells (Aleksandrov 1964). In 40 °C treatments causing complete field mortality (72 and 96 h durations), needle damage was quite obvious when seedlings were removed from storage boxes. Our results agree with Colombo and Timmer (1992) that where damage to needles is visible directly after heat exposure this is accompanied by volatile emissions. As with those workers we did not qualify these emissions by other

than smell, but ethanol and acetaldehyde are known to increase under such conditions (Hawkins and Binder 1990).

We believe that at the higher temperature/time duration treatment combinations injury was consistent with direct membrane damage, and this was the main cause of seedling mortality observed after planting. However, with black spruce, Colombo and Timmer (1992) report 46 °C for 3 h was the lowest temperature/time combination at which they could detect direct damage. A 3 h exposure at 40 °C failed to cause any direct injury. They do, however, report observing 40% indirect damage in seedlings exposed to 40 °C for 3 h, but again, could not show such damage after 3 h at 20 °C. These workers assessed direct damage as visible blanching of needles 2–5 mins after heat treatment and indirect heat damage by dry weight differences 3 weeks after the treatment. The difference in our temperature results, suggesting direct damage at 40 °C and even 30 °C, compared to those of Colombo and Timmer (1992) must be the amount of time seedlings were exposed. In support of this McCreary and Duryea (1987) have shown that exposing Douglas-fir seedlings, in bags, for only 2 days at 32 °C would reduce first year field survival by 15% while a 4 day treatment at that temperature resulted in less than 10% survival. Admittedly, the development of gray leaf mold resulting in almost entire needle drop was a strong influencing factor for the mortality values observed. We found no molds developed during any of our experimental treatments. We consider visible needle damage which developed weeks after planting the result of indirect injury.

Flushing of surviving heat-treated seedlings after planting

No seedlings had flushed 14 days after planting, although the majority of terminal and lateral buds were swelling. After 28 days, the percentage of non-flushing terminal buds was $\leq 10\%$, among plants exposed to 5, 10 and 20 °C for up to 96 h, 30 °C for up to 24 h, and 40 °C for up to 12 h, but increased with duration of exposure at 30 and 40 °C (Fig. 2, data for 10 and 20 °C not shown). Several seedlings with deformed, or partially flushed buds were also observed.

In conifers, the flushing of buds is a two stage process, consisting of a cell division phase and a cell elongation phase (Owens et al. 1985). Heat stress during either phase could account for decreased bud flushing at the higher temperature treatment combinations. Furthermore, of all cell membrane types, nuclear membranes show the least thermostability, so heat injury may well result in loss of ability for cell division, without affecting other life requiring processes governed by the plasma, mitochondrial, and chloroplast membranes (Berry et al. 1975; Levitt 1980). Temperature stresses in excess of 30 °C may, therefore, allow bud meristematic cells to carry on normal metabolic

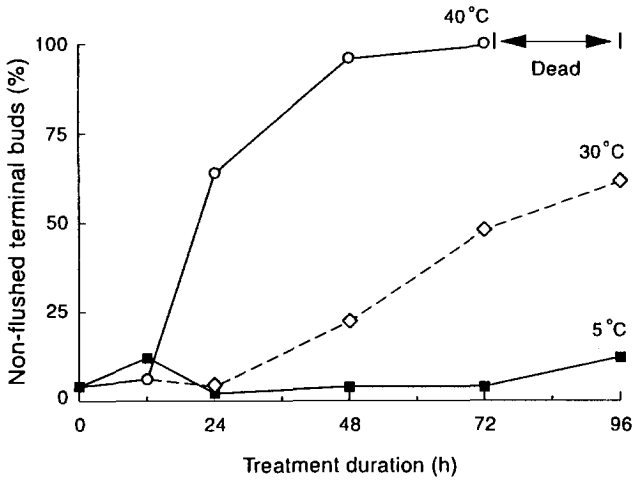

Fig. 2. Percent (%) non-flushed terminal buds of white spruce seedlings 28 days after planting. Heat treatment combinations were 5, 30 and 40 °C for up to 96 h applied after a 7 day thawing treatment at 5 °C from cold storage. Data for 10 and 20 °C are not included since these did not differ from the 5 °C treatment. Seedlings exposed to 40 °C for 72 h or longer did not survive. $n = 50$.

processes but be incapable of division. However, we also observed that many seedlings having lost most of their needles flushed and grew, particularly at the shorter duration of the 40 °C temperature exposure. Similar results were reported by Koppenaal and Colombo (1988), who showed that one-year-old black spruce seedlings were able to flush after exposure to 55 °C for 10 min, even though all foliage was killed. Because buds are protected by scales, bud tissue temperature may not equilibrate with ambient conditions when exposure time is short.

Performance of heat-treated seedlings after one field season

Seasonal growth measured as shoot extension, stem diameter, and dry weight of shoots and roots of heat-treated seedlings was unaffected by pre-planting temperature treatments of 5, 10 and 20 °C for up to 96 h (Fig. 3A, B, C, and D). Control values were 10.0 cm for shoot extension, 6.6 mm for stem diameter, 7.2 g for shoot dry weight, and 5.3 g for root dry weight. Exposure to 30 °C for 48 h significantly ($\alpha = 0.05$) reduced final shoot extension. Final shoot extension was reduced to 7 and 6 cm after 72 and 96 h pre-planting treatment respectively. Stem diameter and shoot dry weight were affected after 72 h and 96 h respectively (Table 2a, b, c; Fig. 3A, B, C). Stem diameter was 5.5 mm after 96 h and shoot dry weight decreased by about one third.

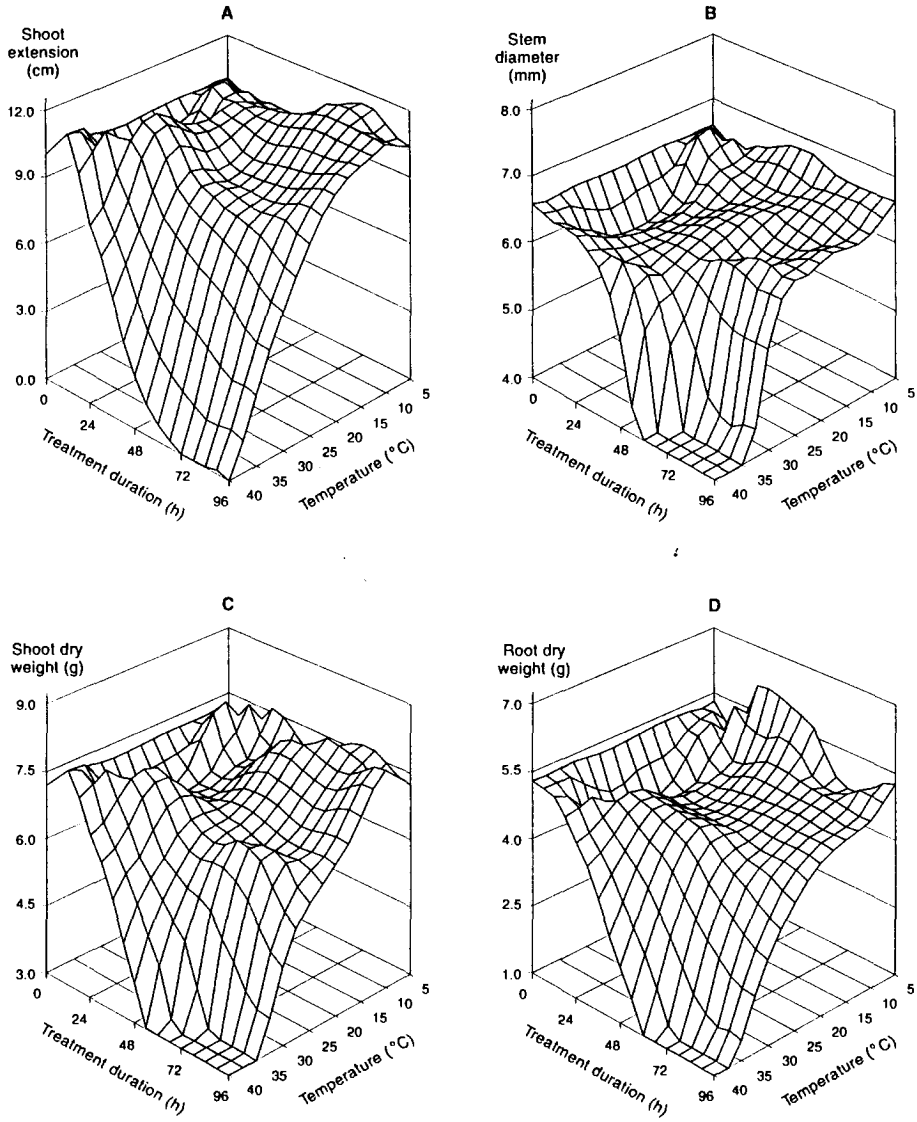

Fig. 3. Interpolated 3D surfaces of temperature treatment duration (h) (X axis), temperature intensity (°C) (Z axis) and seedling field performance response (Y axis) after one field season. Seedling field performance measures are: A) average shoot extension (cm), B) stem diameter (mm), C) shoot dry weight (g), and D) root dry weight (g) of white spruce seedlings at harvest. Preplanting heat treatments were 5, 10, 20, 30 and 40 °C for 0, 12, 24, 48, 72, and 96 h, applied after a 7 day thawing treatment at 5 °C.

Table 2a. Analysis of variance and orthogonal contrasts of selected treatment combinations for shoot extension of white spruce seedlings planted after heat treatments. Initial height was used as a covariate in the analysis. Treatments 72 and 96 h at 40 oC suffered complete mortality and were excluded from the analysis. ($\alpha = 0.05$).

Analysis of variance model for the variable shoot extension				
Source	df	MS	F	p
Shoot extension	27	109.53	3.46	0.00008
Error	28	31.64		
Total	55			
Contrasts of duration		at temperature (°C)		p
0 h vs. 48		30		0.0255
0–48 h vs. 72 h		30		0.3556
48 h vs 96 h		30		0.0025
0–12 h vs. 24 h		40		0.4995
24 h vs. 48 h		40		0.0062
0 h vs. 48 h		40		0.0027
Contrasts of temperature		at duration (h)		
5, 10, 20 °C vs. 30 °C		96		0.0277
5, 10, 20 °C vs. 40 °C		24		0.6928
5, 10, 20 °C vs. 40 °C		48		0.0005

The 30 °C treatment was significantly different from 5, 10 and 20 °C at 96 h for shoot extension (Table 2a, Fig. 3A), and stem diameter (Table 2b, Fig. 3B), but not for shoot dry weight (Table 2c, Fig. 3C). Root dry weight showed similar treatment trends to other performance measures but the ANOVA was statistically non-significant, because of the large variation between individual seedling root weights (Table 2d, Fig. 3D). Predictably, all storage treatments of 40 °C had the severest effect on growth of surviving seedlings. Shoot extension, stem diameter and shoot dry weight were significantly affected by treatment durations of 24 h or longer at 40 °C (Table 2a, b, c; Fig. 3A, B, C). Final shoot extension was 5.5 and 2.0 cm when treated for 24 and 48 h, respectively. The 48 h treatment at 40 °C resulted in a stem diameter of 5.3 mm at time of harvest, and reduced shoot and root dry weight by one half. Treatments longer than 72 h at 40 °C were lethal to all seedlings. The 40 °C treatment was significantly different from 5, 10, 20 and 30 °C after

Table 2b. Analysis of variance and orthogonal contrasts of selected treatment combinations for stem diameter of white spruce seedlings planted after heat treatments. Initial height was used as a covariate in the analysis. Treatments 72 and 96 h at 40 °C suffered complete mortality and were excluded from the analysis. ($\alpha = 0.05$).

Analysis of variance for the variable stem diameter				
Source	df	MS	F	p
Shoot diameter	27	8.94	1.95	0.0423
Error	28	4.58		
Total	55			
Contrasts of duration		at temperature (°C)		p
0 h vs. 48		30		0.2134
0–48 h vs. 72 h		30		0.8976
48 h vs 96 h		30		0.0401
0–12 h vs. 24 h		40		0.2902
24 h vs. 48 h		40		0.0999
0 h vs. 48 h		40		0.0252
Contrasts of temperature		at duration (h)		
5, 10, 20 °C vs. 30 °C		96		0.0386
5, 10, 20 °C vs. 40 °C		24		0.9672
5, 10, 20 °C vs. 40 °C		48		0.0115

48 h for shoot extension, stem diameter, and shoot dry weight, but not for root dry weight (Table 2a, b, c, and d). Shorter durations at 30 °C (24 h) and 40 °C (12 h) appeared to mildly stimulate growth, but the biological significance of this effect is obscure. Perhaps short term metabolic changes, or increased thermotolerance (Burr et al. 1993) due to synthesis of stable heat shock proteins (Koppenaal et al. 1991) are involved.

Physiological testing of seedlings exposed to temperature treatments

a) *Electrolyte leakage from stem and needle segments*

An increase in electrolyte leakage from cells is evidence of excessive stress to tissues, and is considered one of the first signs of direct injury to cell membranes (Levitt 1980; Tal and Shannon 1983; Ruter 1993). With conifers electrolyte leakage has been used to detect freezing stress (Burr et al. 1990), predict vitality (field survival) from root integrity after cold storage (McKay

Table 2c. Analysis of variance and orthogonal contrasts of selected treatment combinations for shoot dry weight of white spruce seedlings planted after heat treatments. Initial height was used as a covariate in the analysis. Treatments 72 and 96 h at 40 °C suffered complete mortality and were excluded from the analysis. ($\alpha = 0.05$).

Analysis of variance table for the variable shoot dry weight				
Source	df	MS	F	p
Shoot extension	27	31.53	1.95	0.0421
Error	28	16.15		
Total	55			
Contrasts of duration		at temperature (°C)		p
0 h vs. 48		30		0.0771
0–48 h vs. 72 h		30		0.7316
48 h vs 96 h		30		0.0349
0–12 h vs. 24 h		40		0.2116
24 h vs. 48 h		40		0.0706
0 h vs. 48 h		40		0.0082
Contrasts of temperature		at duration (h)		
5, 10, 20 °C vs. 30 °C		96		0.1434
5, 10, 20 °C vs. 40 °C		24		0.3502
5, 10, 20 °C vs. 40 °C		48		0.0016

Table 2d. Analysis of variance for root dry weight of white spruce seedlings planted after heat treatments. Initial height was used as a covariate in the analysis. Treatments 72 and 96 h at 40 °C suffered complete mortality and were excluded from the analysis. (α 0.05). No contrasts were made because the model was not significant.

Analysis of variance for the variable root dry weight				
Source	df	MS	F	p
Shoot extension	27	25.92	1.54	0.1303
Error	28	16.81		
Total	55			

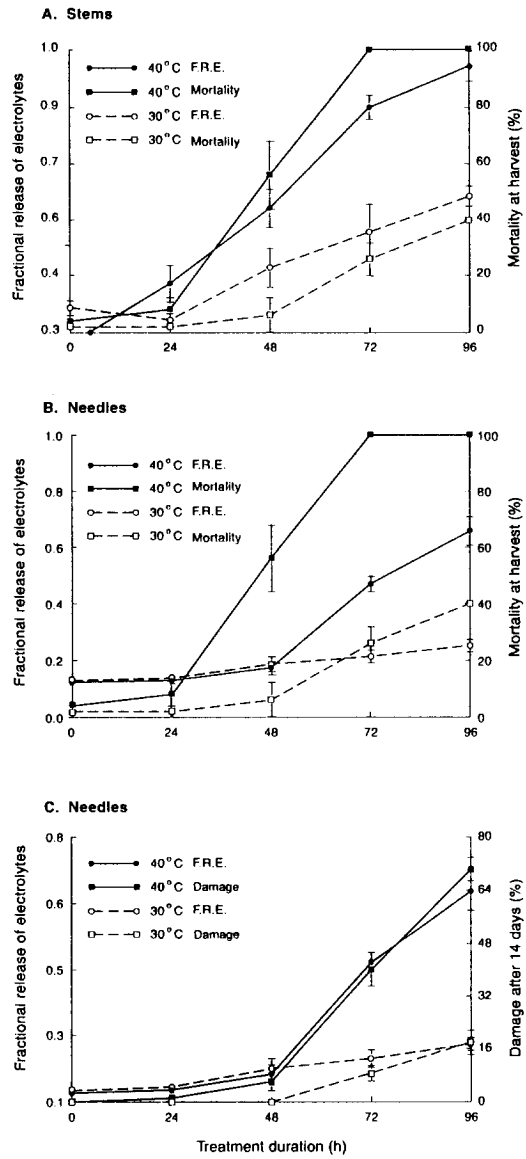

Fig. 4. Preplanting fractional release of electrolytes (FRE) from stem and needle segments after heat treatments of 40 and 30 °C for up to 96 h, and planted seedling mortality (%) at harvest, or visible damage (%) to needles 14 days after planting. Heat treatments were applied after a 7 day thawing treatment at 5 °C. Results for 5, 10 and 20 °C treatments for up to 96 h are not shown as they did not differ from the control treatment. A) Stem FRE shown with mortality (%) at harvest, B) Needle FRE shown with mortality (%) at harvest, C) Needle FRE shown with damage after planting. (FRE testing was done immediately after the short-term storage treatment). Error bars ± 1 SE, $n = 3$.

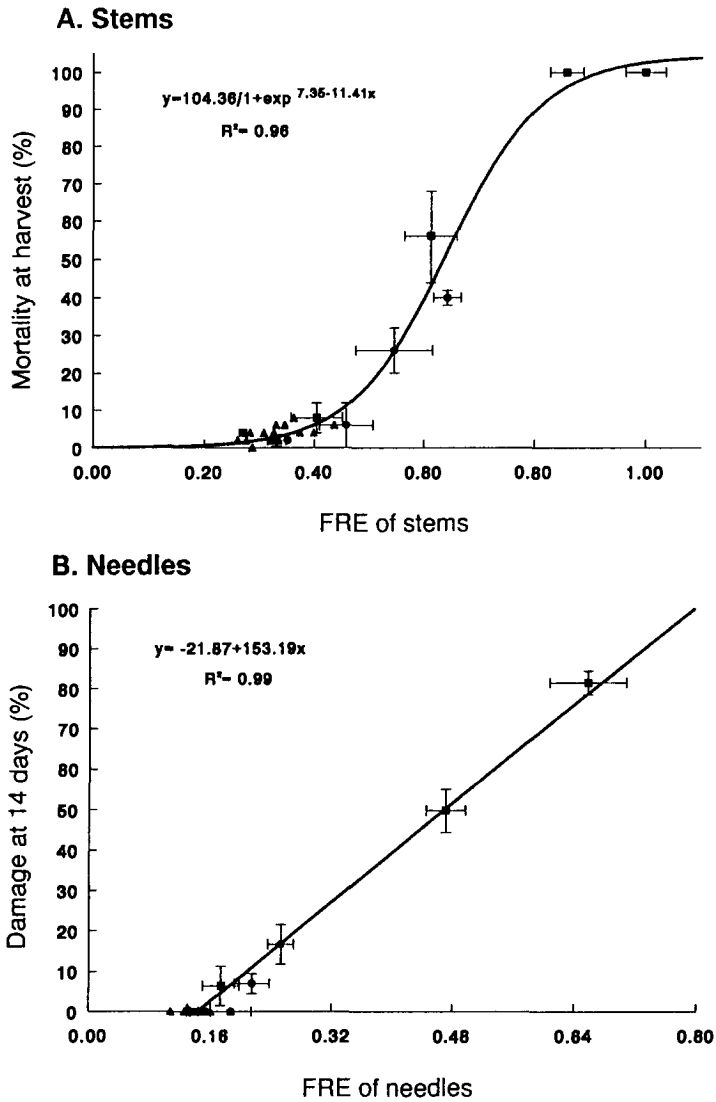

Fig. 5. Logistic regression curve A) for preplanting fractional release of electrolytes (FRE) from stems, and mortality (%) at harvest of planted seedlings; and B) linear regression between preplanting FRE of needles and visible damage (%) seen in needles 14 days after planting. Following a 7 day thawing period at 5 °C from cold storage preplanting treatment combinations included 5, 10, 20, 30 and 40 °C for 0, 24, 48, 72 and 96 h. Vertical bars are ± 1 SE for mortality ($n = 2$). Horizontal bars are ± 1 SE for FRE ($n = 3$). ■ = 40 °C, ● = 30 °C, and ▲ = 5, 10, or 20 °C temperature treatments.

and Mason 1991; McKay 1992) and determine thermotolerance of physiologically distinct (i.e. young, mature and frost hardy) types of needle tissue (Burr et al. 1993). We found close agreement between damage indicated by fractional release of electrolytes (FRE) from stem and needle segments before planting, and field planting results on similarly treated seedlings. Regression analyses of tissue FRE against mortality and needle damage were consistent with the graphical representation in Fig. 4. The FRE from stem segments was greater and conformed more closely to the expression of mortality at harvest time, than the FRE from needle segments (Fig. 5A and B respectively). Mortality (%) at time of harvest versus electrolyte leakage from stems was best fit to a logistic curve ($r^2 = 0.98$, MSE = 19.629) (Fig. 5A) while a linear regression fit the data less well ($y = -45.7 + 147.67\times$, $r^2 = 0.92$, MSE = 68.287) (not shown). The sigmoidal response curve we found to fit best is also described as typical for direct membrane injury due to heat stress (Blum and Albercon 1981; Ruter 1993). A linear regression of mortality at harvest against needle FRE could account for only 82% (MSE = 141.287) ($y = -23.53 + 214.02\times$) of the variation (not shown). Electrolyte leakage from needle segments followed the trend of visible needle damage 14 days after field planting (Fig. 4C). A linear regression model of pre-planting FRE from needles against needle damage 14 days after planting accounted for 99% (MSE = 8.446) of the variation of the mean values (Fig. 5B). For stem FRE against needle damage 14 days after planting the linear equation was $y = -30.32 + 87.05\times$ ($r^2 = 0.86$, MSE = 49.572) (not shown).

The relatively smaller amount of electrolytes released from needles compared to the stem sections may have been due to ion efflux being restricted only to the smaller diameter cut ends of the needle segments. However, the difference cannot entirely be due to physical means because stem segments also showed a greater sensitivity of response to the treatments, although variation was also greater. Fractional release of electrolytes from stem segments at the 40 °C treatment increased after 24 h while for needles only after 48 h, suggesting needles were either more tolerant of heat or able to partly avoid damage at this level of heat stress. This difference at equivalent treatment combinations may suggest a higher thermotolerance of needles. Considering that, in nature, needles are subjected to more direct thermal radiation from the sun than stems, this suggestion seems reasonable. We believe, that for preplanting heat stress, this difference in thermotolerance also indicates the main probable cause of seedling mortality after planting. We suggest direct membrane damage to stem tissues (i.e. cambium and living phloem conducting tissues) rather than sublethal damage to needle cells may be more important to whether the seedling lives or dies. However, the lack of distinct electrolyte leakage increase from needles at 40 °C

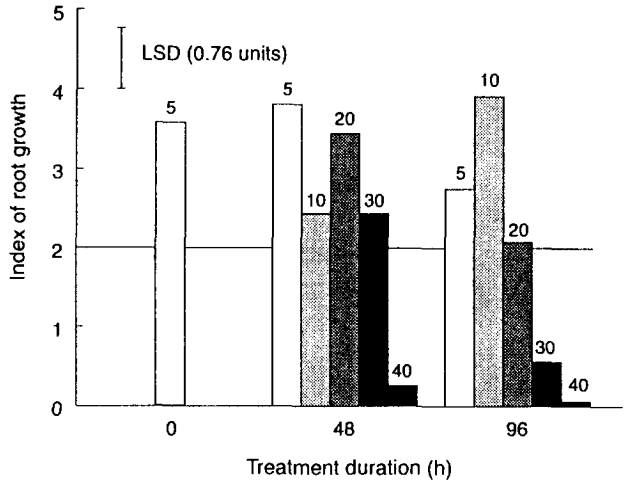

Fig. 6. Root growth potential of white spruce seedlings after exposure to heating treatments of 5, 10, 20, 30 and 40 °C for either 48 or 96 h. Numbers over bars indicate heat treatment temperatures. The RGP value shown for 0 h treatment duration was carried out at the time heat treatments were started following seedling cold storage and thawing for 7 days at 5 °C. The index of root growth (IRG) scale used follows Burdett (1979). IRG values above 2 (shown as a horizontal line) indicate good field survival for white spruce (Binder et al. 1988). Variation is shown at least significant difference ($n = 16$).

after 48 h, and 30 °C after 96 h, (Fig. 4B), resulting in significant mortality values (60% at 40 °C and 40% at 30 °C), could, in isolation be interpreted as suggesting indirect damage to needles eventually results in mortality of the seedling after several weeks. However, the close agreement between pre-planting stem FRE and mortality at harvest (Fig. 4A) suggests that overall survival of seedlings is more dependent upon the ability to move water and solutes through healthy conducting tissues then on sublethal damage to needles. On the other hand, since the observed damage to needle tissues 14 days after planting was best predicted by FRE of needles measured before planting (Fig. 5B), we suggest this measure may provide an indication of potential reduction in growth performance if seedlings are heat stressed prior to planting.

b) *Root growth potential of heat-treated seedlings*

Root growth potential is a well known, much used, test of conifer seedling physiological condition (Burdett 1987; Ritchie and Dunlap 1980; Ritchie 1985, 1990). Root growth potential of the thawed control, expressed as an index of root growth [IRG] (Burdett 1979), was 3.6 (Fig. 6). IRG of seedlings exposed to 5, 10, 20 and 30 °C, either after 48 or 96 h, was quite variable,

but the 20, 30 and 40 °C treatments decreased IRG significantly at the 48 and 96 h exposures. The 40 °C treatment resulted in significantly lower IRG than all other treatments at both 48 and 96 h, except for the 30 °C treatment at 96 h. The 40 °C treatment resulted in a decrease of IRG to 0.5 after 48 h, and no new roots were seen at the 96 h treatment, indicating that these seedlings were already severely damaged or dead. Poor root growth may have been caused by root injury in the most severe heat treatments, but no test of root cell viability was performed. In this regard electrolyte leakage could provide useful information on the condition of living cell membranes of roots (McKay and Mason 1991; McKay 1992). Needle and stem damage was probably detrimental to the production and translocation of photosynthates and plant growth regulators necessary for root growth. Furthermore, root growth is known to require current photosynthate (van den Driessche 1987; Binder et al. 1990), and photosynthetic processes important for root and shoot growth are known to be destroyed by temperatures which are 10 to 12 °C lower than those which cause rapid ion flux from tissues (Goodwin and Mercer 1972; Berry et al. 1975; Pearcy 1977; Levitt 1980).

The variability in the 5 and 10 °C temperature treatments at 48 and 96 h duration in Fig. 6 could be interpreted as suggesting RGP has little predictive value for seedling vitality after planting. However, under optimum environmental conditions large variations in test results have been a general observation for RGP. The theoretical implications of how this relates to any post-planting predictive value remains moot (Burdett 1987; Landis and Skakel 1988; Binder et al. 1990; Ritchie 1990), and beyond the scope of the present discussion. Nevertheless, good agreement between RGP and seedling field survival and height growth has been reported (McKay and Mason 1991; McKay 1992). McCreary and Duryea (1987) report RGP proved to be a good (r^2 = 0.70) predictor of first-year height growth and survival (r^2 = 0.65) in Douglas-fir after exposure to 32 °C for up to 4 days. We found a least squares regression log n fit of the dependent variable mortality at harvest, and the independent variable IRG demonstrated a good relationship (r^2 = 0.98, MSE = 17.77) (Fig. 7). We note, however, that only 2 points (means) out of 12 have more than 10%, but less than 100% mortality. The ability to point out only very good, or very poor quality trees has been considered a generally weak aspect of the IRG test (Binder et al. 1988). On a positive note other studies suggest that, for practical purposes, a root growth index (Burdett 1979) of 2 or higher (shown as a horizontal line in Fig. 6) usually results in acceptable planting survival (Simpson et al. 1988; Binder et al. 1988). We conclude that RGP does have application as a pre-planting indicator of heat stress in boxed white spruce seedlings.

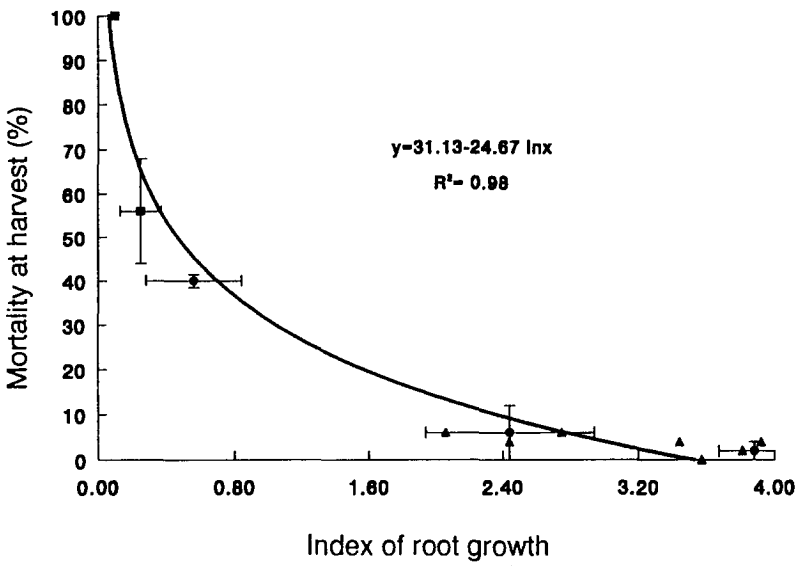

Fig. 7. Natural log function regression of root growth potential (IRG according to Burdett 1979) and mortality (%) at harvest. Data includes a treatment control (i.e. seedlings that had been thawed for 7 days at 5 °C), and preplanting temperature treatments of 5, 10 and 40 °C for 48 and 96 h, and 20 and 30 °C for 24, 48 and 96 h. Vertical bars are ± 1 SE for mortality ($n = 2$). Horizontal bars are ± 1 SE for RGP ($n = 16$). ■ = 40 °C, ● = 30 °C, and ▲ = 5, 10 or −20 °C temperature treatments.

Conclusion

Heat stress caused damage to needles and stems of white spruce seedlings, in boxes, when applied at the end of the cold storage period following thawing for 7 days at 5 °C. Findings indicate that external box temperatures of 30 and 40 °C caused a reduction in the quality of planted stock, and that the degree of damage was dependent on the duration of the heat treatment. At the critical treatment combinations, field needle damage and mortality were increased, while bud flushing, shoot extension, stem diameter, and root and shoot biomass were reduced. Electrolyte leakage from needles and stems was closely related to visible needle damage and field mortality respectively. These observations suggest the field mortality of seedlings at the 30 and 40 °C temperatures, after critical durations, was due to processes correlated with membrane damage. Indirect injury to needles after heat exposure which is not detected by electrolyte leakage is also possible and could significantly affect survival growth performance. However, we found that temperatures up to 20 °C for up to four days did not have any measurable effect on survival, growth, or ability of buds to flush. Possible deleterious metabolic

changes for longer periods at 20 °C, or lower, were not tested. Although high levels of stress resistance after a period of cold storage have been previously suggested for conifer seedlings (Ritchie 1989; Burr et al. 1990) any extension of these data to nursery or field operational situations must err on the side of caution. We discourage exposure of seedlings to temperatures greater than 5 °C for durations that would result in thawing of cold-stored stock, or after thawing but before planting. If, however, it is suspected that stock has received potentially detrimental heat exposure, this study suggests FRE and RGP could equally well establish whether seedling quality was affected. The method of choice will depend on the availability of equipment and technical expertise. FRE does have the advantage of a shorter testing time.

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References

Aleksandrov, V. Y. 1964. Cytophysiological and cytoecological investigations of heat resistance of plant cells toward the action of high and low temperature. Q. Rev. Biol. 39: 35–77.

Berry, J. A., Fork, D. C. and Garrison, S. 1975. Mechanistic studies of thermal damage to leaves. Carnegie Inst. Washington, Yearb. 74: 751–759.

Binder, W. D., Scagel, R. K. and Krumlik, G. J. 1988. Root growth potential: Facts, myths, value?, pp. 111–118. In: Proceedings, Combined Meeting of the Western Forest Nursery Association USDA Gen. Tech. Rep. RM-167.

Binder, W. D., Fielder, P., Scagel, R. K. and Krumlik, G. J. 1990. Temperature and time-related variation of root growth in some conifer tree species. Can. J. For. Res. 20: 1192–1199.

Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21: 43–47.

Burr, K. E., Wallner, S. J. and King, R. M. 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiol. 6: 351–369.

Burr, K. E., Wallner, S. J. and Tinus, R. W. 1993. Heat tolerance, cold hardiness, and bud dormancy relationships in seedlings of selected conifers. J. Amer. Soc. Hort. Sci. 118: 840–844.

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.

Burdett, A. N. 1987. Understanding root growth capacity: Theoretical considerations in assessing planting stock quality by means of root growth tests. Can. J. For. Res. 17: 768–775.

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

Colombo, S. J. and Timmer, V. R. 1992. Limits of tolerance to high temperatures causing direct and indirect damage to black spruce. Tree Physiol. 11: 95–104.

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

Flint, H. L., Boyce, B. R. and Beattie, D. J. 1967. Index of injury – a useful expression of freezing injury to plant tissues as determined by the electrolytic method. Can. J. Plant Sci. 47: 229–230.

Goodwin, T. W. and Mercer, E. I. 1972. Introduction to Plant Biochemistry. Pergamon Press, Oxford.

Hawkins, C. D. B. and Binder, W. D. 1990. Chapter 8 – State of the Art Seedling Stock Quality Tests Based on Seedling Physiology. In: Rose, R., Campbell, S. J. and Landis, T. D. (Eds) Target Seedling Symposium; Proceedings, Combined Meeting of the Western Forest Nursery Associations. USDA For. Ser. Gen. Tech. Rep. RM-200.

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

Koppenaal, R. S. and Colombo, S. J. 1988. Heat tolerance of actively growing, bud initiated, and dormant black spruce seedlings. Can. J. For. Res. 18: 1103–1105.

Koppenaal, R. S., Colombo, S. J. and Blumwald, E. 1991. Acquired thermotolerance of Jack pine, white spruce and black spruce seedlings. Tree Physio. 8: 83–91.

Landis, T. D. and Skakel, S. G. 1988. Root growth potential as an indicator of outplanting performance: Problems and perspectives. USDA For. Ser. Gen. Tech. Rep. RM-167: 106–110.

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

McCreary, D. D. and Duryea, M. L. 1987. Predicting field performance of Douglas-fir seedlings: comparison of root growth potential, vigor and plant moisture stress. New Forests 3: 153–169.

McKay, H. M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. Can. J. For. Res. 22: 1371–1377.

McKay, H. M. and Mason, W. L. 1991. Physiological indicators of tolerance to cold storage in Sitka spruce and Douglas-fir seedlings. Can. J. For. Res. 21: 890–901.

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

Owens, J. N., Webber, J. E., Ross, S. D. and Pharis, R. P. 1985. Interaction between Gibberellin A4/7 and root-pruning on the reproductive and vegetative processes of Douglas-Fir. III Effects on anatomy of shoot elongation and terminal bud development. Can. J. For. Res. 15: 354–364.

Pearcy, R. W. 1977. Acclimation of photosynthesis and respiratory carbon dioxide exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. Plant Physiol. 59: 795–799.

Ritchie, G. A. 1985. Root growth potential: Principles, procedures, and predictive ability, pp. 93–105. In: Duryea, M. L. (Ed) Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests. Dept. of Forest Science, Oregon State University, Corvallis, OR.

Ritchie, G. A. 1989. Integrated growing schedules for achieving physiological uniformity in coniferous planting stock. Forestry 62(suppl.): 213–227.

Ritchie, G. A. 1990. Root growth potential and the target seedling. In: Rose, R., Campbell, S. J. and Landis, T. D. (Eds) Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations. USDA For. Ser. Gen. Tech. Rep. RM-200.

Ritchie, G. A. and Dunlap, J. R. 1980. Root growth potential: Its development and expression in forest tree seedlings. N. Z. J. For. Sci. 10: 218–248.

Ruter, J. M. 1993. High-temperature-induced electrolyte leakage from excised leaves and roots of three hollies. HortSci. 28: 927–928.

Seidel, K. W. 1986. Tolerance of seedlings of ponderosa pine, Douglas-fir, grand fir and Engelmann spruce for high temperatures. Northwest Sci. 60: 1–7.

Shirazi, A. M. and Fuchigami, L. H. 1993. Recovery of plants from "Near-Lethal" stress. Oecologia 93: 429–434.

Simpson D. G., Vyse, A. and Thompson, C. F. 1988. Root growth effects on field performance, pp. 111–118. In: Proceedings, Combined Meeting of the Western Forest Nursery Associations. USDA For. Ser. Gen. Tech. Rep. RM-167.

Sutinen, M.-L., Palta, J. P. and Reich, B. R. 1992. Seasonal differences in freezing stress resistance of needles of *Pinus nigra* and *Pinus resinosa*; evaluation of the electrolyte leakage method. Tree Physiol. 11: 241–254.

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.

Tal, M. and Shannon, M. C. 1983. Effects of dehydration and high temperature on the stability of leaf membranes of *Lycopersicon esculentum, L. cheesmanii, L. peruvianum* and *Solanum pennellii*. Z. Pflanzenphysiol. 112: 411–416.

Treshow, M. 1970. Environment and Plant Response. McGraw-Hill Book Co., New York.

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

van den Driessche, R. 1987. Importance of current photosynthate to new root growth in planted conifer seedlings. Can. J. For. Res. 17: 776–782.