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ORIGINAL ARTICLE

Frost tolerance of two-year-old *Picea glauca* seedlings grown under different irrigation regimes in a forest nursery

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Abstract
This study examined the impact of increased irrigation efficiency on the hardening and frost tolerance of 2-year-old containerized white spruce seedlings in the context of groundwater protection, irrigation management and the maintenance of seedling quality in northern climates. The seedlings were grown under three different irrigation regimes (IR = 30%, 40% and 55% v/v; cm^3 H_2O/cm^3 substrate) and were hardened under conditions of natural photoperiod and temperature. After being subjected to artificial frost tests on four sampling dates during autumn, the seedlings were compared for bud development and frost tolerance. IR had no influence on frost tolerance as determined by measurements of physiological (electrolyte leakage, root water loss) and morphological (shoot damage, root initiation) variables. At the end of the second growing season, there was no significant difference between IRs in seedling height, root collar diameter, shoot dry mass and root dry mass. The results indicate that the amount of water applied to large-dimension 2-year-old white spruce seedlings during the growing season can be significantly decreased without prematurely impeding their growth or hindering their acquisition of frost tolerance.

Keywords: *Cultural practices, freezing damage, hardening, irrigation.*

Introduction

Frost damage is one of the principal reasons that millions of seedlings are rejected each year in northern forest nurseries (Colombo, 1997). In the province of Québec in eastern Canada, seedlings are kept outside during winter and annual rejection rates due to freezing damage can reach 5–30% (Lamhamedi et al., 2005). Seedlings are sorted and classified in the nursery, but some damage to roots from freezing cannot be readily detected without destructive sampling (Bigras & Dumais, 2005). This is why plants whose roots have been damaged by frost are sometimes still delivered to reforestation sites (Bigras, 1995). Severe root damage has negative effects on growth and physiology of seedlings after outplanting (Coursolle et al., 2002; Dumais et al., 2002).

The acquisition of seedling frost tolerance begins with the cessation of height growth and the initiation of bud formation (Sakai & Larcher, 1987). Manipulation of the irrigation regimes (IRs) and the use of water stress in containerized tree seedling nurseries have been found to be one of the most effective cultural practices to initiate the hardening of seedlings (Landis et al., 1989). Towards the end of the growing season in the autumn (fall), seedling producers decrease fertilization and irrigation in temperate and boreal regions of North America. This practice induces bud formation and supports hardening (Landis et al., 1989; Silim & Lavender, 1994). However, it is still not clear to what extent the

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practice of decreasing substrate water content and imposing water stress promotes the frost hardening of shoots (Bigras et al., 2001) or roots (Bigras & Dumais, 2005). Environmental factors, most notably photoperiod (van den Driessche, 1969; Timmis & Tanaka, 1976; Blake et al., 1979), substrate fertility and temperature (van den Driessche, 1969; Young & Hanover, 1978) and their interactions, play important roles. This means that the effect of water stress is different depending on the level and timing of its imposition during the growing cycle (Young & Hanover, 1978). A mild water stress applied before the natural decrease in photoperiod was found to increase the cold hardening capability of Douglas fir seedlings (Timmis & Tanaka, 1976; Blake et al., 1979), while a severe stress had a detrimental effect (Blake et al., 1979).

Maintaining reduced substrate water contents during the entire growing season can result in substantial savings in the quantity of water used for irrigation as well as a reduction in the leaching of nutrients (Lamhamedi et al., 2001, 2003). Increasing irrigation efficiency can reduce the negative environmental impact of plant production practices, but hopefully should not compromise seedling hardening. White spruce seedlings have been shown to set bud earlier during their first growing season when kept under water stress in a dry (15% v/v) peat substrate (Lamhamedi et al., 2001; Stowe et al., 2001). Growth cessation is a prerequisite to cold acclimatization in woody plants (Weiser, 1970), but is not in itself a guarantee of adequate frost tolerance. In addition, results obtained on 1-year-old spruce seedlings may not apply to the larger, 2-year-old seedlings that are delivered to the field because seedlings switch from indeterminate growth in the first growing season to partially determinate growth in the second growing season (Grossnickle, 2000). Two-year-old seedlings have more needles and a larger transpiring surface than 1-year-old seedlings and therefore require different target substrate water contents.

The objective of this study was to compare the effect of growing season IR on the frost tolerance of shoots and roots of 2-year-old white spruce seedlings. The overall goal of this study was to determine whether the amount of water applied to the seedlings during the growing season could indeed be lowered without affecting negatively their growth, their hardening and their subsequent frost tolerance.

Materials and methods

Seeds from a first generation white spruce seed orchard located in Wendover arboretum (45°59′N, 72°30′W), 80 km east of Montréal, Québec, Canada, were sown on 24 May 2001 into IPL 25-350A air-slit containers (25 cavities per container, 350 cm^3 per cavity, 35 × 37 cm; IPL®, Saint Damien, Bellechase, Québec, Canada) filled with a peat:vermiculite (3:1) growing medium. The seedlings were cultivated in an unheated polyethylene tunnel at Pampev Inc., a private forest nursery located in Saint Louis de Blandford, Québec (46°25′N, 72°00′W), and subjected to standard cultural practices during their first year of growth. Containers were raised on pallets during the growing season and placed on the ground for overwintering. The tunnel was left uncovered between October 2001 and April 2002. A thick layer of natural snow covered the seedlings during the winter.

A completely randomized block experiment consisting of six repetitions was installed in April 2002, at the beginning of the second growing season. Three IRs, in which the substrate water content was maintained at target levels (30, 40 and 55% v/v; cm^3 H_2O/cm^3 substrate) throughout the experimental period, were randomly distributed within each block. The lowest water content treatment was not as dry as that used by Lamhamedi et al. (2001) or Stowe et al. (2001) for 1-year-old seedlings (15% v/v) because the purpose of the study was not to impose a severe water stress on the seedlings, but rather to determine the lowest operationally feasible IR that would not affect seedling hardening. Although the 30% IR did not affect 1-year-old seedling growth, it could have a significant impact on 2-year-old seedlings because their larger foliar area induces a greater transpiration. At the other extreme, the 55% IR corresponds to operational conditions and is the usual target irrigation level in peat: vermiculite substrates (Lamhamedi et al., 2001). For each IR, 168 containers (28 containers/block/IR) were used for the six blocks of the experimental design. Each experimental unit was composed of four rows of seven containers, for a total of 28 containers. Adjacent units were separated by a buffer of 28 containers. A buffer of two containers was left between the experimental area and the side of the tunnel, while a one-container buffer was left between the experiment and the tunnel's central aisle. The plastic covering on the tunnel was removed on 4 October 2002, as is customary at the end of the growing season, to promote hardening.

Substrate water content was monitored and adjusted using an MP-917 soil moisture system (ESI Environmental Sensors, Victoria, BC, Canada) which is based on the principles of time domain reflectometry (TDR) (Kirkham, 2005). Target water contents [55%, 40% and 30% (v/v)] were attained using the procedure described in Lamhamedi et al. (2001) and

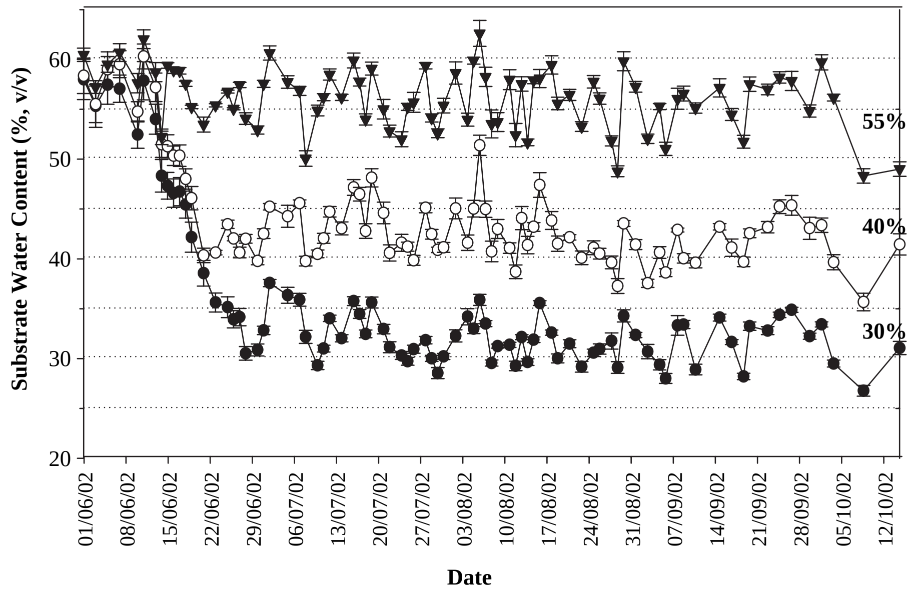

Figure 1. Control of volumetric water content in the rhizosphere of air-slit containerized (2+0) white spruce seedlings grown under three irrigation regimes (30, 40 and 55% v/v). Error bars are standard errors ($n=6$).

Stowe et al (2001). Substrate water content was measured six times a week throughout the experimental period, between June and October 2002 (Figure 1). Irrigation uniformity was assured through the use of a mobile boom irrigation system (Aquaboom; Harnois Industries, Saint Thomas de Joliette, Québec, Canada; coefficient of uniformity 95%).

Substrate fertility was maintained at the same level for all three IRs for the duration of the experiment. This was done to avoid confounding the effects of fertilization, particularly nitrogen (Bigras et al., 2001), with the effect of IR on the acquisition of frost tolerance of the white spruce seedlings. Substrate fertility was monitored and adjusted once every 2 weeks using Plantec (Langlois & Gagnon, 1993), fertilization-scheduling software which takes into account concentration of mineral nutrients in the substrate, plant growth parameters and mineral nutrient needs. The concentration of mineral nutrients in the substrate was determined every 2 weeks over the course of the growing season by sampling and preparing one composite sample of substrate for each of the six repetitions of the three IRs. Substrate mineral concentrations were determined from a filtered extract obtained through compression. Urea fertilizers were used at the beginning of the growing season, whereas nitrate fertilizers were applied during the hardening phase.

Rain gauges (TE52M; Texas Instruments Dallas, TX, USA) were used to monitor the quantity of water applied to the plants during fertilization and irrigation. At the end of the second growing season (10 October 2002), the cumulative volumes of water used to maintain the three IRs were 283, 235 and 224 l m^{-2} for IR55%, IR40% and IR30%, respectively. The cumulative amount of nitrogen applied to each plant cavity, for example, was 167.76, 157.62 and 148.80 mg under IR55%, IR40% and IR30%, respectively.

Temperatures were measured at 2 m above the ground, at the substrate surface and within the rhizosphere between July and October 2002, and recorded at 15 min intervals using a datalogger (model CR10X; Campbell Scientific Corp., Edmonton, Alberta, Canada) (Figure 2). During the sampling period (16 September to 28 October), the average daily air temperature was below 0°C on two occasions, 22 October (−1°C) and 23 October (−0.2°C) (Figure 2). The minimum average daily substrate temperature was 0.5°C. Air temperature dropped below freezing for the first time during the night of 6 October (minimum = −1.1°C). The lowest air temperature during the experimental period was recorded on the morning of 15 October (−7°C). On the same morning, the temperatures at the substrate surface and in the rhizosphere were −4.6°C and 2.2°C, respectively.

Seedling morphology and growth

Seedling growth was monitored every 2 weeks over the course of the growing season. On 23 May, 6 and 20 June, 4 and 18 July, 1, 15 and 29 August, and 12 September 2002, one container per repetition was selected from each of the three IRs. The seedlings were also sampled on 24 October 2002 to obtain an accurate measure of the morphology at the end of the growing season. On each occasion, five randomly

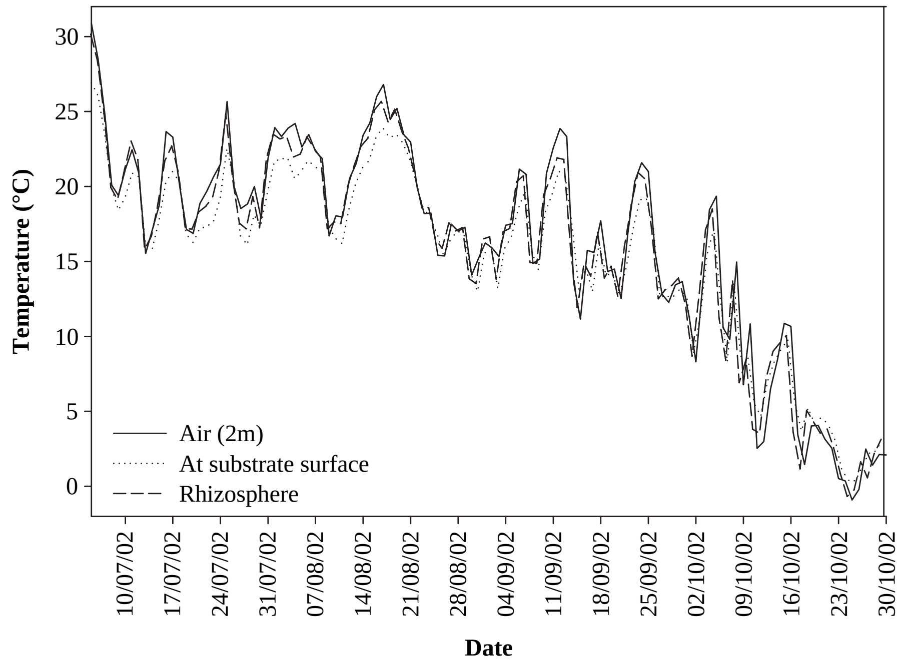

Figure 2. Variation in average daily air temperatures 2 m above the ground, at the substrate surface and in the rhizosphere from 5 July to 30 October 2002 during the second growing season of air-slit containerized white spruce seedlings at Pampev Inc., a private forest nursery located in Saint Louis de Blandford, QC, Canada (46°25′ N, 72°00′ W).

selected seedlings were harvested from each of these containers. Care was taken to evacuate the entire contents of the cavities, and to ensure that all of the fine roots were accounted for. The loose substrate was gently shaken from the root ball and kept for analysis of soil fertility. Upon arrival at the laboratory, the remaining substrate was washed from the roots under running water and the height and root collar diameter of each of the 90 seedlings (five seedlings/container/block/IR) were measured. The seedlings were then severed at the root collar and the above-and below-ground portions of the seedlings were oven-dried for 48 h at 68°C. The dry biomass of each sample was then determined.

Bud formation

Bud formation on the 25 seedlings in each of the 18 containers containing the TDR probes was monitored on a weekly basis, beginning on 1 August 2002. The seedlings were considered to have initiated buds once the exterior scales on the terminal bud were pale yellow in colour (Bigras & D'Aoust, 1993).

Artificial frost treatments

Several tests were conducted during the hardening period in September and October 2002 to determine the frost tolerance of the seedlings in autumn. The number and part (root, shoot) of the seedlings used to quantify frost damage on each sampling date are summarized in Figure 3.

Artificial frost treatments were applied to seedlings harvested on 16 September, 30 September, 14 October and 28 October 2002 using a programmable freezer. The frost treatment protocol was similar to that used by Zhu et al. (2002) for intact plants with root plugs. Given the limited amount of freezer space and the size of the seedlings, four of the six blocks in the experimental design were randomly selected for the artificial frost treatments. On each sampling date, 240 seedlings (20 seedlings/block/IR) were harvested. The 20 seedlings/block/IR were divided into five groups of four seedlings. Each group was subjected to a different frost temperature. The 350 cm^3 root plugs were maintained intact around the root system to simulate natural conditions. Before each frost test, the containers of seedlings were immersed in water for 2 h to ensure uniform substrate water content.

In preparation for electrolyte leakage tests, the apices (last 5 cm of the apical shoots, including the entire terminal bud) of two seedlings/block/IR/frost treatment were cut and placed in 125 ml Erlenmeyer flasks. Four seedlings/block/IR were grouped in a hermetically sealed plastic bag and exposed to one of four temperatures: $T_1 = -4°C$, $T_2 = -8°C$, $T_3 = -12°C$ and $T_4 = -20°C$. The apices and the seedlings subjected to the control temperature ($T_0 = +4°C$) were kept in a refrigerator. The artificial frost treatments (T_1, T_2, T_3 and T_4) were

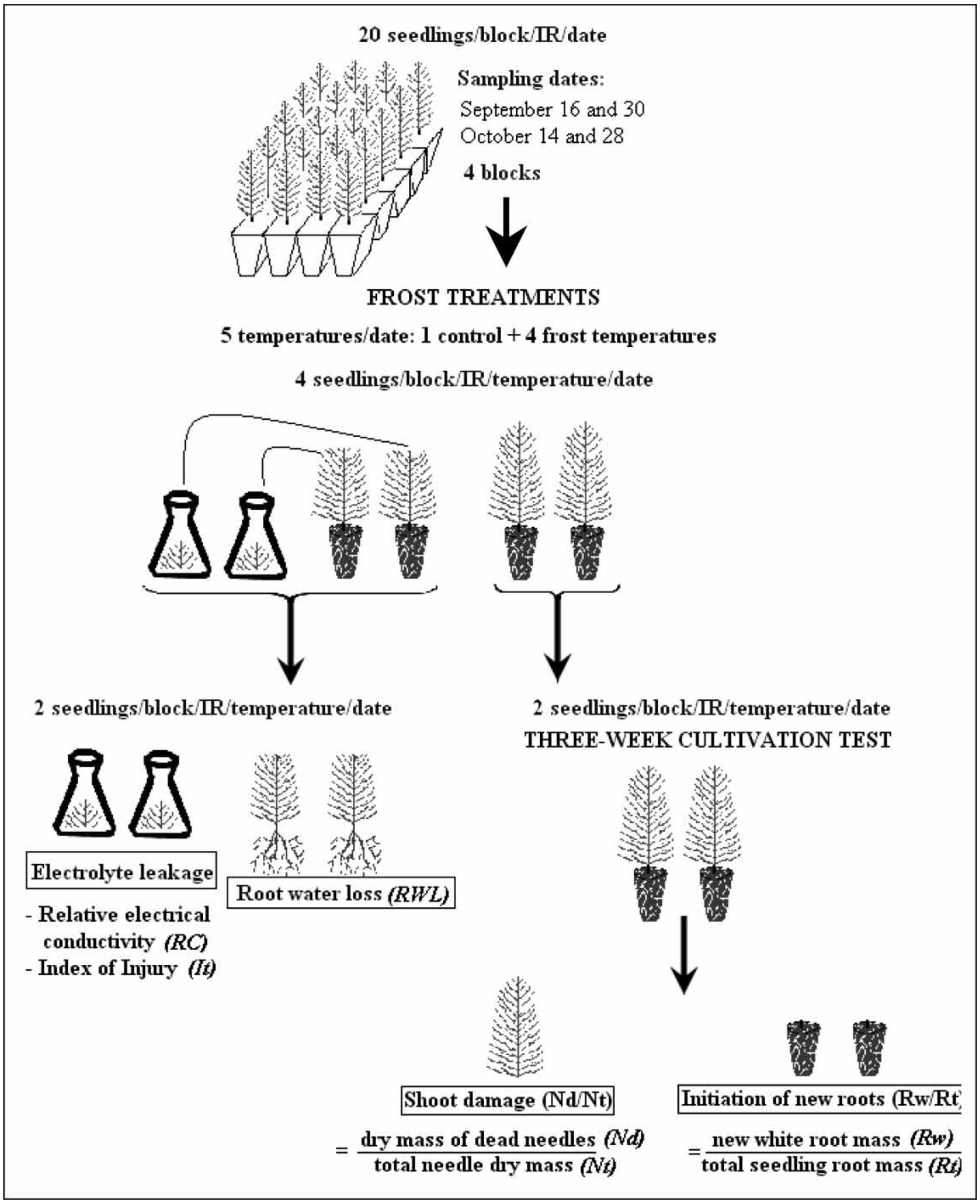

Figure 3. Number of seedlings and seedling parts used to quantify root and shoot frost damage on the four sampling dates. IR = irrigation regime.

administered in a freezer (model T20RS; Tenney Environmental, Williamsport, PA, USA) equipped with a programmable controller (model Versa Tenn II; Union, New Jersey, USA). One temperature probe (model 107B; Campbell Scientific Corp., Edmonton, Alberta, Canada) was placed in an empty Erlenmeyer flask to measure the air temperature around the shoot. A second probe was inserted into the root plug of one of the four seedlings scheduled to be frozen at -20°C to verify the temperature inside the root plug. Temperatures were recorded at 3 min intervals.

The seedlings were first chilled to -2°C for 40 h to ensure that all of the substrate in the root plugs attained a frozen state (Lindström & Stattin, 1994; Zhu et al., 2002). The temperature was then gradually decreased at a rate of 2°C h^{-1} (Levitt, 1980). Once the air temperature around the shoot tissue reached one of the target levels (-4°C, -8°C, -12°C or -20°C), it was kept constant for 1 h. When the 16 Erlenmeyer flasks per IR and the 16 seedlings per IR reached the particular target temperature, they were removed from the freezer and placed in a refrigerator at $+4^{\circ}$C. Because the root plugs were left intact and saturated with water before the artificial frost treatments, the rhizosphere temperatures were always several degrees higher than those experienced by the shoot tissue. During the frost treatments, the rhizosphere temperatures were +4°C (control), −1°C, −4°C, −8°C and −18°C, whereas air temperatures around the shoots were +4°C, −4°C, −8°C, −12°C and −20°C during the same treatments.

The damage caused by the artificial frost treatments was quantified as described below and in Figure 3.

Index of injury

The electrical conductivity was evaluated according to the procedure described by Colombo et al. (1984). After the frost tests were completed, two apices/block/IR were immersed overnight (18 h) in 100 ml of demineralized water at +4°C. The following morning, the Erlenmeyer flasks were shaken vigorously by hand (10 s) and the electrical conductivity of this solution (EC_1) was measured immediately using a conductivity meter (model 160; Orion Research, Boston, MA, USA). The samples were then placed in an autoclave for 15 min at 121°C to destroy the cell membranes and maximize electrolyte liberation. After autoclaving, the solution was left to stabilize overnight (18 h) at 4°C. The following morning, the solution was vigorously shaken before its electrical conductivity was remeasured (EC_2). The relative electrical conductivity (RC) was calculated using the following formula:

$$RC = \frac{EC_1}{EC_2} \times 100$$

Frost hardiness to a given temperature (t) was expressed using an index of injury (I_t) calculated on a percentage basis (Flint et al., 1967):

$$I_t = \frac{RC_{\text{frozen(t)}} - RC_{\text{control}}}{1 - (RC_{\text{control}}/100)}$$

where I_t indicates the amount of injury as a function of frost severity. A high I_t signifies a low level of frost tolerance (Flint et al., 1967).

Root water loss tests

The percentage of water lost from the root systems (RWL) of two seedlings/block/IR/temperature was evaluated following the procedure described by Ritchie (1990) and adapted for (2+0) white spruce seedlings by Coursolle et al. (2000). Root water loss (RWL) was calculated as the ratio of extruded water mass to total root system mass × 100. A greater RWL value indicates greater frost damage.

Initiation of new roots and shoot damage

Once the artificial frost tests had been completed, two seedlings/block/IR/temperature (120 seedlings) were repotted into 3 litre plastic pots filled with a moist peat:vermiculite (3:1) mixture and subsequently grown under optimal greenhouse conditions for 21 days (photoperiod = 14 h; day/night temperature = 25°C/18°C ± 3°C). Overhead high-pressure sodium (HPS) lamps (400 W; P.L. lighting systems) were used to extend the photoperiod from 16 h 00 to 21 h 00 each day to ensure a 14 h photoperiod. During each of the four cultivation tests, the seedlings were watered regularly, but were not fertilized.

Twenty-one days after repotting, all of the substrate was gently washed from the seedlings' root systems. All white roots (≥1 cm long) that had grown both outside and inside the original root plug during the cultivation test were subsequently removed, dried and weighed. The older, lignified roots, formed before the cultivation test, were also dried and weighed. The initiation of new roots during the period of the cultivation test was quantified by calculating the ratio of new white root mass (R_w) to total seedling root mass (R_t) (i.e. R_w/R_t).

Dead foliage that fell off the branches during the cultivation test was also collected. At the end of the 21 day period, shoot damage resulting from the frost treatments was quantified by calculating the ratio of the dry mass of the dead needles (N_d) to the total needle dry mass (N_t) (i.e. N_d/N_t).

Experimental design and statistical analyses

A split-strip plot design was used for the study of seedling growth. The main plots consisted of the three IRs, which were completely randomized within each of the six blocks in the nursery tunnel (experimental unit = 28 plant containers). Subplots consisted of the 10 sampling dates (experimental unit = one plant container). The effect of IR and sampling date, as well as the interactions between the two factors, were analysed using PROC MIXED (SAS Institute, Cary, NC) (Littell et al., 1996; Bernier-Cardou & Bigras, 2001). Regime, sampling date and their interactions were considered to be fixed effects, while the effect of block and all associated interactions were considered to be random effects. The random part of the model was reduced following the method described by Bernier-Cardou and Bigras (2001). Simple effects were considered significant at a threshold of 5%, double interactions at a threshold of 1% and triple interactions at a threshold of 0.1%.

A split-strip-strip plot design was used for artificial frost experiment (Bernier-Cardou & Bigras, 2001). The main plots consisted of the three IRs, which were completely randomized within each of the six blocks in the nursery tunnel (experimental unit = 28 plant containers). Subplots consisted of the four sampling dates (experimental unit = one plant container), while the sub-subplots were associated with the five artificial frost temperatures (experimental unit = group of four plants). The analyses were performed using PROC MIXED (SAS Institute) (Littell et al., 1996; Bernier-Cardou & Bigras, 2001). Irrigation, date and temperature effects, as well as their interactions, were considered as fixed effects, and the effects of blocks and their interactions as random effects. The random part of the model was reduced following the method described by Bernier-Cardou and Bigras (2001). Simple effects were considered significant at a threshold of 5%, double interactions at a threshold of 1% and triple interactions at a threshold of 0.1%. R_w/R_t was log-transformed to meet the statistical assumptions of normality and homoscedasticity. The effect of date on bud formation was characterized by polynomial contrasts.

In the case of the variables measured after the artificial frost treatments (*RC*, N_d/N_t, *RWL* and R_w/R_t), if a date × temperature interaction proved to be significant at 1%, an analysis by date was conducted both to characterize the interaction more precisely and to clarify the gradual progression of seedling hardening with respect to the frost temperatures used for the evaluation (Milliken & Johnson, 1984). For each date, a Fischer test was used to determine whether at least one difference existed among the five temperatures. If the test was found to be significant, a Student's *t*-test was conducted to compare the temperatures two by two (SLICE and PDIFF options of the LSMEANS statement of PROC MIXED) (Littell et al., 1996).

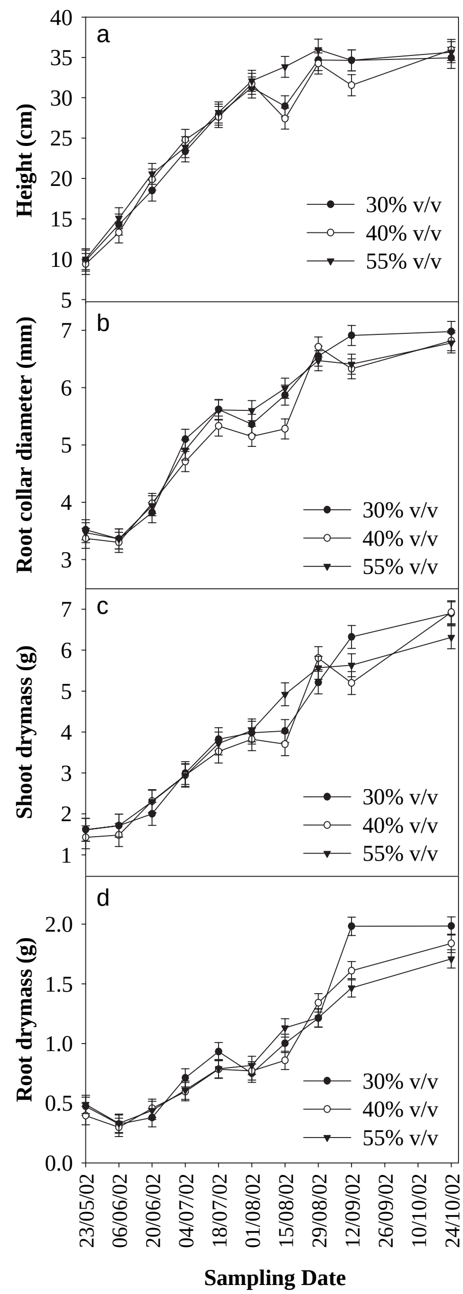

Figure 4. Evolution of (a) height, (b) root collar diameter, (c) shoot dry mass, and (d) root dry mass of (2+0) white spruce plants subjected to three irrigation regimes (IR: 30%, 40% and 55% v/v; cm^3 H_2O/cm^3 substrate) and sampled 10 times (23 May, 6 and 20 June, 4 and 18 July, 1, 15 and 29 August, 12 September and 24 October, 2002) during their second growing season. On each date, five randomly selected seedlings were harvested from one container per block. Each point corresponds to the average value for the six blocks × five = 30 seedlings. The bars represent the standard errors associated with each point ($n = 30$).

Results

Effects of irrigation regimes on seedling growth

During the second growing season, sampling dates significantly affected all the measured morphological variables ($p < .0001$) (Figure 4). IR did not influence height growth ($p = 0.3897$) or shoot dry mass increment ($p = 0.6005$) (Figure 4a, c), but did significantly influence root collar diameter growth ($p = 0.0207$) and root dry mass increment ($p = 0.0273$) (Figure 4b, d). The IR effect on root dry mass was different according to the sampling date (interaction IR × date, $p = 0.0050$). Nevertheless, at the end of the second growing season (24 October 2002) there was no significant difference between IRs in seedling height ($p = 0.9180$), root collar diameter ($p = 0.7444$), shoot dry mass ($p = 0.5729$) and root dry mass ($p = 0.3750$).

Effects of irrigation regimes on seedling hardening

IR did not significantly affect bud formation ($p=0.1050$). Seedlings submitted to an IR of 30% or 55% initiated their buds at the same time (data not shown). Bud formation was more rapid at the beginning of August for all three IRs and slowed down as the observation period progressed (quadratic date effect, $p<0.0001$).

IR did not influence the frost tolerance of the apices or needles. The simple effect of IR was not significant for either *RC* ($p=0.4961$) or I_t ($p=0.3964$) measured immediately after the frost treatments, or for N_d/N_t ($p=0.5190$), which was calculated at the end of each of the 3-week bioassays. IR also had no effect on root frost tolerance. The simple effect of IR was not significant for either *RWL* ($p=0.3392$), measured immediately after frost treatments, or R_w/R_t ($p=0.6640$), calculated at the end of each of the 3-week bioassays. Moreover, as neither the interactions between IR and date nor interactions between IR and frost temperature were significant, IR did not significantly influence either the kinetics of the seedling hardening or the level of frost tolerance reached by the seedlings.

Hardening of seedlings

RC, I_t, *RWL*, N_d/N_t and R_w/R_t were significantly affected by frost temperature. The extent of the effect varied with sampling date (temperature $\times$ date significant at a threshold of 1% for all of the variables) (Figure 5). The three IR were pooled because neither the simple effects of IR, nor its interactions, were significant.

Regardless of the sampling date being considered, there was never a significant difference in RC between the control temperature (+4°C) and the −4°C frost temperature (Figure 5a). On the first sampling date, there was a significant difference in RC values between samples that were exposed to the control temperature (+4°C) and those exposed to frost temperatures of −8°C and colder (T_0 vs T_2: $p>|t|=0.0046$). On the second and third sampling dates, the RC values of the seedlings subjected to a −8°C frost were no longer significantly different from the RC values of the seedlings exposed to +4°C (date B: T_0 vs T_2: $p>|t|=0.3794$; date C: T_0 vs T_2: $p>|t|=0.1838$). It was not until the fourth sampling date that there was no significant difference in the RC values among the five artificial frost temperature treatments.

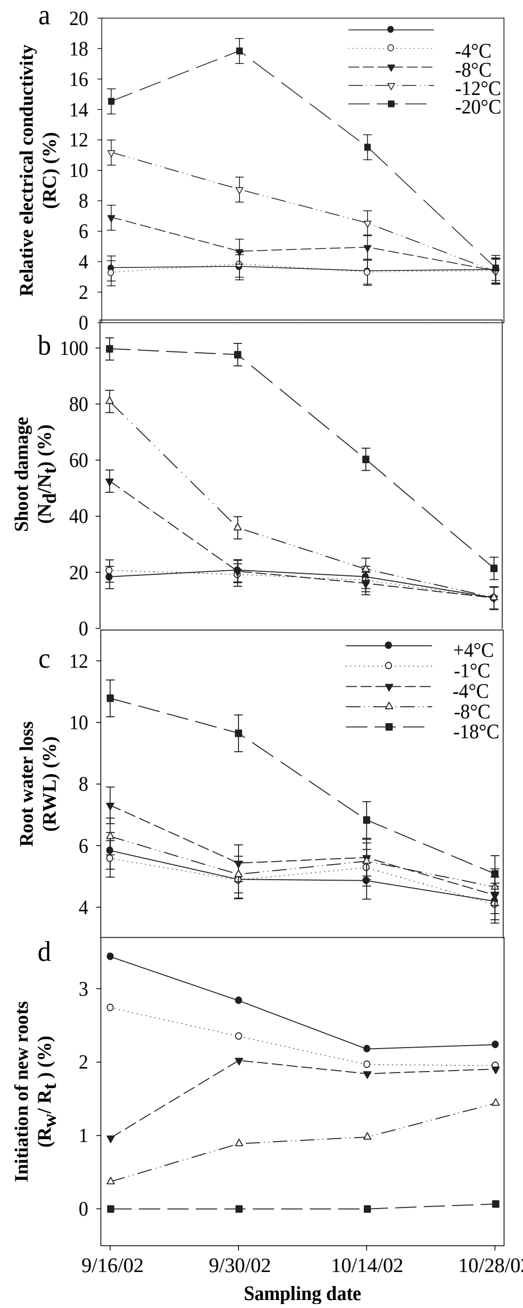

Figure 5. Evolution of (a) relative electrical conductivity (RC), (b) shoot damage (ratio N_d/N_t), (c) root water loss, and (d) initiation of new roots (ratio R_w/R_t) of (2+0) white spruce seedlings subjected to artificial frost treatments (+4, −4, −8, −12 and −20°C for shoot tissue and +4, −1, −4, −8 and −18°C for root tissue) on four sampling dates (16 and 30 September, 14 and 28 October 2002). Since neither the simple effect of irrigation regime (IR) nor its interactions were significant, the three IRs were pooled for each date $\times$ temperature. Each point corresponds to the average value for the four blocks $\times$ three IRs $\times$ two seedlings $=24$ seedlings (except for b, where one point $=$ four blocks $\times$ three IRs $\times$ one seedling $=12$ seedlings). The bars represent the standard errors associated with each point. The legend in (a) pertains to graphs (a) and (b), while the legend in (c) pertains to graphs (c) and (d).

Results were similar when damage to the aerial part is considered (Figure 5b). The only difference was observed on the third sampling date (14 October), where seedlings subjected to frost treatments of $-4°C$, $-8°C$ and $-12°C$ did not sustain a significantly different amount of damage from those exposed to the control treatment (T_0 vs T_3: $p>|t|=0.5962$).

For the first two sampling dates, only seedlings subjected to the lowest temperature ($-18°C$) exhibited RWL values significantly superior to those of the control temperature ($+4°C$) ($p<0.0001$ for both dates) (Figure 5c). Beginning on the third sampling date (14 October), there was no longer a significant difference in RWL among the artificial frost temperature treatments.

Results obtained for initiation of new roots (R_w/R_t) were quite different (Figure 5d). On the first sampling date (16 September), R_w/R_t diminished significantly with temperature, beginning at $-4°C$ (T_0 vs T_2: $p>|t|<0.0001$). On the second (30 September) and third (14 October) sampling dates, the ratio decreased significantly beginning at $-8°C$ (date B: T_0 vs T_3: $p>|t|<0.0001$; date C: T_0 vs T_3: $p>|t|=0.0007$). No new white roots were formed after a $-18°C$ frost treatment, regardless of the degree of hardening.

Discussion

Maintaining substrate water content at 30% v/v, rather than 55% v/v, throughout the second growing season of air-slit containerized white spruce seedlings did not significantly affect the end of the season values for growth (height, diameter, dry masses), bud formation or any of the variables used to estimate the kinetics of frost tolerance acquisition in the autumn. However, the water savings were substantial. Maintenance of substrate water content at 30% v/v, rather than 55% v/v, required 26% less water (224 vs 283 $l\ m^{-2}$). This strategy also limits leaching, as shown by Lamhamedi et al. (2001), who exposed white spruce seedlings to four IRs (IR-60%, IR-45%, IR-30% and IR-15% v/v) during their first growing season under similar experimental conditions (tunnel, air-slit containers, mobile boom irrigation system).

Under natural conditions, conifer seedlings initiate dormancy in mid-summer with the formation of the terminal buds, largely in response to moisture stress (Lavender & Cleary, 1974). To mimic these conditions, low substrate water contents are commonly used to induce shoot growth cessation and initiate hardening by triggering budset in containerized seedlings grown under conditions of natural day length (Grossnickle, 2000; Landis et al., 1989). Timmis and Tanaka (1976) and Blake et al. (1979) found that a mild moisture stress, imposed on Douglas fir seedlings before there was a natural decrease in photoperiod, increased their degree of cold hardening. Similarly, Macey and Arnott (1986) found that periodic and moderate moisture stress effectively induced terminal bud formation in 1-year-old white spruce seedlings. In these last three studies, moisture stress also influenced seedling growth, whereas the results of the present study show that maintaining substrate water content at 30% v/v, rather than 55% v/v, throughout the second growing season did not influence growth, bud formation or frost hardening of 2-year-old white spruce seedlings. The lack of effect of IR-30% on 2-year-old white spruce seedlings can then be explained by the fact that this level of substrate water content was high enough to induce neither a moderate nor an episodic water stress on 2-year-old white spruce seedlings (Timmis & Tanaka, 1976; Blake et al., 1979; Macey & Arnott, 1986). However, it is likely that a lower water content would influence seedling growth, and hasten budset and hardening, in the same way as an IR of 15% (v/v) caused water stress (Lamhamedi et al., 2001) and induced budset in 1-year-old white spruce seedlings (Stowe et al., 2001).

An effect of the highest moisture content (55% v/v) on the development of shoot frost tolerance was also expected. Van den Driessche (1969) found that reducing photoperiod had a greater positive effect on the hardening of well-watered Douglas fir seedlings than on seedlings receiving a reduced water supply. Nursery irrigation that is either too abundant or too frequent may generate a second flush of growth in late summer or early autumn (Lavender & Cleary, 1974) if other environmental variables are favourable. For example, Khan et al. (1996) found that Douglas fir seedlings grown for 12 weeks under very high soil moisture conditions (65% v/v) were 84% more likely to resume growth than those grown in the driest soil. As noted previously, growth cessation is a prerequisite to cold acclimatation in woody plants (Weiser, 1970). In the present study, an IR of 55% v/v did not influence bud formation or induce a second growth flush. According to van den Driessche (1969), seedlings growing with an IR of 30% or 40% were as well watered as those growing with an IR of 55%. The absence of second flush during the autumn can be explained by the fact that seedlings were not under an imposed dormancy induced by a nutrient or a moisture stress which could have been broken by relief of the stress (Young & Hanover, 1978). Rather, the seedlings in the present study were under a primary dormancy induced by the natural diminution of photoperiod and temperature (Weiser, 1970), which cannot be lifted until a sufficient number of cold degree days has accumulated.

High substrate moisture content has apparently little detrimental impact on root frost hardiness of white spruce seedlings. While cessation of shoot growth and shoot hardening are induced by decreasing photoperiod and cold temperatures under boreal conditions, cessation of root growth and root hardening are induced by substrate temperature (Bigras & Dumais, 2005). As is common in a forest nursery, the seedling containers in the current study were raised above the ground on pallets. Pallets allow air circulation under and around containers during early autumn and provide appropriate conditions for root hardening (Bigras & Dumais, 2005). In cold climates, root growth usually stops at temperatures near 2–5°C (Lyr & Hoffmann, 1967) and roots subsequently attain a maximum level of hardening (Bigras & Dumais, 2005). The average daily substrate temperature went down to 5°C beginning on 10 October (Figure 2). This may explain why, when the IRs are pooled, roots appear to have tolerated a frost temperature of $-18°C$ without any apparent damage beginning on the third sampling date (14 October) (Figure 5c.). The fact that no new roots formed after the exposure to $-18°C$ (Figure 5d) on the third (14 October) and the fourth (28 October) sampling dates does not contradict this explanation. Indeed, RWL gives an estimation of the frost damage on the roots, and the roots only. In contrast, R_w/R_t depends on the state of the roots and on the state of the needles where current photosynthates needed for root growth are produced (van den Driessche, 1987; Pellicer et al., 2000). On the third sampling date, shoot damage (N_d/N_t) in seedlings subjected to the lowest frost temperature ($-20°C$) was 61% (Figure 5b). Only one-third of the needles were therefore available to produce the photosynthates necessary to meet the physiological needs of the entire seedling. On the last sampling date (28 October), there was no additional shoot damage (Figure 5b), but a sufficient number of cold degree days had accumulated to lift primary dormancy. Therefore, when the seedlings were placed under optimal growing conditions, they broke bud (Lamhamedi et al., 2005). Flushing buds are more important sinks for current photosynthates than are roots (Waring & Pitman, 1985).

In conclusion, it is possible to reduce significantly substrate water content, from 55% (v/v) to 30% (v/v), as well as the quantities of water applied to containers without prematurely interrupting height growth or hindering the acquisition of frost tolerance of 2-year-old white spruce seedlings. The extension of such studies to other seedling cultures may help to reduce the environmental impact of nursery operations, and produce substantial savings in irrigation and fertilization costs.

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