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Impacts of nursery cultural treatments on stress tolerance in 1 + 0 container white spruce (*Picea glauca* [Moench] Voss) seedlings for summer-planting

Weixing Tan

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Abstract Impacts of nursery cultural treatments (T) on stress tolerance of greenhouse-grown 1 + 0 container white spruce (*Picea glauca* [Moench] Voss) seedlings (mean height 24 cm, root collar diameter 3.1 mm) for summer planting were studied. Seedlings were subjected to 12-h short-day treatments of 0 (T0), 3 (T3), 7 (T7), 10 (T10), or 15 (T15) days, followed by 0, 7, 17, 40, or 46 days of reduced N supply, respectively. Relevant physiological and morphological factors were examined concurrently. Foliar N concentrations exceeded optimal levels and differed little among treatments, suggesting a minor confounding role for N reduction. Both frost and drought tolerance increased incrementally from T0 through T15. Electrolyte leakage index decreased steadily from T0 (25% for roots, 17% for needles) to T15 (1% for roots, 2% for needles) after 2-h exposure of fine roots to -2°C and of needles to -8°C. Withholding soil watering for 19 days caused 80% mortality among seedlings in T0, 50% in T3, and $< 10\%$ in T7–T15. The transpiration decline curve suggested that enhanced drought tolerance was largely attributable to quicker stomatal closure during water stress and lower cuticular transpiration rate. The treatments increased root growth capacity on a per-seedling, but not per-root-mass, basis. Needle primordia were developed in all T7-T15 seedlings but not in T0 and T3 treatments, suggesting that nurseries may need no more than 7 days of blackout application for conditioning spruce seedlings for summer planting. Shoot dry weight fraction increased gradually from T0 through T15 and was linearly correlated with needle specific weight and frost tolerance, and may thus be useful in monitoring progress of conditioning treatments.

Keywords Short-day · Conditioning · Drought tolerance · Frost tolerance · Physiology

W. Tan (✉)
Department of Science, Grande Prairie Regional College, 10726 – 106 Avenue, Grande Prairie, Alberta, Canada T8V 4C4
e-mail: wtan@gprc.ab.ca

Introduction

Reforestation by summer planting of container stock has become increasingly common in boreal Canada (Revel et al. 1990; Mitchell et al. 1995). Whereas stock for spring-planting is normally lifted in fall and stored over-winter (e.g. Grossnickle 2000), stock for summer planting is usually grown in winter through spring or early summer, lifted, and ''hot-planted'' without interim storage. Such stock must be able to tolerate stresses commonly generated in newly outplanted seedlings by conditions at the planting site, including drought and non-optimal temperatures (Blake and Sutton 1987; Tan 1992; Grossnickle 2000).

Stress tolerance has been developed by means of cultural conditioning treatments applied in the fall to stock for spring planting, or in summer to stock for summer planting. These treatments include manipulation of light (e.g. short day), nutrient, moisture, and/or temperature regimes (Grossnickle 2000; Paterson et al. 2001). Extensive research has enabled appropriate operational guidelines and practices to improve stress tolerance in stock for spring planting, but much less is known about this in summer stock (Grossnickle 2000). In spruces, for example, seedling freezing tolerance has been enhanced commonly through a period of short-days (e.g. Christersson 1972; Bigras and D'Aoust 1992; Hawkins et al. 1996; Coursolle et al. 1998); while the results were variable under reduced N or water availability (e.g. Young and Hanover 1978; Blake et al. 1979; Macey and Arnot 1986; Calmé et al. 1993; Bigras et al. 1996). A high level of uncertainty, nevertheless, remains as to how different mechanisms may have mediated the changes in stress tolerance during the cultural treatments (Sutton 1988; Grossnickle 2000).

Response of summer stock to conditioning treatments may differ from that of spring stock and need to be ascertained in order to develop conditioning guidelines appropriate for summer planting stock (Grossnickle 2000). The present study examines the degree to which certain cultural treatments impart drought and frost tolerance to white spruce stock for summer planting and explores physiological changes associated with changes in tolerance.

Materials and methods

Seedling production, cultural treatments, and sampling for tests

Stratified white spruce (*Picea glauca* [Moench] Voss) seeds from registered seedlot No. MDF12-92-25-5-95SW (Alberta; 56°57′N, 117°53′W; elevation, 670 m) were sown in 315B Styrofoam® containers (Beaver Plastics Ltd., Edmonton, Canada) using peat moss as a growing medium on a different date (Table 1) for each of five cultural treatments (T). Different sowing dates aimed to limit variation of seedling size and allow concurrent testing of impacts after the completion of the cultural treatments which varied in duration. About 1200 seedlings for each treatment were grown at the Pacific Reforestation Technology Beaverlodge Nursery, Alberta. All experimental containers grouped by treatment were situated in a central area in the greenhouse surrounded by other non-experimental white spruce seedlings.

The seedlings were grown under the following conditions: photoperiod of 23 h with the natural light being supplemented by 600 W high pressure sodium vapor

Table 1 Sowing date, short-day exposure (photoperiod-h and days of exposure) and sampling date for five nursery cultural treatments (T) in white spruce seedlings

Date	Nursery cultural treatment				
	T0	T3	T7	T10	T15
29 Dec 1998					Sown
6 Jan 1999				Sown	
25 Jan			Sown		
5 Feb		Sown			
18 Feb	Sown				
15 May					12-h for15 days, then 15.5-h for 22 days
26 May				12-h for 10 days, then 15.5-h for 16 days	
21 June			12-h for 7 days, then ambient for 17 days	Ambient for 24 days	Outside ambient for 24 days
5 July		12-h for 3 days, then ambient for 7 days			
15 July 1999	Same sampling date for all treatments				

The 'ambient' refers to the environment inside the unheated greenhouse under natural daylength; while the 'outside ambient' refers to the environment outside the greenhouse The seedlings received a 23-h photoperiod unless otherwise indicated

lamps (General Electric); daily average air temperature of 20–23°C; and relative humidity of > 40%. From June 21, 1999, the daily average temperature was allowed to fluctuate between 13 and 17°C. Nutrient solution was applied 1–3 times weekly via a sprinkler system similar to that used by Khasa et al. (2001) whenever the seedling containers dried to approximately 70% of their saturated weight. Through the actively growing period, seedlings were fertilized at 150 ppm N (N:P:K 2:1:2 with adequate micronutrients). The N concentration was reduced to 100 ppm 2 weeks before, and then to 70 ppm during, the short-day application. Thereafter, the concentration of N was changed to 0, 50 and 70 ppm during the 1st, 2nd and 3rd week, respectively. This sequence was repeated every 3 weeks until the sampling date.

After reaching an average height of ~20 cm, the seedlings in each of the five treatments were subjected to 0 (T0), 3 (T3), 7 (T7), 10 (T10), or 15 days (T15) of short-day application by completely excluding light from the seedlings with blackout curtains for 12 h per day from 20:00 to 8:00 (Table 1). After the blackout the seedlings in T3–T15 were further conditioned through 7, 17, 40, or 46 days of reduced N fertilization, respectively. The T10 cultural regime is normal practice in the Beaverlodge Nursery for growing white spruce for summer planting.

About 100 seedlings were sampled, without bias, from half of the containers (4–5) in each treatment on one sampling date at the end of treatments (Table 1). Subsamples were taken, again without bias, for tests, as detailed below. For testing survival under controlled water stress, 15 seedlings in each treatment, all about 24 cm tall for uniform transpiration water loss, were selected directly from the nursery.

Seedling morphology and nutrient content

Fifteen seedlings from each treatment were measured for shoot height (cm), root collar diameter (mm), shoot fresh weight (g) under well-watered conditions, and shoot and root dry weight (g) after oven-drying at 80°C for 48 h. Root to shoot ratio (g g^{-1}) was calculated as the ratio of root dry weight to shoot dry weight; shoot dry weight fraction (g g^{-1}, SDWF) as the ratio of shoot dry weight to shoot fresh weight (Grossnickle et al. 1991). Specific needle weight (g m^{-2}) was measured on a ~5 cm section of the terminal shoot as needle dry weight/total needle surface area. The total needle surface area was measured using a volume displacement technique (Johnson 1984; Tan et al. 1992).

The number of needle primordia on each embryonic shoot was estimated on eight seedlings from each treatment following the procedure of Templeton et al. (1993).

Total needle nitrogen (N), phosphate (P), and potassium (K) concentrations (% of the dry weight) were analyzed in each of seven seedlings from each treatment using the technique of Huang and Schulte (1985) for P and K, and that of the Association of Official Analytical Chemists (1995) for N (Enviro-Test Laboratories, Saskatoon, Saskatchewan).

Seedling physiological conditions

Needle and root electrolyte leakage in response to freezing temperatures

Eight seedlings from each treatment were washed with tap water and rinsed twice with distilled water. Two needle and two root samples were prepared from each seedling with one sample being subjected to freezing and the other held at room

temperature as a control. Each needle sample was prepared by removing 10 fully expanded needles from the terminal shoot (approximately 4 cm from the terminal bud). Root samples were prepared by cutting ten unbroken fine roots 2 cm from the root tip. The samples were dipped twice into double-distilled water to remove surface electrolytes (Tan and Blake 1993), blotted dry with filter paper, and placed in a vial containing 0.5 ml double-distilled water.

After initial cooling to 5°C, samples were cooled at 6°C per hour to either – 2°C for roots or – 8°C for needles and the temperatures were then maintained for 2 h. Samples were placed in a Styrofoam® cooler in a refrigerator and allowed to thaw gradually overnight. The control samples were left in a dark refrigerator during the entire period.

After adding 3.5 ml double-distilled water in each vial, the samples were shaken in the dark at room temperature (20 ± 2°C) for 24 h (Tan and Blake 1993). The conductivity of the solution in each vial was measured using a conductivity meter (YSI 3100 Conductivity Meter, Simpson Co., IL). All samples were then autoclaved for 20 min, let cool overnight, and then a second conductivity measurement was made to estimate the total electrolytes in each sample (Tan and Blake 1993). Relative electrolyte leakage index (I_s) (Martin et al. 1987; Tan and Blake 1993) was calculated as:

$$I_s = \frac{100 \times (R_s - R_0)}{(1 - R_0)}$$

where R_s is the ratio of electrical conductivity measured after freezing divided by that measured after autoclaving; R_0 is the average ratio of electrical conductivity of unstressed roots or needles (controls) from each treatment measured after refrigeration divided by that measured after autoclaving.

Transpiration control

The seedling's capacity to control water loss was examined by measuring transpiration and/or stomatal conductance in well-watered (favorable) conditions and during dehydration. The instantaneous transpiration rate (T_v, μmol m^{-2} s^{-1}) and stomatal conductance (C_s, mmol m^{-2} s^{-1}) of eight well-watered seedlings from each treatment were determined by measuring vapor loss with a LI-1600 Porometer (Li-Cor Inc., Lincoln, NE) inside a well-ventilated room (to prevent CO_2 buildup) under an air temperature of 22 ± 1°C, RH of 25 ± 5%, and photosynthetically active radiation (PAR) of 450 μmol m^{-2} s^{-1} provided by 1000 W MH1000U Multi-Vapor lamps (Philips). The measurement was made on the top ~5 cm portion of the terminal shoots following the procedures of Tan et al. (1992).

These same seedlings were also used to determine transpiration decline curves during dehydration following the modified procedures of Quisenberry et al. (1982). Briefly the top ~5 cm portion of the terminal shoot from each seedling was cut and re-cut under water and kept dark overnight with only the cut surface under water to allow a full re-hydration (Tan and Blake 1997). After drying with filter paper, each shoot was weighed immediately to obtain the initial weight. The shoots were then allowed to dehydrate on a bench under controlled conditions (air temperature of 22 ± 1°C, RH of 25 ± 5%, and PAR of 310 μmol m^{-2} s^{-1}) and re-weighed approximately 1, 3.5, 5.5, 7.5, and 10.5 h after the initial weighing. Needle areas were

determined after the final weighing using a volume displacement method (Johnson 1984). The average transpiration rate (T_w, μmol m^{-2} s^{-1}) over each sampling period was calculated based on the weight loss, time and needle area for each sample.

Survival under controlled water deficit stress

The 15 seedlings from each treatment selected directly from the nursery were transplanted at 10 × 10 cm spacing into a box of water-washed fine sand ~35 cm deep, and surrounded by one row of border seedlings in a greenhouse which provided a 20-h photoperiod with the natural light supplemented by 1000 W Multi-Vapor lamps (MH1000U, Philips) reaching a PAR of 200 μmol m^{-2} s^{-1} at seedling height; daily average air temperature of 18–21°C; and no control of relative humidity. The seedlings were watered to full soil saturation on each of the 3 days after the transplanting. Water was then withheld for 19 days by which time many seedlings were severely wilted. Mortality was then assessed after one week of daily watering.

Pre-dawn xylem pressure potential (Ψ_x) was measured on an upper lateral branch of four seedlings in each treatment (Tan et al. 1992) with a Scholander–Hammel pressure chamber (PMS Instruments Co., Corvallis, USA) 6, 14 and 19 days after water was withheld. Soil water content (%, g H_2O 100 g^{-1} dry soil) at these times and after saturation and free drainage overnight in a plastic bag (equivalent approximately to initial soil water content) was also determined with soil dried in an oven at 105°C for 48 h.

Root growth capacity (RGC)

Root growth capacity (RGC) was estimated as total number of new roots generated (1) per seedling and (2) per g root dry weight after 2 weeks at two different root (soil) temperatures (i.e. 20 ± 2°C and 9 ± 1°C). For testing RGC at 20°C, 15 seedlings from each treatment were washed and their roots dyed with methylene blue for 10 s to facilitate determination of new roots (Zwolinski and Peterson 1994). The seedlings were rinsed carefully and then transplanted into pots 23 cm in diameter containing 3:1 peat moss and perlite mixture. Five seedlings, one from each treatment, were planted in each pot and placed in a controlled environment with an air temperature of 20 ± 2°C, 18-h photoperiod, PAR of 200 μmol m^{-2} s^{-1} at seedling height (1000 W MH1000U Multi-Vapor lamps, Philips), and no control of humidity. The seedlings were watered daily for 2 weeks with a balanced nutrient solution for conifers containing 100 ppm N (Tan and Hogan 1995). New roots > 1.0 cm were then counted and new root dry weight determined.

In order to control the root temperature at 9 ± 1°C, 12 seedlings from each treatment were placed in a solution culture (hydroponic) system, similar to the technique used by Grossnickle et al. (1991), after careful washing, root dyeing, and rinsing, as described previously. The same balanced nutrient solution of Tan and Hogan (1995) was used (100 ppm N) and changed every 4 days. The hydroponic system was placed under the same conditions as the potted seedlings with the solution temperature controlled by a chiller (Aqua Chiller, Jewel Industry Co., Chicago, IL). After two weeks no new roots > 1 cm long were observed in any seedling. The number of new roots > 0.1 cm long was counted, and new root dry weight determined.

Data analysis

Differences between treatments were examined by one-way analysis of variance, and when significant, a Duncan multiple range test was used to determine significant differences among treatment means ($P = 0.05$). The standard error of the mean was calculated and presented for each treatment mean. Linear regression analysis was used to examine the relationship between some parameters. Statistical analyses followed General Linear Model or Regression Procedures in a SAS-PC software (SAS 1988). The survival rate under the controlled water stress was compared among treatments ($P = 0.05$) using a z-test statistic in a pair-wise fashion (Weimer 1993).

Results and Discussion

Impacts of nursery cultural treatments on seedling morphology

Development of needle primordia

Needle primordial development in white spruce seedlings was significantly influenced by cultural treatments. Terminal buds and needle primordia were not formed in T0 and T3, but were developed by all seedlings in T7-T15. The number of needle primordia increased significantly from 79 in T7 to 164 in T10 and T15, which is comparable to the 100–230 found in other conditioned 1 + 0 conifer seedlings under different cultural regimes (e.g. Macey and Arnott 1986; Grossnickle 2000). An increasing number of needle primordia has been associated with the natural progression of dormancy development in white spruce (Macey and Arnott 1986) and western hemlock (Grossnickle et al. 1991).

The T7 treatment, which included 7 days of 12 h photoperiod and a subsequent 17 days of reduced N supply, was thus sufficient to induce terminal bud formation for white spruce. Similarly, Coursolle et al. (1998) found that an 8-day short-day treatment was adequate to induce fall conditioning in four white spruce provenances. The greater number of short-days proposed by others (Macey and Arnott 1986; Krasowski et al. 1993; Grossnickle et al. 1991; Hawkins and Draper 1991; Odlum et al. 2001) as necessary for inducing growth cessation in spruces would therefore appear unnecessary.

Shoot dry weight fraction (SDWF)

Compared with the actively growing seedlings in T0, SDWF in white spruce seedlings increased gradually and significantly in response to the increasing intensity of cultural treatments from T3 to T15 (Table 2). Consistent with these results, a steady increase in SDWF has been reported in other spruce species during their fall acclimation and/or cultural treatments (Colombo 1990; Calmé et al. 1993), and is believed to be a common plant response to conditioning (e.g. Levitt 1972; Blum 1988). A decline in plant tissue water content may aid in osmoregulation and delay intracellular ice formation and cellular dehydration, leading to an increasing tolerance to freezing and other stresses (Levitt 1972; Blum 1988; see also Fig. 1).

Table 2 Morphology and needle N concentration of white spruce seedlings after conditioning by five nursery cultural treatments (T)

Treatment	Height (cm)	Total dry weight (g)	Root collar diameter (mm)	Root/shoot ratio (g g^{-1})	Shoot dry weight fraction (g g^{-1})	Specific needle weight (g m^{-2})	N concentration (%)
T0	20.8c (0.67)	1.89c (0.18)	3.21a b (0.13)	0.327c (0.014)	0.265d (0.00049)	34.9c (1.11)	2.69bc (0.068)
T3	24.3b (0.82)	2.08bc (0.15)	3.29a (0.12)	0.294c (0.014)	0.272d (0.0032)	35.2c (1.38)	2.66bc (0.079)
T7	23.8b (0.69)	2.21bc (0.16)	3.20ab (0.12)	0.343bc (0.019)	0.299c (0.0046)	40.7c (2.94)	2.87b (0.082)
T10	23.4b (0.78)	2.47b (0.24)	2.88b (0.12)	0.379ab (0.017)	0.356b (0.0060)	53.6b (2.89)	3.25a (0.072)
T15	28.2a (1.10)	3.41a (0.25)	3.13ab (0.09)	0.416a (0.019)	0.419a (0.0115)	60.9a (2.37)	2.44c (0.157)

Standard error of mean (n = 15 for morphological parameters, n = 7 for N) is included in the bracket. Within one column, means with the same letter do not differ significantly as determined by a Duncan multiple range test (P = 0.05)

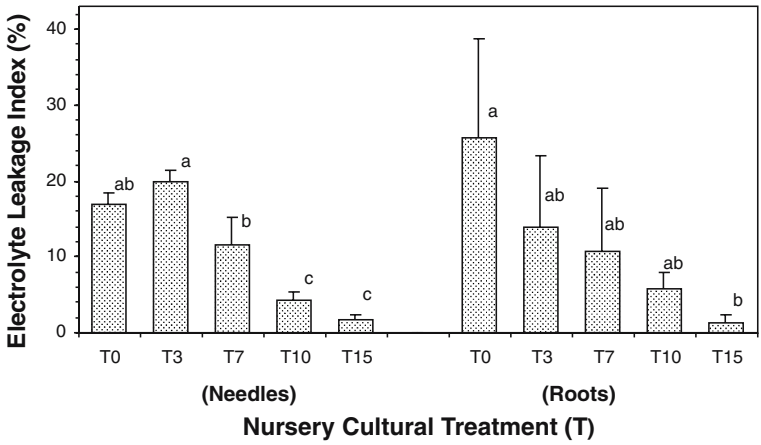

Fig. 1 Electrolyte leakage index of white spruce needles or roots from five nursery cultural treatments (T) after exposure to a freezing temperature of – 2°C for roots or – 8°C for needles over 2 h. Vertical bars are standard error of mean (n = 8) and means with the same letter within each respective group do not differ significantly as determined by a Duncan multiple range test (P = 0.05)

Other morphological characteristics and nutrient conditions

With an average height of 24 cm, total dry weight of 2.4 g, and root collar diameter of 3.1 mm, all seedlings were within the morphological ranges suggested for 1 + 0 spruce stock for summer-planting (Grossnickle 2000). There were no substantial differences in seedling size among T3, T7 and T10, although the seedlings in T15 tended to be bigger while the T0 seedlings were smaller (Table 2). This is likely due to a more intensive cultural regime and longer growing period for T15 seedlings.

Root/shoot ratio tended to increase gradually from T0 to T15 (Table 2). Other studies have repeatedly shown that the short-day treatment of conifers promotes growth of roots more than of shoots, thereby increasing root/shoot ratio (e.g. Grossnickle et al. 1991; Hawkins et al. 1996), which may increase plant drought tolerance by providing more favorable root water absorption relative to transpiration (e.g. Levitt 1972; Tan 1992).

The needle concentrations of N, P and K in all seedlings were above 2.44%, 0.38% and 0.98%, respectively for all treatments, i.e. within the suggested optimal ranges for conifers (e.g. Swan 1970; Tan and Hogan 1995). The differences among the treatments in N concentration were largely small (Table 2), suggesting that the reduced N supplies may have not played a major confounding role in impacting white spruce responses to the overall cultural treatments.

Impacts of nursery cultural treatments on physiological conditions

Needle and root electrolyte leakage after freezing

From T0 to T15, the electrolyte leakage index decreased gradually for both roots and needles (Fig. 1), suggesting a gradual increase in post-freezing membrane integrity and function. The percent leakage in T15 seedlings was near zero, indicating no damage at – 2°C or – 8°C for roots or needles, respectively. Similar reports of

Table 3 Mean instantaneous needle transpiration rate (T_v) and stomatal conductance (C_s), determined through vapor loss with a LI-1600 Porometer, under well-watered conditions of white spruce seedlings subjected to five nursery cultural treatments (T)

	Treatment				
	T0	T3	T7	T10	T15
T_v (dry weight basis) (μmol g^{-1} s^{-1})	12.3a (0.76)	11.7a (0.98)	13.8a (0.95)	11.1a (1.03)	7.8b (0.57)
T_v (area basis) (μmol m^{-2} s^{-1})	434.9c (39.9)	405.5c (22.4)	556.6ab (44.5)	587.9a (48.8)	471.6bc (27.7)
C_s (mmol m^{-2} s^{-1})	19.7c (1.91)	19.1c (1.09)	27.4ab (2.49)	29.2a (2.55)	22.4bc (1.46)

Standard error of mean (n = 8) is included in the bracket below each mean. Within each row, means with the same letter do not differ significantly as determined by a Duncan multiple range test (P = 0.05)

enhanced seedling cold hardiness in white spruce and other conifers through cultural treatments involving short-day applications have been published by Colombo et al. (1982), Bigras and D'Aoust (1992), Hawkins et al. (1996), and Ryyppö et al. (1998).

A consistent increase in frost tolerance from T0 to T15 suggests that frost tolerance may develop incrementally during the cultural processes well before the terminal bud is fully developed. This is inconsistent with the suggestion that the frost tolerance begins to increase only after an initial period of conditioning/acclimation development (Nissila and Fuchigami 1978; Colombo 1990). More studies are needed to clarify this important issue, especially in a range of other commercial tree species, since it may have profound impacts on our nursery cultural practices.

Transpiration control

Instantaneous needle transpiration rate (T_v, determined by vapor loss) on a dry weight basis under normal conditions was similar for the white spruce seedlings in

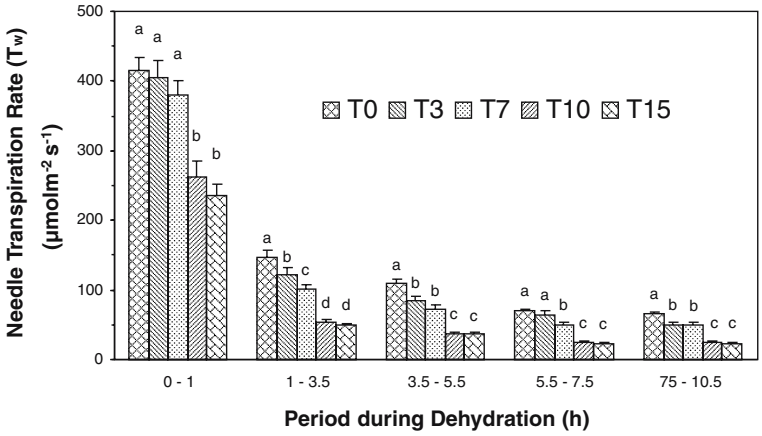

Fig. 2 Average needle transpiration rate (μmol m^{-2} s^{-1}) of detached shoots, determined through weight loss (T_w), after different periods (h) of dehydration in white spruce seedlings subjected to five nursery cultural treatments (T). Vertical bars are standard error of mean (n = 8) and means with the same letter within each time period do not differ significantly as determined by a Duncan multiple range test (P = 0.05)

T0 to T10 but was significantly lower in T15 (Table 3). On an area basis, however, T0 and T3 appeared to have a lower T_v and stomatal conductance (C_s) compared with T7-T15, apparently relating to a gradual increase in needle specific weight from T0 to T15 (Table 1).

On the onset of initial dehydration, however, the T10 and T15 seedlings were able to reduce their transpiration rate (T_w, determined by weight loss) far more quickly, and to maintain a much lower T_w over the entire dehydration periods compared with T0 and T3; while T7 seedlings were consistently in the middle (Fig. 2). In general, the seedlings in T3 maintained a lower T_w than in the T0. This suggests that the cultural treatments, induced by blackout treatment for a period as short as 3 days, may have incrementally promoted the stomatal sensitivity to water stress, allowing a quicker stomatal closure during drought, thus ameliorating the severity of water stress.

T_w appeared to stabilize after 7.5 h dehydration in all treatments (Fig. 2), and therefore the T_w at this time period is considered equivalent to the cuticular transpiration rate since the stomata may be fully closed (Quisenberry et al. 1982; Vanhinsberg and Colombo 1990). The cuticular transpiration rate (i.e. T_w during 5.5–7.5 h) in the T0 seedlings was almost three times as much as in the T10 and T15, 40% more than T7, and about 10% more than T3. This result is consistent with the finding of Vanhinsberg and Colombo (1990) in black spruce that the needle cuticle thickness doubled or tripled over the process of cultural treatments (or maturation). A low rate of cuticular transpiration may benefit the conditioned seedlings under severe drought stress or during winter desiccation.

Survival under controlled water stress

After watering ceased, the water content in the fine sand declined gradually from 21% initially, to 7.5% after 6, 8% after 14, and eventually to $< 1\%$ after 19 days, which may represent a soil water potential of < -1.5 MPa, normally considered to

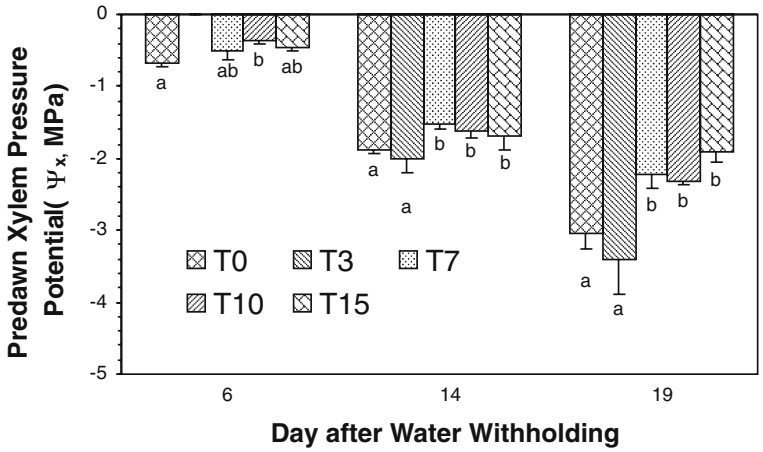

Fig. 3 Change of pre-dawn xylem pressure potential (Θ_w, MPa) of white spruce seedlings from five nursery cultural treatments (T) 6, 14 and 19 days after withholding water from the soil. Vertical bars are standard error of mean ($n = 4$). Means with the same letter on each day do not differ significantly as determined by a Duncan multiple range test ($P = 0.05$)

be the permanent wilting point for most plants (Juma 2001). Consequently average pre-dawn xylem pressure potential (Ψ_x) in the seedlings decreased significantly from higher than – 1.0 MPa on Day 6 to between – 2.0 and – 3.0 MPa on Day 19 (Fig. 3), an indication of severe water stress for conifers (Tan 1992).

After 19 days without watering, only 20% of the seedlings survived in T0, about 50% in T3, but more than 90% in T7–T15, suggesting a greater drought tolerance of seedlings in T7, T10 and T15. This improved drought tolerance may be largely due to transpiration control under water stress (i.e. dehydration avoidance), since a slightly less negative Ψ_x was developed in T7, T10 and T15 seedlings during the water stress compared with T0 and T3 (Fig. 3). Transpiration rate did not differ greatly among treatments under well-watered conditions (Table 3). The enhanced dehydration avoidance capacity in T7–T15 seedlings is in part attributable to a quicker stomatal closure during the initial development of water stress and lower cuticle transpiration water loss after stomatal closure (Fig. 2). The potential contribution of a higher root/shoot ratio in the T7–T15 seedlings (Table 2) could not be assessed in this study since the randomization of all seedlings in one single sand box may have ensured a quick soil water equilibrium (i.e. similar soil water conditions for all treatments), and no significant new root growth was observed during the final survival assessment. Adjustments in water relations may also contribute to an enhanced drought tolerance in spruces during acclimation/dormancy development (Colombo 1990).

Root growth capacity (RGC)

At a root temperature of 9°C for 2 weeks, no seedling had produced any new roots longer than 1.0 cm. New roots > 0.1 cm long were few (5–17 per plant) and did not differ significantly among treatments (data not shown). Under 20°C, RGC appeared to increase gradually from T0 to T10 when calculated on a per seedling basis, but the differences were no longer existent on the basis of root dry weight (Fig. 4). This

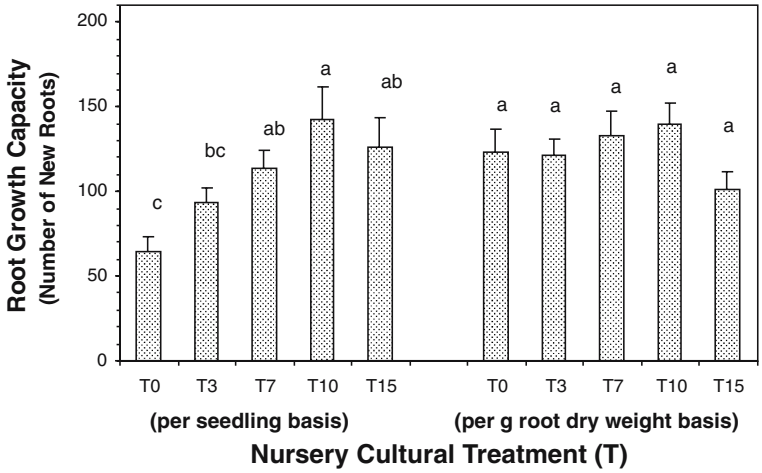

Fig. 4 Root growth capacity (RGC) of white spruce seedlings subjected to five nursery cultural treatments (T) represented on either a per seedling basis or per gram of root dry weight basis. Vertical bars are standard error of mean (n = 15). Means with the same letter within each respective group do not differ significantly as determined by a Duncan multiple range test (P = 0.05)

indicates that a higher RGC was due to a larger root system, suggesting that the cultural treatments increased white spruce RGC mostly through enhancing root mass growth. Greater RGC has also been previously associated with greater original root size in pine (Williams et al. 1988) and interior spruce (Grossnickle and Major 1994). These results highlight the importance of considering root size when presenting and interpreting the results of RGC.

Use of SDWF to monitor nursery conditioning progress

The SDWF has been suggested as a useful tool for nurseries to monitor the process in cultural conditioning treatments for spruce seedlings, and to determine the optimum lifting dates for winter storage (Calmé et al. 1993). This suggestion can also be applied to spruce summer planting stock as supported by five observations from this study: (1) SDWF values increased gradually with the increasing duration/intensity of the conditioning treatments (Table 2), (2) SDWF was linearly and highly correlated with specific needle weight (R^2 = 0.98; refer to Table 2) which provides a more direct mechanistic linkage to the function of other physiological and biochemical processes in leaves, such as photosynthesis; (3) SDWF was highly correlated with frost tolerance in roots (R^2 = 0.90; refer to Table 2 and Fig. 1) and needles (R^2 = 0.75); (4) SDWF is simple and speedy (2 days) to determine, and does not require expensive instruments; and (5) SDWF showed small variation within replications (Table 2). Colombo (1990), however, urged caution in using this criterion to predict seedling readiness for storage because of its potential insensitivity to the lower range (10–30%) of seedling damage. Further studies are certainly needed.

Conclusions

This study increases our knowledge of how nursery cultural treatments may impact the stress tolerance of white spruce seedlings prepared for summer planting. About 3–15 days of short-day application followed by reduced N supply significantly and incrementally increased bud formation and tolerance to frost and drought in white spruce seedling. The increase in drought tolerance was largely attributable to enhanced dehydration postponement (avoidance) resulting from quicker stomatal closure during water stress and a lower cuticular transpiration rate. Since 7 days of short-day application followed by 17-day reduced N supply were obviously sufficient to induce terminal bud formation and cessation of height growth in white spruce, nurseries may not need the longer blackout periods commonly prescribed for conditioning summer-planted spruce. Shoot dry weight fraction may be used to monitor nursery conditioning progress, since it was closely correlated with development of stress tolerance during cultural treatments, and its measurement is simple and speedy.

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