Irrigation Control and Physiological Responses of Nursery-grown Black Spruce Seedlings (1+0) Cultivated in Air-slit Containers

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Abstract. Air-slit containerized (IPL 25-350A) black spruce [*Picea mariana* (Mill) BSP; 1+0] seedlings, were subjected to four irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, by volume, cm³ water/cm³ substrate) under tunnel conditions in a forest nursery. Irrigation regimes were controlled by time-domain reflectometry during the first growing season. With exception of midday water potential, water relation and gas exchange variables were unaffected by substrate water content. There were no significant differences in height, diameter or number of needle primordia of the seedlings grown under IR-15%, IR-30%, and IR-45%. Seedlings grown in a substrate with a high water content (IR-60%) had lower oven-dry biomass and higher cuticular transpiration rates than seedlings grown under the three other irrigation regimes. Root hydraulic conductivity was not affected by irrigation regime, but showed a general decrease towards the end of the growing season. Variation in root hydraulic conductivity was most likely related to root growth and degree of lignification. Seedlings that had been exposed to IR-60% during the first growing season remained smaller than the other seedlings throughout the second growing season. Results of this study indicate that nursery managers can reduce the quantity of irrigation water used without significantly affecting the growth or physiology of air-slit containerized black spruce seedlings (1+0) grown under tunnel conditions.

This study was part of a research program whose goal is to develop an irrigation strategy for the tunnel production of air-slit containerized spruce seedlings using time-domain reflectometry (TDR) to monitor substrate water content (Lamhamedi et al., 2001, 2002; Stowe et al., 2001). Tunnel production is the most common method of containerized forest seedling production in Quebec. Containers are placed under a unheated polyethylene-covered structure immediately after seeding (mid-May) and remain there until early October, when the cover is removed and the seedlings are transferred outside.

Although smaller quantities of fertilizers are used in forest nurseries than in agriculture or horticulture, a potential risk of nutrient leach-

ing still exists (Bilderback, 2002; Juntunen et al., 2002). Reducing the quantity of water used for irrigation not only lowers costs associated with irrigation and fertilization, but also reduces percolation of fertilizer solution into the groundwater. The goal of this program is to achieve these benefits without compromising the morphological and physiological characteristics of seedlings destined for reforestation. Black spruce (*Picea mariana* (Mill) BSP) and white spruce (*Picea glauca* (Moench) Voss) are the two most commonly planted species in the province of Quebec. In 1999–2000, they represented 53% (80.1 million) and 22% (33.7 million plants) of total Quebec forest seedling production, respectively (Gagnon et al., 2000).

Due to low evapotranspiration rates under

tunnel conditions and the relatively low leaf area of (1+0) seedlings, it may not be necessary to maintain an elevated water content in growing medium during the first growing season (Lamhamedi et al., 2001, 2003; Stowe et al., 2001). Maintenance of a lower rhizosphere water content increases substrate aeration, which may improve the morpho-physiological quality of seedlings (Heiskanen, 1993). A lower substrate water content also may reduce nutrient leaching (Cresswell, 1995; Lamhamedi et al., 2001) and incidence of pathogenic fungi (Bilderback and Jones, 2001). Reduction of substrate water content at the end of the growing season has been shown to slow growth, improve frost tolerance, and favor bud formation and cuticle development (Landis et al., 1999; Stowe et al., 2001).

Cultural requirements of white spruce are very different from those of black spruce, particularly with respect to irrigation and fertilization. Lamhamedi et al. (2001) and Stowe et al. (2001) showed that the morphology and physiology of white spruce seedlings (1+0) were negatively affected by the maintenance of a 15% (by volume) water content, while these variables were not adversely affected by a high substrate water content (60%, by volume). Conversely, Lamhamedi et al. (2003) found that the morphology of first-year black spruce seedlings was unaffected by substrate water contents as low as 15%, but was negatively affected by water contents of 60%. Studies carried out on white spruce led Lamhamedi et al. (2001) to propose an irrigation strategy for the culture of this species in air-slit containers. Similar knowledge is necessary for black spruce cultivation if a proper irrigation strategy is to be developed under nursery conditions.

The objective of the present study was to investigate the growth, gas exchange and root hydraulic conductivity of containerized black spruce seedlings in response to maintenance of substrate water contents within the range of 15% to 60% by volume throughout the first growing season. An additional objective was to determine if treatment effects would carry

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over from the first to the second growing season, when seedlings were exposed to standard nursery cultural practices.

Material and Methods

Plant material and experimental design. Black spruce seeds (provenance N0-031-30) were sown into IPL 25-350A air-slit containers (25 cavities/container, 350 cm³/cavity, IPL, Saint-Damien-de-Bellechasse, Que.) containing a standard 3 peat : 1 vermiculite (by volume) mixture. The cavities have square-shaped openings (5.9 × 5.9 cm) and taper with depth. Prior to seeding, bulk density of the peat mixture was adjusted to 0.11 g·cm⁻³. During germination phase, volumetric water content of the substrate was maintained at approximately 50% to 60%. After thinning seedlings to one individual per cavity, containers were installed in a randomized complete block design in an unheated tunnel at the Centre de Production de Plants Forestiers de Québec (CPPFQ, Sainte-Anne-de-Beaupré, Que., 47°02'N, 70°55'W). Four irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, by volume; cm³ water/cm³ substrate) were constantly maintained for 15 weeks during the first growing season (mid-July to the end of October 1999). Within each of six blocks, each irrigation regime was applied to seedlings growing in three consecutive rows of nine containers. Experimental units were separated by buffer zones each containing an additional 27 containers. The row of containers forming the border around the outside of the experimental area also served as a buffer and was not used for sampling. All details on plant material and experimental design were described by Lamhamedi et al. (2001, 2003) and Stowe et al. (2001).

Control of irrigation and environmental variables. The MP-917 soil moisture measurement system (Environmental Sensors Inc., Victoria, B.C.), which is based on time-domain reflectometry (TDR) technology (Topp and Davis 1985), was used to monitor and adjust the substrate water contents of the four irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%). TDR probes were installed horizontally through the five central cavities of twenty four (4 IR × 6 repetitions) seedling containers, passing alternatively through substrate and air. The probes remained in place for the duration of the experiment. The containers selected for sampling were judged to have substrate water contents closest to the mean value for their respective experimental units. On each sampling date, two successive measurements of substrate water content were made to determine the irrigation prescription for each individual experimental unit. A motorized robot, mounted on a ground rail and equipped with 22 nozzles (Aquaboom, Harnois Industries, Saint-Thomas-de-Joliette, Que.), was used for seedling irrigation. The coefficient of uniformity of the robot varied between 95% and 98% and each pass increased the substrate water content by 0.8% to 1.0%. Thirty minutes after the completion of irrigation, the water content of the substrate was verified. Substrate nitrogen content was maintained at 250 ppm, using PLANTEC fertilization-scheduling software

(Girard et al., 2001; Langlois and Gagnon, 1993). Measurement and adjustment of substrate water content were described by Lamhamedi et al. (2001, 2003) and Stowe et al. (2001).

Substrate water content was monitored and adjusted three times a week (Monday, Wednesday, and Friday). Target substrate water contents were reached by mid-July and maintained for the remainder of the growing season (15 weeks), with the exception of IR-60%, which tended to be slightly lower than the target level (Lamhamedi et al., 2003).

Relative humidity and air temperature in the tunnel at two meters above ground as well as temperature of the rhizosphere were recorded every 5 min using a data acquisition system, (CR10X; Campbell Scientific, Edmonton, Alberta). Rain gauges (model TE 525M; Texas Electronics, Dallas, Texas), placed in the center of one experimental unit per irrigation regime, were used to measure the quantity of irrigation water applied. During seedlings' active growing phase (July and August), average air temperature in the tunnel varied between 14 and 26 °C. Relative humidity varied between 60% and 100% during the same time period.

Seedling development—first growing season. Growth of seedlings in their first growing season was reported in Lamhamedi et al. (2003). Total number of needle primordia in the terminal buds was determined for three seedlings per IR per block, harvested on 20 Oct. 1999. The buds were dissected according to the method described by Templeton et al. (1993).

Water relation variables of the shoots and roots. Midday xylem water potential (Ψ_{xd}) was determined for one seedling per IR per block on 29 Aug., 12 and 25 Sept., and 10 Oct. 1999 between 11:30 and 12:30 using a pressure chamber (model 600; PMS Instruments, Corvallis, Ore.) and the method described by Ritchie and Hinckley (1975). To ensure that seedling stems were adequately lignified and had large enough root collar diameters to be inserted into the rubber seals of the pressure chamber, sampling dates after the end of the active growing phase were selected. This approach also permitted us to evaluate the morphophysiological effects of maintaining different irrigation regimes over a prolonged period.

Pressure-volume (PV) curves were constructed for shoots and root systems of the seedlings harvested on each of the four aforementioned sampling dates. The composite method (Parker and Colombo, 1995; Stowe et al., 2001) was used for pressure-volume analysis of the shoots. At each sampling date, five seedlings per IR per block (30 seedlings per IR) were used to construct a PV curve for each irrigation regime. Seedlings were left to air dry in the lab. Every 20 min, the water potential of one randomly selected seedling was measured. Each seedling was used only once. This process continued until a maximum water potential of -3.0 MPa was reached. The sap expression technique (Parker and Colombo, 1995) was used for the pressure-volume analysis of the root systems. Tissue dehydration was achieved by repeatedly applying an over-pressurization of 2.0 MPa for 10-min intervals. Six seedlings (one seedling per IR per block) were used on

each sampling date to produce one PV curve for each IR.

Nonlinear regression was used to estimate the following water relation variables from the PV curves: osmotic potential at turgor loss ($\Psi_{\pi_{tp}}$) and at tissue saturation ($\Psi_{\pi_{sat}}$), relative tissue water content at turgor loss (R_0) and maximum modulus of elasticity (ϵ_{max}). Because only one estimate of the relevant data was available per date for each shoot PV curve, the four sampling dates were used as replicates to determine the effect of irrigation regime on the shoot water relation variables. Three to six curves per IR per date were used for statistical analysis of the root system variables.

Root hydraulic conductivity. Root hydraulic conductivity was quantified using the method described by Colombo and Asselstine (1989). One seedling per IR per block (24 seedlings) was harvested on the same sampling dates used for analysis of the water relation variables. Seedlings were shielded from the light and rehydrated overnight in a dilute fertilizer solution (35 ppm N; 20-8-20 formulation, Plant Products Ltd., Bramalea, Ont.). The following morning, the severed root system was immersed in a bottle of 35 ppm N solution that had been placed in a pressure chamber. Pressure in the chamber was then increased in increments of 0.2 MPa. After each pressure increase, fluid exuding from the cut surface was collected for a 10 min in preweighed pieces of tissue paper-filled Tygon tubing (Saint-Gobain Performance Plastics Corporation, Akron, Ohio). Volume of the root system was determined by the volume displacement method (Burdett, 1979). Fresh biomass of the root system was measured immediately after it had been removed from the pressure chamber. The sample was then oven dried for 48 h at 65 °C before measurement of dry root biomass. Flux of water exuded from the root system at each pressure level, (J_v ; $\mu\text{L}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ of root volume) was calculated, assuming that 1 mg of exuded fluid was equivalent to 1 mL of water. For each sampling date, data from four seedlings/IR (total of 16 seedlings) were used to construct a curve describing the relationship between J_v and the applied pressure. A separate curve was produced for each IR regime. Root hydraulic conductivity (L_p) was derived from the slope of the linear portion of each curve (Colombo and Asselstine, 1989).

Four pressure chambers were used simultaneously for pressure-volume and root hydraulic conductivity measurements. The four irrigation treatments were randomly assigned to the chambers on each sampling date using a Latin-square design. Latin-square assignment minimized data acquisition errors inherent in the use of multiple pressure chambers (Zine El Abidine et al., 1993).

Gas exchange measurements. Gas exchange variables were measured on 18 July; 1, 15, and 29 Aug. and 12 and 25 Sept. 1999. Measurements were made using a portable open-flow gas analyzer equipped with a cylindrical conifer cuvette and a portable light unit (models LCA-4, PLC4-C, and PLU-002, respectively; ADC Co., Hoddesdon, U.K.). One seedling per IR per block was exposed to saturated light conditions ($> 900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Twelve successive pho-

Table 1. Analysis of variance of midday xylem water potential (Ψ_{xd}), relative tissue water content at the turgor loss point (R_0), osmotic potential at tissue saturation (Ψ_{sat}), osmotic potential at the turgor loss point (Ψ_{tulp}), maximum modulus of elasticity (ϵ_{max}), and root hydraulic conductivity (L_v) of black spruce seedlings produced in air-slit containers. Values in bold typeface indicate statistically significant effects.

Source	df	Ψ_{sd} (MPa)	PV curves								L_v ($\mu\text{L}\cdot\text{min}^{-1}\cdot\text{MPa}^{-1}\cdot\text{mL}^{-1}$)
			Shoots				Roots				
			R_0 (%)	Ψ_{rsat} (MPa)	Ψ_{rtlp} (MPa)	ε_{max} (MPa)	R_0 (%)	Ψ_{rsat} (MPa)	Ψ_{rtlp} (MPa)	ε_{max} (MPa)	
First growing season ^z											
Irrigation regime (IR)	3	0.0017	0.1179	0.2707	0.1813	0.1517	0.9091	0.6235	0.8821	0.9701	0.1301
IR × date (D1)	9	0.6142	---	---	---	---	0.0007	0.0177	0.4151	0.0031	0.0010
Contrasts											
IR-45% vs IR-60%	1	0.0197	---	---	---	---	0.8929	---	---	0.8660	0.1998
IR-30% vs IR-45%	1	0.0123	---	---	---	---	0.9193	---	---	0.7744	0.5676
IR-15% vs (IR-30% & IR-45%)	1	0.0009	---	---	---	---	0.5700	---	---	0.9542	0.1173
Second growing season ^y											
Date (D2) ^x	3	---	0.5358	0.0230	0.0230	0.0020	---	---	---	---	---

^zDuring the first growing season, the seedlings were grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, by volume).

^yDuring the second growing season, the seedlings were grown under standard nursery cultural practices.

^xThe presented values compare the effect of irrigation regime on the shoot PV curve variables for the last sampling date of the first growing season with those of the single sampling date in the second growing season. The irrigation regimes were used as repetitions.

tosynthesis measurements were taken for each sample. Only the final eight measurements were used for statistical analyses. Data were corrected as a function of projected surface area, which was measured using WinSeedle image analysis software (Regent Instruments Inc., Quebec, Que.). Net photosynthesis (A_n), transpiration rate (E), stomatal conductance to water vapor (g_{sw}) and water use efficiency (WUE), are all expressed on a unit-leaf area basis.

Cuticular transpiration. Cuticular transpiration rates were determined at the end of the first growing season using the artificial dehydration method described by Kerstiens (1996) and Stowe et al. (2001). On 20 Oct. 1999, three seedlings per IR per block were randomly selected and rehydrated in darkness overnight at 21 °C. The following day, the shoots were left to dehydrate at ambient conditions on the lab bench for a total of 440 min. Every 30 min the shoots were weighed. The difference in fresh mass between successive measurements was assumed to be due to the amount of water lost over the time period. Water loss curves [$\text{g of water lost (g of plant dry mass)}^{-1}\cdot\text{min}^{-1}$] were constructed for each irrigation regime. The cuticular transpiration rates were determined using the data from the linear portion of the water loss curves, which represents the water transpired from the needles after stomatal closure (elapsed time 245 to 430 min) (Slavik, 1974a, 1974b).

Second growing season. When seedlings were moved outside the tunnel at the end of the first growing season, care was taken to preserve the initial experimental design. Seedlings were grown under standard nursery conditions during their second growing season. Three types of comparative measurements were made at the end of the second growing season. First, daily evolution of xylem water potential (Ψ_x) was evaluated by measuring the Ψ_x of one seedling per IR per block every two hours between 6:00 a.m. and 8:00 p.m. on 22 Aug. 2000. Second, PV curves were constructed for the aboveground portion of the seedlings harvested on 18 Oct. 2000 using the composite method described previously. Data obtained from these curves were compared to those obtained from the last sampling date of the first growing season (10 Oct. 1999). Irrigation regimes were used as replicates

for this analysis because seedlings harvested from each block were pooled to construct one PV curve per IR. Third, height, root collar diameter and dry biomass of the shoots and roots of 12 seedlings per IR per block, harvested on 27 Oct. 2000, were measured.

Statistical analyses. Effects of irrigation regime (whole plot) and sampling date (sub-plot) on the midday water potential, hydraulic conductivity, root water relation variables, and gas exchange variables were analyzed using a general linear model for a split-plot ANOVA design as described by Lamhamedi et al. (2003). Time of measurement was used as sub-plot in diurnal water potential curve analyses. A completely randomized block model was used to analyze morphological variables and those obtained from PV curves for shoots. Differences between irrigation treatments were determined by *a priori* contrasts when main effects were found to be significant (Steel and Torrie, 1980). When IR $\times$ date interactions were found to be

significant, contrasts by date and polynomial contrasts for each IR (all dates combined) were performed (Milliken and Johnson, 1984). Results for significant interactions are included in the text where appropriate.

Cuticular transpiration was analyzed using a repeated measures analysis of variance, as described in Stowe et al. (2001). In addition to computing F tests for the main effects, irrigation regime (IR) and desiccation time (T) as well as the T $\times$ IR interaction, *a priori* contrasts were made to determine the difference in cuticular transpiration rates among irrigation regimes.

To insure normality of residuals and homogeneity of variance, certain variables were subjected to logarithmic transformations. The GLM procedure of SAS (Version 8.01; SAS Institute Inc., Cary, N.C.) was used for all statistical analyses. Significance levels of $\alpha = 0.05$ and $\alpha = 0.01$ were used for the main effects and interactions, respectively. Given the objective of this study, emphasis was placed on

Fig. 1. Means and standard errors of midday shoot xylem water potential (Ψ_{xd}) by irrigation regime of root systems of (1+0) black spruce seedlings produced in air-slit containers in a tunnel and subjected to four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, by volume); $n = 24$.

the effect of irrigation regime and its interaction with sampling date.

Results

Control of irrigation regimes. The cumulative amounts of water that were applied to maintain the desired substrate water contents in IR-15%, IR-30%, IR-45%, and IR-60% were 60, 69, 95, and 253 mm, respectively.

Water relations of root and shoot tissues. Irrigation regime had a significant effect on midday xylem water potential (Ψ_{xd} , Table 1) over the four sampling dates. Seedlings grown under IR-45% had significantly higher (less negative) Ψ_{xd} values than seedlings grown under IR-30% and IR-60%. Ψ_{xd} values from IR-15% were significantly lower (more negative) than the average of the values of IR-30%

and IR-45% (Fig. 1). Near the end of the 1999 growing season, IR-15% and IR-45% exhibited the most negative (-0.84 MPa) and least negative (-0.71 MPa) Ψ_{xd} values, respectively. By contrast, osmotic potential at saturation ($\Psi_{\pi_{\text{sat}}}$) and at turgor loss ($\Psi_{\pi_{\text{tp}}}$), relative tissue water content at turgor loss (R_0), and maximum modulus of elasticity (ϵ_{max}) of the shoots remained unchanged between treatments and throughout the first growing season, with average values of -1.36 MPa, -1.67 MPa, 0.20 and 8.04 MPa, respectively (Table 1).

There was no significant simple effect of irrigation regime on the water relation variables of the root systems (Table 1). A strong irrigation regime $\times$ sampling date interaction was found for ϵ_{max} and R_0 . However, contrasts showed no significant difference among irrigation regimes for these two variables over the growing season. Contrast by date analyses showed that the ϵ_{max} value under IR-15% was significantly higher than averaged values under IR-30% and IR-45% on 29 Aug. ($P = 0.0006$), and that the IR-60% value was significantly lower than IR-45% value on 12 Sept. ($P = 0.0044$). Polynomial contrasts failed to show significant relationships between each irrigation regime and sampling dates for elasticity values (Fig. 2A, $P > 0.01$). For R_0 , IR-15% value was significantly higher than IR-30% and IR-45% averaged values on 29 Aug. ($P = 0.0054$). Polynomial contrasts showed a first-order ($P = 0.0004$) and a second-order ($P = 0.0010$) relationship for IR-60% and IR-15% over sampling dates, respectively (Fig. 2B). Average $\Psi_{\pi_{\text{sat}}}$ and $\Psi_{\pi_{\text{tp}}}$ values for a given irrigation regime varied during the sampling period from -0.65 to -0.77 MPa and from -1.03 to -1.12 MPa, respectively.

Root hydraulic conductivity. Root hydraulic conductivity showed a strong IR $\times$ D interaction (Table 1, Fig. 2C). Contrasts by date indicated that the L_v value under IR-15% was significantly higher than averaged values under IR-30% and IR-45% on 25 Sept. ($P = 0.0001$). Polynomial contrasts showed significant linear ($P < 0.0001$) and cubic ($P < 0.0001$) relations for IR-30% and IR-15% over the sampling period, respectively.

Gas exchange. Irrigation regime had no significant effect on A_n , E , g_{sw} , WUE. Mean values are $12.16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $3.39 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $0.189 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and $3.90 \mu\text{mol CO}_2/\text{m}^2/\text{s per mmol H}_2\text{O}/\text{m}^2/\text{s}$, respectively.

Cuticular transpiration. Irrigation regime significantly affected the rate of cuticular transpiration (Table 2, Fig. 3). Repeated measures analysis of variance did not show a significant irrigation regime by desiccation time interaction. Main effects contrasts indicate that seedlings

grown under IR-60% had a significantly higher rate of cuticular transpiration than seedlings from IR-45% ($P = 0.0133$), but there was no difference among IR-15%, IR-30% and IR-45%. Cuticular transpiration decreased with elapsed desiccation time (Table 2, Fig. 3).

Needle primordia. There was no effect of substrate water content on the number of needle primordia formed. Terminal buds of the black spruce seedlings in our study contained an average of 205 primordia.

Seedling growth during the second growing season. Irrigation regimes imposed on seedlings during their first growing season had no significant effect on the height or diameter of the seedlings measured during their second growing season (Table 3). However, contrasts showed that dry shoot biomass of seedlings grown under IR-60% were lower than those from treatment IR-45% at the end of the second growing season (Table 3). Similarly, Lamhamedi et al. (2003) reported that the shoot biomass of the IR-60% treatment at the end of the first growing season was lower than for the other three treatments.

Seedling physiology during the second growing season. Irrigation regimes did not have a significant effect on the daily variations of xylem water potential (Ψ_x) of the seedlings (Fig. 4). There was a significant difference between the first and second growing season for $\Psi_{\pi_{\text{sat}}}$, $\Psi_{\pi_{\text{tp}}}$ and ϵ_{max} (Table 1). The $\Psi_{\pi_{\text{sat}}}$ and $\Psi_{\pi_{\text{tp}}}$ values were 156% and 161% more negative, respectively, during the second growing (-2.0 and -2.7 MPa) season than during the first growing season (-1.36 and -1.67 MPa). The maximum modulus of elasticity (ϵ_{max}) increased by 246% from the first (5.5 MPa) to the second growing season (13.5 MPa). No difference between the two growing seasons was detected for R_0 .

Discussion

Results of this experiment complement those obtained by Lamhamedi et al. (2003), who found that an irrigation regime of 15% (by volume) did not affect growth or mineral status of black spruce seedlings (1+0) under nursery conditions. In the present study, gas exchange and water relations variables of black spruce seedlings grown in air-slit containers were unaffected by substrate water contents maintained at values as low as 15% during the first growing season. Nursery growers can significantly reduce the quantity of water applied to black spruce seedlings during their first growing season under tunnel conditions. Tolerance of black spruce to low substrate water contents is also supported by field observations of black spruce seedlings following wetland drainage (MacDonald and Lieffers, 1990; Roy et al., 1999).

The maintenance of a high substrate water content (60% by volume) reduced growth of black spruce seedlings during their first growing season (Table 3). High substrate water content generally decreases availability of oxygen in the rhizosphere (Kozłowski, 1997). Conditions of poor substrate aeration can impede root development and initiation of fine roots, thereby diminishing a seedling's capacity to absorb water and nutrients (Heiskanen, 1993; Kozłowski,

Fig. 2. Means and standard errors of (A) maximum modulus of elasticity (ϵ_{max}), (B) relative tissue water content at turgor loss point (R_0), and (C) root hydraulic conductivity (L_v) of (1+0) black spruce seedlings produced in air-slit containers in a tunnel and subjected to four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, by volume). For A and B, n varies from 3 to 6; for C, n = 6.

Table 2. Summary of repeated measures analysis of variance of the cuticular transpiration rate of excised shoots from black spruce seedlings (1 + 0) produced in air-slit containers under four different irrigation regimes in a tunnel (IR-15%, IR-30%, IR-45% and IR-60%, by volume). Values in bold typeface indicate statistically significant effects.

Source	df	P
Irrigation regime (IR)	3	0.0501
Contrasts		
IR-45% vs IR-60%	1	0.0133
IR-30% vs IR-45%	1	0.8550
IR-15% vs (IR-30% & IR-45%)	1	0.3378
Desiccation time (T)	6	<0.0001
IR × T	18	0.3689

Fig. 3. Means and standard errors of the cuticular transpiration rate of excised shoots from 1+0 black spruce seedlings produced in air-slit containers in a tunnel and subjected to four different irrigation (IR-15%, IR-30%, IR-45%, and IR-60%, by volume); n = 18.

Table 3. Analysis of variance and means (± standard deviations) of the morphological characteristics of black spruce seedlings produced in air-slit containers at the end of the second growing season^a (27 Oct. 2000). Values in bold typeface indicate statistically significant effects.

Source	df	Ht (cm)	Diam (mm)	Shoot biomass (g)	Root biomass (g)
Irrigation regime (IR)	3	0.3814	0.6212	0.0589	0.5895
Contrasts					
IR-45% vs IR-60%	1	---	---	0.0377	---
IR-30% vs IR-45%	1	---	---	0.5606	---
IR-15% vs (IR-30% & IR-45%)	1	---	---	0.2618	---
Means ± standard deviations (n = 47)					
IR-15%		38.2 (±4.6)	5.56 (±1.14)	6.697 (±1.600)	4.067 (±1.479)
IR-30%		40.2 (±4.7)	5.85 (±1.14)	7.230 (±1.474)	4.403 (±1.460)
IR-45%		40.2 (±4.3)	5.64 (±1.14)	6.960 (±1.314)	3.798 (±1.673)
IR-60%		38.6 (±4.0)	5.64 (±1.16)	6.092 (±1.163)	4.013 (±1.756)

^aDuring the first growing season the seedlings were grown under four different irrigation regimes (IR -15%, IR-30%, IR-45%, and IR-60%, by volume). During the second growing season the seedlings were grown under standard nursery cultural conditions. Analysis of growth during the first growing season is reported in Lamhamed et al. (2003).

1997). Lamhamed and Bernier (1994) reported that root growth is impeded when seedlings are grown under saturated soil conditions.

Comparison of our results with those obtained for white spruce reveals that black spruce may be more tolerant of low rhizosphere water contents under nursery conditions. When subjected to a similar irrigation protocol, white spruce growth was reduced under IR-15% as a result of an apparent stomatal limitation to gas

exchange (Lamhamed et al., 2001). However, white spruce seedlings seem unaffected by substrate water contents as high as 60% by volume. Lamhamed et al. (2001, 2003) showed that leaching of mineral nutrients from the substrate increases exponentially as a function of an increase in the rhizosphere water content. Therefore, maintenance of high substrate water contents is undesirable, even if seedlings can tolerate this condition.

Osmotic potentials at saturation ($\Psi\pi_{sat}$) and at turgor loss ($\Psi\pi_{tl}$) of both roots and shoots were not influenced by irrigation regime (Table 1). Moreover, the capacity of xylem conductive tissue to retain water was similar among the treatments. Zine El Abidine et al. (1994a, 1994b) reported similar values for the osmotic potentials of black spruce seedlings and mature trees. These authors also failed to find osmotic adjustments in black spruce seedlings. Absence of changes in cell wall elasticity and osmotic adjustment in the current study indicates that low substrate water contents do not induce a severe stress in black spruce seedlings during the first growing season. Water relations measurements also indicate that seedling response to water availability changes with time (Table 1, Fig. 2A and B).

The degree of lignification and permeability of roots to water and solutes may help to explain variations noted for maximum modulus of elasticity (ϵ_{max}), water content at turgor loss (R_0) and hydraulic conductivity (L_v) in black spruce seedling roots. No differences among irrigation regimes were observed for ϵ_{max} and R_0 on last two sampling dates, suggesting that increased lignification after mid-September inhibited any irrigation regime effect. This is supported by significant linear trends in R_0 and L_v over time, for IR-60% and IR-30%, respectively. Although we observed some variations in cell wall elasticity (Fig. 2A), ϵ_{max} values for shoot and root tissues during the first growing season remained low (<10 MPa), probably due to limited xylem development.

The hydraulic conductivity of a root system is related to its architecture (Blake and Sutton, 1987; Colombo and Asselstine, 1989; Grossnickle and Blake, 1985). Observed variations in L_v at the end of the first growing season could be related to the kinetics of root growth and the development of root hairs and nonlignified roots. The $L_v \times IR$ interaction may result from differences in translocation of photosynthates between the shoots and roots. Fine root development in conifer species, especially during the hardening phase, is largely dependent on the allocation of current photosynthates (Lippu, 1998; Marshall, 1985). Kinetics of absorption at the soil/root interface is controlled by the presence of root hairs. Extremes in soil moisture impede the development of these absorptive surfaces and have a pronounced effect on their viability. Episodes of drought may increase the proportion of lignified roots (Pallardy et al., 1995), thus negatively affecting the absorption capacity and viability of fine roots and root hairs as well as the resistance to water flow at the soil–root interface (Passioura, 1988; Grossnickle, 2000). Elevated substrate moisture reduces the amount of oxygen in the rhizosphere, discouraging fine root development and affecting gas exchange and the absorption of water and mineral nutrients.

Seedlings grown under IR-60% had significantly higher rates of cuticular transpiration than those grown under other irrigation regimes (Fig. 3). This response may be attributed to the presence of a thinner epicuticular wax layer as a result of this treatment. In a parallel study, Stowe et al. (2001) associated high cuticular

Fig. 4. Means and standard errors of xylem water potential (Ψ_x) as a function of time of day for 2+0 black spruce seedlings produced in air-slit containers and grown outside. During the second growing season, the seedlings were subjected to standard nursery cultural practices.

transpiration rates with high tissue water contents of white spruce needles.

The number of needle primordia present in terminal buds at the end of a growing season is an indication of height growth potential the subsequent year. Favorable environmental conditions during the period of bud development may increase the number of primordia formed (Colombo, 1997; Templeton et al., 1993). Our results, which are comparable with those obtained by Colombo (1997) for black spruce seedlings cultivated in a heated greenhouse, suggest that rhizosphere water content can be maintained at a low level during the first growing season without compromising growth potential of seedlings in the following year.

Finally, measurements carried out during the second year in the nursery demonstrate the importance of carry-over effects between growing seasons. Although all seedlings were exposed to a similar cultural regime during the second growing season, seedlings exposed to the wettest (60%) irrigation regime remained smaller than other seedlings. This effect persisted in spite of physiological changes and significant differences in various water relations parameters between the two growing seasons.

This and other studies of nursery-grown air-slit containerized spruce seedlings (Lamhamedi et al., 2001, 2003; Stowe et al., 2001), show that black spruce is more tolerant to low substrate water contents than white spruce. Substrate water contents can be decreased to 15% (by volume) without affecting growth, morphology and physiological processes of container-grown black spruce seedlings during the active growth phase of their first growing season. Quantity of irrigation water applied can be reduced, thereby minimizing leaching of mineral nutrients (Lamhamedi et al., 2001, 2003) and preserving groundwater quality.

Substrate water content during the first growing season has a persistent effect on seedling growth in the nursery and perhaps, also in the field after planting.

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