Water relations, cuticular transpiration, and bud characteristics of air-slit containerized *Picea glauca* seedlings in response to controlled irrigation regimes

Debra C. Stowe, Mohammed S. Lamhamedi, and Hank A. Margolis

Abstract: White spruce seedlings (*Picea glauca* (Moench) Voss) were grown in air-slit containers (IPL 25-350A) in a tunnel under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v; (cm $H_2O)^3$·(cm substrate)$^{-3}$). At the end of the first growing season the water-relation variables of the shoots were determined. Seedling morphology, the rates of cuticular transpiration and terminal bud development, as well as the number of needle primordia were also measured. Irrigation regime had no significant effect on any of the water-relation variables. Seedlings grown under the IR-15% were smaller and completed bud development more quickly than seedlings grown under IR-30%, IR-45%, and IR-60%. The formation of needle primordia was enhanced under IR-30%. Both the irrigation regime and the amount of time the detached shoots were left to transpire had a significant effect on the rate of cuticular transpiration. A comparison of the water-relation variables at the end of the first (1998) and second (1999) growing seasons showed that the younger seedlings had more negative osmotic potential at tissue saturation and greater maximum modulus of elasticity values. None of the other water-relation variables were significantly affected by seedling age, but the absolute values of all the variables were greater in the younger (1+0) seedlings.

Résumé : Des semis d'épinette blanche (*Picea glauca* (Moench) Voss) (1+0), produits dans des récipients à parois ajourées ont été cultivés dans un tunnel sous quatre régies d'irrigation différentes (RI-15%, RI-30%, RI-45% et RI-60%, v/v; (cm $H_2O)^3$·(cm substrat)$^{-3}$). À la fin de la première saison de croissance les caractéristiques hydriques des parties aériennes ont été déterminées. La morphologie des semis, le taux de transpiration cuticulaire, la cinétique de la formation des bourgeons apicaux et le nombre de primordia foliares ont aussi été mesurés. Aucune des variables hydriques n'a été significativement affectée par la régie d'irrigation. Les semis de la régie RI-15% étaient plus petits et ont complété la formation des bourgeons plus rapidement que les semis soumis aux autres régies d'irrigation. La formation de primordia foliares a été significativement augmentée sous la régie RI-30%. Le taux de transpiration cuticulaire a été influencé significativement par la régie d'irrigation et par le temps de dessiccation des pousses détachées. La comparaison des variables hydriques à la fin de la première (1998) et la deuxième saison de croissance (1999) a montré que les jeunes semis ont un potentiel osmotique à saturation plus négatif et des valeurs élevées du module d'élasticité. Aucune des autres variables hydriques n'a été significativement affectées par l'âge des semis, mais leurs valeurs absolues étaient plus grandes chez les jeunes semis (1+0).

Introduction

Large seedlings have been shown to have a competitive advantage over noncrop species in reforestation programs (Overton and Ching 1978; Newton et al. 1993; Lamhamedi et al. 1997, 1998; Jobidon et al. 1998). With growing environmental concerns, the use of large seedlings has been proposed as an alternative to herbicides in plantation establishment (Perreault et al. 1993; ministère de l'Énergie et des Ressources 1994). The IPL 25-350A container (25 cells per container, 350 cm^3 per cell; IPL®, Saint-Damien, Bellechasse, Que.) has been used operationally in Quebec since 1994 for the production of large seedlings (Gingras 1993; Gingras and Richard 1999). Its square cavity construction optimizes air-pruning efficiency and avoids the formation of a mass of fine roots at the base of the root plug (Gingras and Richard 1999). There is a tendency to over irrigate air-slit containerized seedlings to compensate for the rapid drying of the substrate (Biernbaum 1992; Ford 1995; Lambany et al. 1997). Nursery managers must also take into account that seedlings are small relative to the size of the cavity during the first growing season and that the seedlings probably do not require all of the water that is retained by the growing medium (Lambany et al. 1997). Adequate irrigation management can control leaching of fertilizers (Cresswell 1995; Pelletier and Tan 1993) and decrease the incidence of disease (Beyer-Ericson et al. 1991). The moisture content of the substrate must be closely monitored throughout the

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growing season to obtain the morphological characteristics that will assure field survival of the seedlings after outplanting.

Irrigation management can be used to improve the hardening and frost tolerance of seedlings (Landis et al. 1989, 1999; Timmis and Tanaka 1976). The cessation of growth, bud development, and premature hardening of coniferous seedlings at the end of the growing season can be easily initiated by maintaining low substrate water contents (Landis et al. 1999; Lamhamedi et al. 2000; Bigras et al. 2001). Low substrate water contents (15–30%, v/v) also favour the rapid formation of epicuticular waxes on the needles of white spruce (1+0) seedlings (Stowe 2001). The improvement of cuticle development through the optimization of cultural practices can improve the resistance of seedlings to winter desiccation, especially when the roots and soil are frozen (Colombo 1997; Jenks and Ashworth 1999; Bigras et al. 2001).

Previous studies have shown the usefulness of time domain reflectometry (TDR) in monitoring the water content of sandy soils and peat substrates (Lambany et al. 1996, 1997; Lamhamedi et al. 1997, 2000). A precise and immediate determination of substrate water content can be made within an accuracy of 2% (Topp and Davis 1985). The current study is the third in a series that has used TDR to monitor the water content of the peat–vemiculite (3:1) substrate of air-slit containerized (IPL 25-350A) white spruce (*Picea glauca* (Moench) Voss) seedlings during their first growing season under controlled conditions. Lamhamedi et al. (2001) showed that the maintenance of a 15% (v/v; (cm $H_2O)^3$·(cm substrate)$^{-3}$) difference between the two irrigation regimes (25 and 40%, v/v) did not have any significant effect on morphological variables (height, root collar diameter, and dry mass of root and shoot tissue) and root architecture (total length, diameter, and surface area of roots). These results suggested that it was possible to maintain a low water content in the rhizosphere without any adverse effects on the growth and physiology of white spruce seedlings. Lamhamedi et al. (2001) investigated the effect of a wider range of substrate water contents (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) on white spruce seedling morphology and physiology and found no significant differences in height, root collar diameter, shoot and root dry mass, root surface, root length, net photosynthesis, and nutrient contents of white spruce seedlings (1+0) grown in peat–vermiculite (3:1) substrates with water contents of 30, 45, or 60%, (v/v).

The major objective of our recent work (Lamhamedi et al. 2000, 2001) has been to optimize irrigation of containerized white spruce (1+0) seedlings under operational conditions without compromising the morphology and development of the seedlings. To complement our previous research, the objective of the current study was to compare the effects of four irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) on the water-relation variables, rates of cuticular transpiration and bud development, and the production of needle primordia of white spruce seedlings during their first growing season. It is important to note that we did not impose a water stress on the seedlings by withholding water (Kwon and Pallardy 1989; Gebre et al. 1994; Zwiazek 1991) or by using osmoregulators such as polyethylene glycol (PEG) (Blake et al. 1991; Tan et al. 1992; Zwiazek and Blake 1989, 1990). Immersing a root system in a liquid solution reduces the availability of oxygen to the roots and may not induce the same physiological response as a stress imposed in a soil substrate. Versules et al. (1998) suggested that even well-aerated PEG solutions require supplemental oxygenation to avoid hypoxia. We avoided the problems inherent in artificial drought-inducing methodologies in our study by monitoring the progressive decrease and maintenance of substrate water content treatments in real time over an entire growing season under operational conditions with a MP-917 soil moisture measurement system, while maintaining the fertility of the substrate at constant and identical levels in the four irrigation regime treatments.

Material and methods

Plant material and experimental design

On May 6, 1998, white spruce seeds of provenance X01-034-96 were sown into IPL 25–350A air-slit containers in a 3:1 (v/v) peat–vermiculite substrate with a density of approximately 0.11 g·cm^{-3}. The density of the substrate was determined by weighing the peat–vermiculite mixture after it had been dried to a constant mass. The water content of the substrate was maintained near field capacity (50–60%, v/v; (cm $H_2O)^3$·(cm substrate)$^{-3}$) for 7 weeks after seeding. Two weeks after germination, the seedlings were thinned to one per cavity. A completely randomized block design consisting of six repetitions of four different irrigation regimes (IR) was installed in an unheated polyethylene production tunnel at Centre de Production de Plants Forestiers du Québec Inc. (CPPFQ), Sainte-Anne-de-Beaupré, Que. (47°02′N, 70°55′W), on June 26, 1998. Seedling production under polyethylene tunnels is specific to the province of Quebec. Because the tunnels are neither heated nor have artificial lighting, cultural practices are less intensive than those required for greenhouse production (Margolis 1987). The irrigation regimes involved the constant maintenance of substrate water contents at one of four levels (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) for a period of 18 weeks from the end of June until the end of October 1998, when the seedlings were moved out of the tunnel. Water-retention curves relating the volumetric water content and water potential of peat substrates used in Quebec nurseries were described in detail by Bernier and Gonzalez (1995) and Paquet et al. (1993).

Within each block, each IR was represented by three rows of nine containers, for a total of 27 containers per irrigation regime per block. A buffer zone of an additional 27 containers separated the treatments from each other. The row of containers around the borders of the experiment was not sampled but served as a buffer. Between seeding and the start of the experiment, all of the containers had been subjected to the same irrigation regime. To install the experimental design, the substrate water content in each of the containers (27 containers × 6 blocks × 4 irrigation regimes) was measured before and after irrigation on three successive days using a MP-917 soil moisture measurement system (ESI Environmental Sensors Inc., Victoria, B.C.). On the fourth day, nine containers per block per irrigation regime were chosen at random for measurement of substrate water content. The MP-917 probes were then installed for the duration of the growing season in the containers that had a substrate water content closest to the mean value for their respective experimental units. Mean substrate water contents in the containers varied from 38 to 53% (v/v) with a SE of 0.8–3.7 within each block. The containers with the MP-917 probes were placed in the centre of each experimental unit.

The arrangement of the original experimental design was maintained when the plants were transferred outside the tunnel at the end of the growing season in late October 1998, so that the effect of substrate water content in 1998 on seedling morphology in 1999 could be determined. The seedlings were grown under the established cultural practices of the CPPFQ during the second growing season and all of the seedlings received the same irrigation and fertilization treatments.

Control of substrate water content and fertility

The irrigation regimes were applied starting on June 26, 1998. After this date, the water content of the substrate in the containers was monitored three times a week (Monday, Wednesday, and Friday) at approximately 09:00. Substrate water content measurements were made with an MP-917, an instrument based on the principles of TDR (Topp and Davis 1985; Lambany et al. 1996, 1997). Twenty-four (4 treatments × 6 repetitions) probes were used. Each probe consisted of two parallel stainless steel rods (40 cm in length, 3.17 mm in diameter) and was fitted with a diode assembly on each end. The probes were inserted horizontally through the five central cavities of the 24 containers, passing alternately through peat and air (Lambany et al. 1996; Lamhamedi et al. 2000). Two successive readings of the substrate water content were taken. Irrigation was carried out using a motorized robot equipped with 22 nozzles and mounted on a ground rail (Aquaboom, Harnois Industries, Saint-Thomas-de-Joliette, Que.). Each pass of the robot increased the water content of the substrate by 0.8–1.0%, v/v. Thirty minutes after the irrigation had been completed, soil water content was again measured with the MP-917. Lambany et al. (1996, 1997) showed that the water content of a peat–vermiculite (3:1, v/v) mixture stabilizes within this time period.

A target substrate fertility level of 250 ppm N was maintained throughout the growing season in all of the irrigation regime treatments, through biweekly adjustments of substrate fertility using PLANTEC, a fertilization-scheduling software (Langlois and Gagnon 1993; Girard et al. 2001). The amount of water applied with the fertilizer was taken into account when calculating that day's irrigation prescription. Rain gauges (TE52M; Texas Instruments, Dallas, Tex.) were used to monitor the quantity of water applied to the plants during fertilization and irrigation. Depending on the irrigation regime, each seedling received the following quantities of nitrogen (N), phosphorus (P), and potassium (K) over the course of their first growing season: 48.54 mg N, 14.23 mg P, and 28.86 mg K for IR-15%; 55.36 mg N, 14.23 mg P, and 28.86 mg K for IR-30%; 61.14 mg N, 14.23 mg P, and 28.86 mg K for IR-45%; 79.15 mg N, 22.90 mg P, and 38.17 mg K for IR-60%.

Seedling growth

To assess the effect of substrate water content on seedling growth after one growing season, 72 seedlings (12 seedlings/block) were randomly harvested on October 13, 1998 (Julian date 286), from each of the irrigation regime treatments. Growth variables, including height, root collar diameter, and dry mass of roots and shoots, were measured. The development of the seedlings' growth over the entire growing season is presented in Lamhamedi et al. (2001).

Shoot water-relation variables

Plant material was harvested during the afternoons of September 7, September 21, October 7, and October 20, 1998 (Julian dates 250, 264, 280, and 293, respectively), for pressure–volume (PV) analysis. Thirty shoots per regime (five shoots from each of the six blocks) were cut below the soil level and placed in plastic bags containing moist paper towels. The samples were transported to the laboratory at the Université Laval in a cooler. Upon arrival at the laboratory, the shoots were recut under water at the root collar to insure that no air was left in the xylem vessels during rehydration. They were then placed in covered containers with their lower ends submerged to a depth of 1.5 cm in water and left to rehydrate overnight (~15 h).

The following morning, the bases of the shoots were trimmed and tissue exterior to the stele was removed on the bottom 2 cm of the stem. The shoots were weighed to determine their saturated mass (SM). Immediately after weighing, when the relative water content of the shoots was judged to be 1.0 (or 100%), three shoots per irrigation regime treatment were selected at random for measurement of their equilibrium balance pressures (**P*). Each shoot was enclosed in a pressure chamber (model 600, PMS Instrument Co., Corvallis, Oreg.) with the base of its stem protruding through the airtight seal. The pressure in the chamber was increased until fluid appeared on the cut surface of the stem, indicating that the pressure in the chamber was equal to the tension with which water was being held in the xylem (**P*). Shoots with **P* greater than 0.5 MPa were rejected. The remaining shoots were left to transpire freely on the laboratory bench. In PV analysis experiments, the theoretical value of **P* = 0 is rarely obtained immediately following tissue rehydration because of incomplete rehydration of the shoot (Kandiko et al. 1980; Ritchie and Schula 1984; Koppenaal et al. 1988).

Pressure–volume analysis was conducted using the composite method described by Parker and Colombo (1995). Every 15–20 min, over the course of the day, a shoot was selected at random, weighed to determine its fresh mass, and placed in the pressure chamber to measure its **P*. Four pressure chambers were used simultaneously as described by Zine El Abidine et al. (1993, 1994*a*). One chamber was used for each of the four irrigation regimes. Each shoot was used only once. The process was repeated with subsequent shoots until **P* values were greater than 2.5 MPa. At the end of the experiment, the shoots were dried at 65°C for 48 h and then weighed to determine their dry mass.

The resulting data pairs of shoot water content (RWC) and the negative inverse of the associated balance pressures (–1/**P*) were graphed to produce a PV curve for each irrigation regime at each sampling date. Pressure–volume curve analysis software (version C) (Schulte 1998) was used to obtain the following water-relation variables: the relative tissue water content at the turgor loss point (RWC_{tlp}); tissue water potential (Ψw); osmotic potential at turgor loss ($\Psi\pi_{tlp}$) and at tissue saturation ($\Psi\pi_{sat}$); as well as the symplastic fraction (SF); modulus of elasticity (ε); and maximum turgor potential (Ψp_{max}).

The same water-relation variables were measured again at the end of the second growing season on October 6, 1999 (Julian date 279). The data obtained for the (2+0) seedlings were used in association with those of October 7, 1998, to determine whether or not the irrigation regime applied during the first growing season had an effect on the water-relation variables during the second year of growth. The data from the two sampling dates was also used to analyze the effect of seedling age on the water-relation variables.

Cuticular transpiration

At the end of the growing season (October 21, 1998), 48 seedlings (2 seedlings × 6 blocks × 4 regimes) were selected at random from the containers containing the TDR probes and subjected to artificial wilting to determine their rate of cuticular transpiration (Fukuda 1935; Hygen 1951, 1953; Kerstiens 1996). The seedlings were cut at the soil level and brought to the laboratory where the base of the stems were trimmed and then rehydrated for 11 h in darkness in a covered beaker of water. The following morning the shoots were trimmed to a length of 5.0 cm, blotted dry, and weighed to determine their saturated mass. The shoots were then left to dehydrate on the laboratory bench. Each sample was reweighed every 20 min for the duration of the experiment (430 min). The difference in fresh mass between successive measurements was assumed to be due to the amount of water lost over the time period. The cumulative amount of water lost over the elapsed time period was calculated. At the end of experiment, the excised shoots were oven-dried at 68°C for 48 h. Water loss curves (g water loss·(g plant dry mass)$^{-1}$·min^{-1}) were constructed for each irrigation regime. Cuticular transpiration rates were determined for the linear portion of the water loss curves after stomatal closure (250–430 min elapsed time) as described by Slavìk (1974*a*, 1974*b*).

Rate of bud development

In each of the six blocks, a container holding 25 healthy seedlings was chosen to be representative of each of the four irrigation regimes. On September 5, 14, 19, 28, and October 6, 20, 1998 (Julian dates 248, 257, 262, 271, 279, and 293, respectively), the percentage of seedlings that had completed bud development was noted. Bud development was considered to be complete when the bud scales of the terminal bud turned from pale yellow to light brown in colour as described for spruce seedlings (Bigras and D'Aoust 1993; Templeton et al. 1993; Bigras et al. 1996).

Number of primordia formed

The terminal shoots of three seedlings per block per irrigation regime were harvested on October 30, 1998 (Julian date 303), after all of the seedlings had completed of bud development. The seedlings were chosen from containers containing the TDR probes. The buds were dissected following the method of Templeton et al. (1991, 1993) under a stereoscopic microscope (Stemi 2000, Zeiss, Göttingen, Germany) equipped with a camera (MC 100 Spot, Zeiss, Göttingen, Germany) on November 2 and 3, 1998 (Julian dates 306 and 307). A shallow incision was made around the circumference of the stem at the base of the bud. The cap of bud scales was then removed to expose the needle primordia. The number of primordia was counted in several short rows to determine a mean number of primordia per row, this value was then multiplied by the number of short rows spiralling from the top of the bud to determine the total number of primordia present in the embryonic shoot.

Statistical analysis

All data were analyzed using the general linear methods (GLM) procedure of SAS (SAS Institute Inc., Cary, N.C.) and a significance level of $p \leq 0.05$ to determine differences between the irrigation regimes. If the effect of irrigation regime was significant, the irrigation regimes were compared using a priori orthogonal contrasts (Steel and Torrie 1980). Given that we were interested in reducing the amount of irrigation without compromising seedling quality, we chose to contrast IR-60% versus IR-45%, IR-45% versus IR-30%, and IR-15% versus the mean of IR-30% and IR-45%. To respect the $p \leq 0.05$ significance level, our experimental design allowed us to use only three orthogonal contrasts. These three contrasts were chosen because seedling growers in Quebec commonly use irrigation regimes similar to IR-60%. We chose to compare 30 and 45%, because we expected the optimum moisture contents to be within this range. The 15 versus 30 and 45% contrast allowed us to determine whether or not white spruce seedlings could be grown in a substrate with very low water content (IR-15%). Before analysis of the water-relation variables, the raw data for RWC_{tlp}, and $\Psi\pi_{sat}$ were transformed using a natural log transformation, and a $\ln(x + 1)$ transformation was used for $\Psi\pi_{tlp}$. These transformations were necessary because the data for these variables showed a non-normal distribution. The adjusted means and standard error values were retransformed for presentation. Because of the nature of the composite PV curves, only one estimate of the relevant water-relation variables was obtained per sampling date for each of the four irrigation regimes (cf. Colombo 1987; Parker and Colombo 1995).

When the water-relation variables from October 7, 1998 (1+0 seedlings), were compared with those of October 6, 1999 (2+0 seedlings), the data from the 2 years were used as repetitions to determine the effect of irrigation regime on the water-relation variables. Since the effect of irrigation regime was not significant, the four irrigation regimes from each of the two sampling dates were then used as repetitions to test the effect of sampling age on the water-relation variables.

The water loss curves for the last half of the cuticular transpiration experiment (elapsed time 250–430 min) were analyzed using a repeated measures analysis of variance. This portion of the water loss curves represents cuticular transpiration after stomatal closure (Slavík 1974*a*, 1974*b*). Repeated measures analysis was also used to analyze the rate of bud development, the polynomial coefficients being calculated by regression because of the unequal spacing of sampling dates (Yandell 1997). The needle primordia data were analyzed with the GLM analysis of variance procedure.

Results

Substrate water contents

The water content of the substrate was maintained at approximately 52% (v/v) during germination and the appearance of the first needles, as practiced in Quebec forest nurseries. Thereafter, the thrice-weekly monitoring and irrigation schedule, established 2 weeks after germination, enabled us to maintain substrate water contents in the rhizosphere at or near the desired levels (v/v) in IR-45% and IR-60% from June 29 and in IR-30% and IR-15% from July 17 and August 3, respectively (Fig. 1). In Fig. 1, each two successive data points show the substrate water content as measured before and after each scheduled irrigation and illustrate the precision with which we were able to maintain the four distinct irrigation regimes with the MP-917. During the growing season, the standard errors for the substrate water contents of the four irrigation treatments varied between 0.2 and 2.2. Sampling for the variables measured in this study occurred during the months of September and October when the substrate water contents were well established.

Growth of white spruce seedlings at the end of their first growing season

Analysis of variance showed that IR had a significant effect on height ($p < 0.0001$), root collar diameter ($p < 0.0001$), and dry mass of the shoots ($p < 0.0001$) and roots ($p < 0.0001$) at the end of the first growing season (Table 1). Orthogonal contrasts revealed that the seedlings grown under IR-15% were shorter and had smaller root collar diameters and shoot and root dry mass than those grown under the other irrigation regimes (Table 1).

Shoot water-relations during the first growing season

During their first growing season, IR did not have a significant effect on RWC_{tlp} ($p = 0.0720$) and $\Psi\pi_{tlp}$ ($p = 0.3938$), $\Psi\pi_{sat}$ ($p = 0.9102$), SF ($p = 0.2691$), maximum modulus of elasticity (ε_{max}) ($p = 0.2038$), water potential at tissue saturation (Ψw) ($p = 0.5360$), or Ψp_{max} ($p = 0.8576$) (Table 2). It was noted, however, that the seedlings grown under IR-45% were able to maintain turgor until a relative tissue water content (RWC) of 0.77, whereas the plants from IR-15%, IR-30%, and IR-60% lost turgor at RWC of 0.90, 0.87, and 0.89, respectively (Table 3).

Fig. 1. Variations in substrate water content (%, v/v) before and after thrice-weekly irrigation of air-slit containerized white spruce seedlings (1+0) grown under tunnel conditions during the 1998 growing season (mean ± SE, *n* = 6).

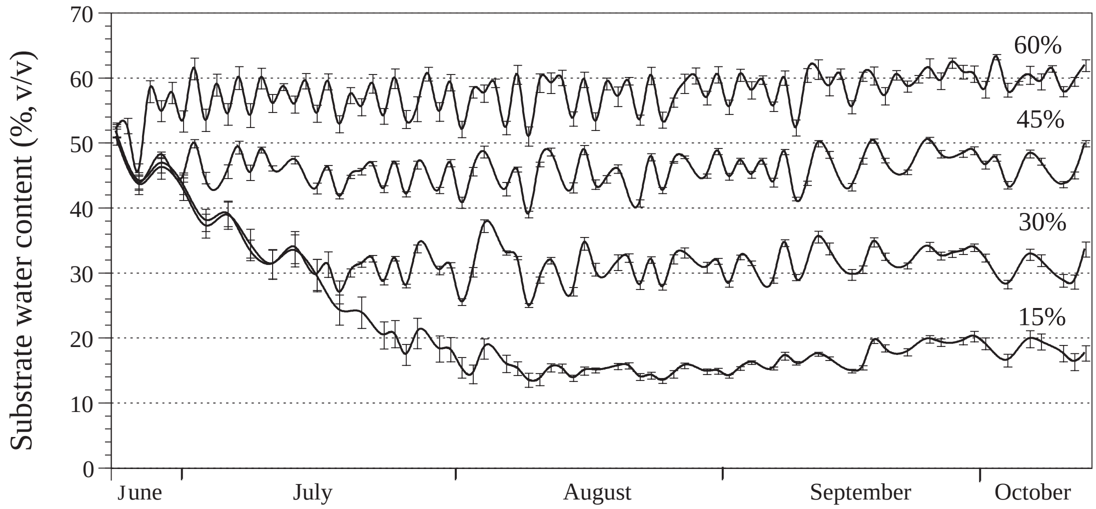


Table 1. Comparison of the (A) mean ± SE and (B) probability values of orthogonal contrasts of the morphological variables of white spruce seedlings (1+0) grown in air-slit containers under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

(A) Means ± SEs.

Irrigation regime (%, v/v)	Height (cm)	Root collar diameter (mm)	Shoot dry mass (mg)	Root dry mass (mg)	Total dry mass (mg)
IR-15	5.7±0.2	1.68±0.1	334±17	198±12	532±28
IR-30	7.2±0.4	2.02±0.1	557±32	266±12	824±43
IR-45	8.0±0.3	2.16±0.1	631±32	290±12	921±73
IR-60	8.4±0.4	2.12±0.1	644±24	270±9	914±31

(B) Orthogonal contrasts.

	Probability values				
Contrast	Height	Root collar diameter	Shoot dry mass	Root dry mass	Total dry mass
IR-30% vs. IR-45%	0.2119	0.3821	0.5366	0.6352	0.5673
IR-45% vs. IR-60%	0.8927	0.4108	0.7161	0.1978	0.5574
IR-15% vs. (IR-30% and IR-45%)	0.0001	0.0001	0.0001	0.0001	0.0001

Note: The sampling date was October 13, 1998.

Comparison of shoot water-relation variables at the end of the first and second growing seasons

In 1998, seedlings had significantly more negative $\Psi\pi_{sat}$ (p = 0.0137) and greater ε_{max} values (p = 0.0518) than in 1999 (Fig. 2). However, none of the other water-relation variables were significantly affected by either the irrigation regime applied during the first growing season or by seedling age (Table 4). The values of RWC_{tlp}, Ψw, $\Psi\pi_{tlp}$, SF, Ψp_{max} varied from 0.74 to 0.91, from –0.08 to –0.21 MPa, from –1.82 to –1.35 MPa, from 0.80 to 0.87 MPa, and from 0.92 to 1.30 MPa, respectively. The absolute values of all of the variables were greater in the younger (1+0) than in the older (2+0) white spruce seedlings.

Cuticular transpiration

Both the irrigation regime (p = 0.0076) and the length of time that the shoots were left to transpire ($p \le 0.0001$) had a significant effect on the rate of cuticular transpiration. A significant interaction between elapsed desiccation time and irrigation regime was also found (p = 0.0174) (Table 5), indicating that slopes of the four cuticular transpiration curves are not parallel. Orthogonal contrasts applied to the data showed that the rate of cuticular transpiration of IR-45% was significantly greater than IR-30% (p = 0.0144) and IR-60% (p = 0.0285) (Fig. 3, Table 5). There was no significant difference between cuticular transpiration of IR-15% and the combined means of IR-30% and IR-45% (p = 0.6726).

Rate of bud development

The rate of bud development was significantly affected by irrigation regime (p = 0.0222) and sampling date ($p \le$ 0.0001) (Table 6). The seedlings grown under IR-15% completed bud development more quickly than the seedlings grown under the other three irrigation regimes (Table 6). As

Table 2. Probability values for analyses of variance of water-relation variables of white spruce seedlings (1+0) grown in air-slit containers under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

Source	df	RWC_{tlp}	$\Psi\pi_{tlp}$	$\Psi\pi_{sat}$	SF	ε_{max}	Ψw	Ψp_{max}
Irrigation regime	3	0.0720	0.3938	0.9102	0.2691	0.2038	0.5360	0.8576
Error	12							
Total	15							

Note: The irrigation regimes were compared using the four dates as repetitions and vice versa. RWC and $\Psi\pi_{sat}$ were transformed by natural log transformation for analysis, while ln(*x* + 1) transformation was used for $\Psi\pi_{tlp}$ (n = 4). RWC, relative tissue water content; $\Psi\pi_{tlp}$, osmotic potential at the turgor loss point; $\Psi\pi_{sat}$, osmotic potential at tissue saturation; ε_{max}, modulus of elasticity at tissue saturation; SF, symplastic fraction; Ψw, total water potential; Ψp_{max}, maximum turgor potential at tissue saturation.

Table 3. Mean values of the water-relation variables for air-slit containerized white spruce seedlings (1+0) grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

	RWC_{tlp}	$\Psi\pi_{tlp}$ (MPa)	$\Psi\pi_{sat}$ (MPa)	SF	ε_{max} (MPa)	Ψw (MPa)	Ψp_{max} (MPa)
Irrigation regime							
IR-15%	0.90	–1.68	–1.52	1.09	19.51	–0.29	1.24
IR-30%	0.87	–1.83	–1.55	0.86	13.28	–0.34	1.22
IR-45%	0.772	–2.07	–1.57	0.96	9.48	–0.22	1.38
IR-60%	0.88	–1.64	–1.45	0.86	17.03	–0.25	1.23
Sampling date							
Sept. 7	0.87	–1.81	–1.54	0.97	15.36	–0.33	1.21
Sept. 21	0.86	–1.69	–1.45	1.07	12.37	–0.30	1.16
Oct. 7	0.89	–1.62	–1.40	0.84	18.94	–0.18	1.23
Oct. 20	0.83	–2.11	–1.73	0.89	13.17	–0.28	1.48

Note: The adjusted means were retransformed for presentation (n = 4), each repetition represents a composite of 30 plants per irrigation regime. The standard errors for irrigation regime were as follows: RWC_{tlp}, 0.22; $\Psi\pi_{tlp}$, 0.22; $\Psi\pi_{sat}$, 0.08; ε_{max}, 3.41; SF, 0.09; Ψw, 0.06; Ψp_{max}, 0.15. The standard errors for sampling date were RWC_{tlp}, 0.27; $\Psi\pi_{tlp}$, 0.21; $\Psi\pi_{sat}$, 0.07; ε_{max}, 3.76; SF, 0.09; Ψw, 0.06; Ψp_{max}, 0.13. RWC, relative tissue water content; $\Psi\pi_{tlp}$, osmotic potential at the turgor loss point; $\Psi\pi_{sat}$, osmotic potential at tissue saturation; ε_{max}, modulus of elasticity at tissue saturation; SF, symplastic fraction; Ψw, total water potential; Ψp_{max}, maximum turgor potential at tissue saturation. RWC and $\Psi\pi_{sat}$ were transformed by ln transformation for analysis, while ln(*x* + 1) transformation was used for $\Psi\pi_{tlp}$.

the season progressed the rate of terminal bud development slowed (Fig. 4). The interaction between irrigation regime and date was not significant (p = 0.6418), indicating a constant effect of irrigation regime on rate of terminal bud development throughout the sampling period (Fig. 4).

Number of primordia formed in the terminal buds

Irrigation regime had a significant effect on total number of primordia per bud (p = 0.0090) (Table 7). The seedlings from IR-30% had the greatest number of rows of primordia and at least 50 more primordia/bud than the seedlings from the other three irrigation regimes (Fig. 5).

Discussion

Control of substrate water content

Lower substrate water content increases aeration of the substrate, thereby improving root growth and discouraging the production of moss and pathogens (Bernier and Gonzalez 1995; Landis et al. 1989; Lamhamedi et al. 2000). The MP-917 has proven useful in controlling irrigation regimes to reach target substrate water contents as reported by Lambany et al. (1996, 1997), Gingras et al. (1999), and Lamhamedi et al. (2000). It provides precise, reproducible measurements quickly and is simple to use. The MP-917 is currently used for both container and bare-root production of seedlings in the Quebec government nurseries in Sainte-Luce, Berthier, and Grandes-Piles. The use of the substrate probes, which remain in place for the duration of the growing season, does not inhibit seedling growth and allows substrate water content to be monitored without disturbing the containers (Paquet et al. 1993; Lambany et al. 1996, 1997). If overwatering can be avoided, the costs of production (labour, fertilizers, and water) can be decreased, pollution of the water table through leaching of nutrients from the containers can be minimized, and the efficiency of fertilization and irrigation treatments can be improved.

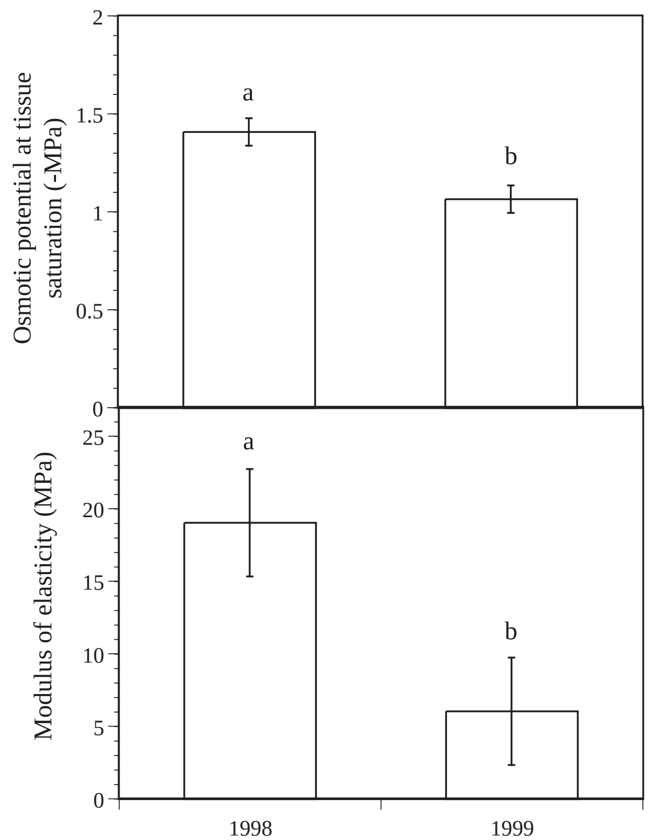
When the experimental design was installed on June 26, 1998, the water content of all of the containers was approximately 52% (v/v) (Fig. 1). The IR-60% treatment was quickly established through irrigation and within 3 days, the substrate in the containers in the IR-45% regime had dried sufficiently for this treatment to also be established. However, the substrate in the IR-30% containers did not dry to

Table 4. Probability values for water-relation variables of air-slit containerized white spruce seedlings grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) during their first growing season and submitted to standard nursery irrigation and fertilization regimes during their second growing season.

Source	df	RWC_{tlp}	$\Psi\pi_{tlp}$	$\Psi\pi_{sat}$	SF	ε_{max}	Ψw	Ψp_{max}
Irrigation regime	3	0.8928	0.7603	0.8743	0.8551	0.7123	0.4345	0.5381
Error	4							
Total	7							
Seedling age	1	0.1092	0.3952	0.0137	0.2170	0.0518	0.0875	0.2272
Error	6							
Total	7							

Note: Seedlings were grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) during the first growing season. All seedlings were subjected to the same standard nursery irrigation regime during the second growing season, but were evaluated according to the IR of the first year. RWC, relative tissue water content; $\Psi\pi_{tlp}$, osmotic potential at the turgor loss point; $\Psi\pi_{sat}$, osmotic potential at tissue saturation; ε_{max}, modulus of elasticity at tissue saturation; SF, symplastic fraction; Ψw, total water potential; Ψp_{max}, maximum turgor potential at tissue saturation.

Fig. 2. Comparison of the osmotic potential at saturation ($\Psi\pi_{sat}$) and modulus of elasticity (ε_{max}) of white spruce seedlings near the end of their first (October 7, 1998) and second (October 6, 1999) growing seasons. The air-slit containerized seedlings were grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) during their first growing season, and they were submitted to standard nursery irrigation and fertilization regimes during the second year. Means with the same letter are not significantly different at $p \leq 0.05$ (n = 4).



the targeted level until July 17, and it took until the August 3 for the substrate in the IR-15% to reach the desired water content (Fig. 1). Despite the delay in establishing IR-30% and IR-15%, the irrigation regimes had been strictly maintained at the desired levels for at least a month before sampling began in September.

Water-relation variables

The four dates chosen for the analysis of water-relation variables were at the end of the growing season when the stems of the seedlings were sufficiently lignified and had large enough root collar diameters to be inserted into the rubber gaskets of the pressure chamber. The effect of irrigation regime on RWC_{tlp} (p = 0.0720) was close to being significant at the $p \leq 0.05$ level when four sampling dates were used.

The objective of this study was to study the effect of substrate water content on the growth and physiology of white spruce seedlings over the entire first growing season as opposed to the effect resulting from a treatment applied over a short duration. Sampling at the end of the growing season enabled us to do this. Several studies published recently (Babu et al. 1999; Farrant et al. 1999) suggested that osmotic adjustment may not occur if the stress is applied too rapidly and that physiological response to water stress is a function of the rate at which the stress is applied. We monitored the progressive decrease and maintenance of substrate water content were over an entire growing season under operational conditions by time domain reflectometry. We know of no other studies that controlled the intensity of water stress within the rhizosphere directly. Other studies have used gravimetric analysis (Timmis and Tanaka 1976; Kwon and Pallardy 1989) to estimate water content and soil water potential.

The RWC_{tlp} did not vary significantly from one sampling date to another (Tables 2 and 3) and was consistent with the values obtained at the end of the growing season in other studies with white spruce (Grossnickle 1989) and black spruce (Colombo 1987). The seedlings grown under IR-45% were able to maintain turgor to a lower RWC than those from the other irrigation regimes (Table 3). The fact that IR-45% seedlings also had the most negative $\Psi\pi_{sat}$ and $\Psi\pi_{tlp}$ indicates that these plants were able to hold water more tightly within their xylem vessels during dehydration. These seedlings also had the greatest osmotic amplitude for turgor

Fig. 3. Rate of cuticular transpiration of excised shoots from white spruce seedlings (1+0) grown in air-slit containers under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v). This figure represents the linear transpiration phase, after stomatal closure. Note that standard errors varied from 1.8×10^{-4} to 2.7×10^{-4} g $H_2O\cdot$(g dry mass)$^{-1}\cdot$min^{-1} (n = 12).

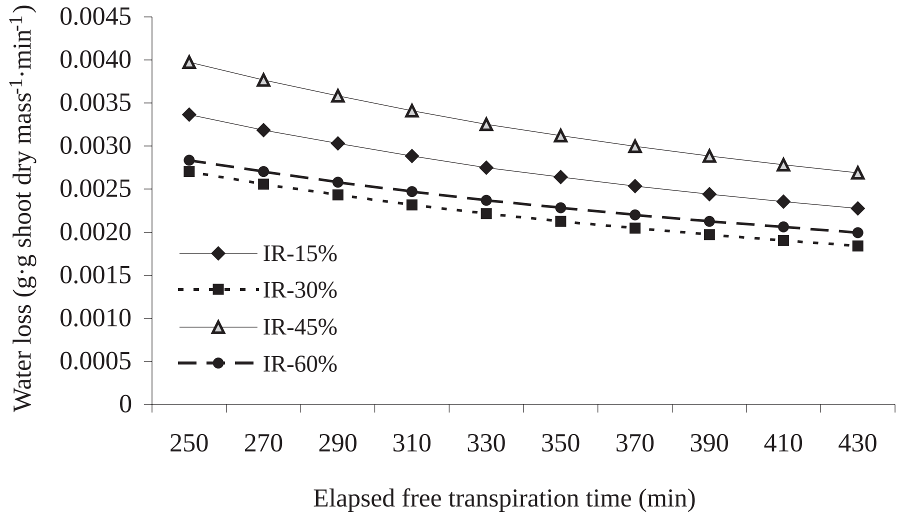


Table 5. Probability values from repeated measures analysis of variance of the cuticular transpiration of detached white spruce shoots (1+0) previously subjected to four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

Source	df	*p*
Irrigation regime (IR)	3	0.0076
Orthogonal contrasts		
IR-30% vs. IR-45%	1	0.0016
IR-45% vs. IR-60%	1	0.0062
IR-15% vs. (IR-30% and IR-45%)	1	0.9480
Desiccation time (T)	9	0.0001*
T × IR	27	0.0174*
Orthogonal contrasts		
IR-30% vs. IR-45%	9	0.0144
IR-45% vs. IR-60%	9	0.0285
IR-15% vs. (IR-30% and IR-45%)	9	0.6726

*MANOVA test criteria using Wilks' Lambda statistic.

Table 6. Analysis of variance for the rate of bud development of white spruce seedlings (1+0) subjected to four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

Source	df	*p*
Irrigation regime (IR)	3	0.0222
Orthogonal contrasts		
IR-30% vs. IR-45%	1	0.7905
IR-45% vs. IR-60%	1	0.6196
IR-15% vs. (IR-30% and IR-45%)	1	0.0082
Sampling date (D)	4	0.0001*
D × IR	12	0.6418*
Error (IR)	20	
Error (D)	80	
Total	119	

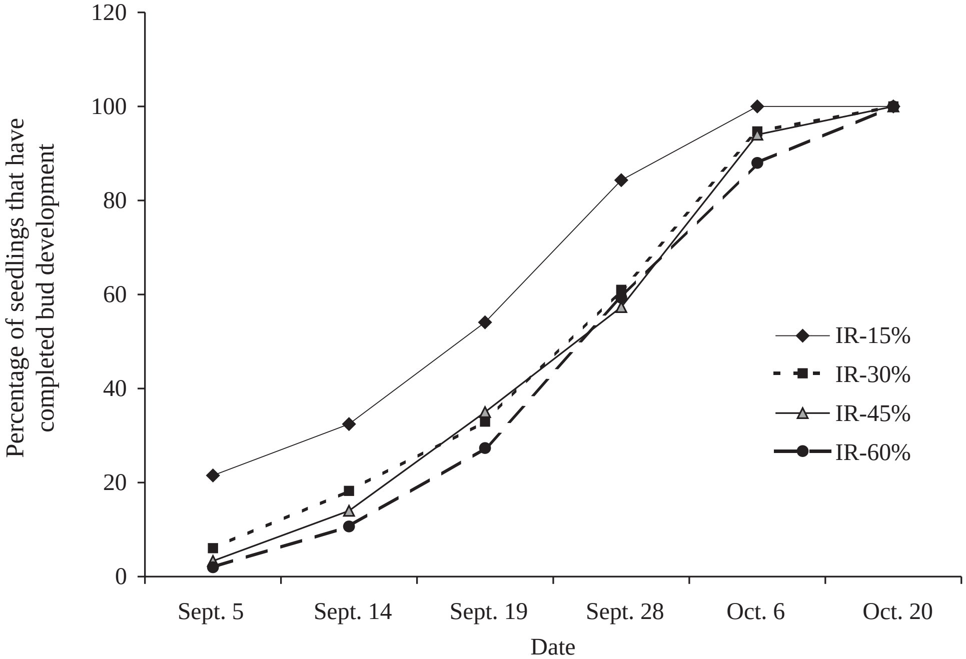
*MANOVA test criteria Wilks Lambda statistic.

maintenance ($\Delta\Psi\pi = \Psi\pi_{sat} - \Psi\pi_{tlp}$). The maintenance of the different substrate water contents had no significant effect on any of the other water-relation variables studied. Similar results were obtained by Zine El Abidine et al. (1994*b*) for both black spruce seedlings and mature trees. Bernier and Gonzalez (1995) found no difference in the water-relation variables of first-year containerized white spruce seedlings grown in peat substrates with differing rhizospheric water contents.

The SF of the seedlings grown under IR-15% exceeded the theoretical value of 1.0 (or 100%) (Table 3), although this is not uncommon in PV analysis (Richter et al. 1980; Cortes and Sinclair 1985; Colombo 1987; Parker and Colombo 1995). Parker and Pallardy (1987) suggested that this phenomenon may be due to the lack of an equilibrium in water potential between the needle and stem tissue. It may also be related to the infiltration of water into the air-filled spaces of the needle and stem tissue during rehydration (Ritchie and Schula 1984; Parker and Pallardy 1987). Overall, SF values in our study at the end of the first and second growing season were comparable with those obtained for other species such as 2-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Ritchie and Schula 1984), 1-year-old red pine (*Pinus resinosa* Ait.) (Parker and Colombo 1995), and 1-year-old white oak (*Quercus alba* L.) shoots (Parker and Pallardy 1987).

The relationship between RWC and Ψp is governed by the elasticity of the cell walls and the Ψπ of the cell (Abrams 1988). The mean ε_{max} values were higher at the end of the first growing season (18.9 MPa) than after the second growing season (6.3 MPa) (Fig. 2), indicating that the older seedlings were better able to adjust to changes in cell water content. Values of ε_{max} tend to peak during the period of maximum primordia formation (Ritchie and Schula 1984; Colombo 1987; Zine El Abidine et al. 1993). During the first growing season, ε_{max} values were higher on October 7, 1998, than on the other three sampling dates (Table 3), indicating that this date most likely fell within the period when needle

Fig. 4. Terminal bud development of white spruce seedlings (1+0) grown under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%) at the end of the first growing season. Note that the last sampling date was not included in the statistical analysis, because all of the seedlings had completed bud development; therefore, there was no variance. The standard error varied between 2.50 and 8.86% (n = 6).



primordia were being rapidly produced. This is consistent with Colombo (1997) who estimated the period of primordia formation to last about 5 weeks after bud initiation in first-year black spruce seedlings.

The water-relation variables for October 7, 1998, when the seedlings were being grown under controlled conditions in the tunnel, were used for comparison with those of October 6, 1999, after the seedlings had spent their second growing season outside under standard nursery cultural conditions. The environmental conditions in a tunnel are very different than the growing conditions outside. It is likely that the cycle of phenological development under natural conditions during the second growing season was more advanced than in the tunnel (Colombo 1997), and that the majority of primordia formation had already occurred in preparation for the third growing season. The ε_{max} values obtained after the second growing season are consistent with studies with white spruce and other conifer species (Renault and Zwiazek 1997).

The maximum modulus of elasticity coincided with the point of maximum turgor, when the tissue was fully saturated. A linear increase of ε with increasing turgor (response type I) is the type of response most often described in the literature (Roberts et al. 1981). A high ε value indicates that the cell walls are rigid. As the water content of the cell decreases, the cell walls become more elastic to maintain cell turgidity.

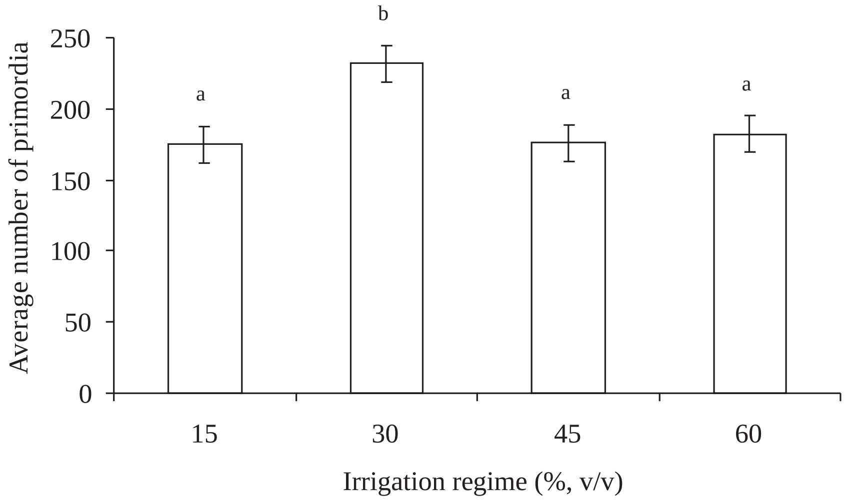
Cuticular transpiration

Rehydration of excised shoots may have artificially elevated the rate of cuticular transpiration in both IR-45% and IR-15% shoots. The seedlings grown under these irrigation regimes showed higher SF values (0.96 and 1.09, respectively) after PV analysis (Table 2) than did IR-30% (0.86) and IR-60% (0.86). Tissue rehydration may permit water to infiltrate the cellular and intercellular spaces not normally filled with water, thus affecting the saturated mass (SM) of the shoot. A proportionally large amount of water is lost when the shoots are initially exposed to ambient conditions during dehydration, as in our cuticular transpiration and PV analysis experiments (Parker and Pallardy 1987). Therefore, calculations of cumulative water loss are artificially elevated. The rate of cuticular transpiration was highest in shoots grown under IR-45%. These shoots had an average tissue water content (SM – DM/DM, where DM is dry mass) of 69% compared with a water content of 67% in the shoots from the other three irrigation regimes. Transpiration rate is related to tissue water content (Fukuda 1935; Hygen 1951, 1953). The rate of change in the cuticular transpiration rate

Table 7. Probability values ($p > F$) for primordia variables of fully formed white spruce (1+0) buds grown in air-slit containers under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v).

Source	df	Total primordia
Block	5	0.1088
Irrigation regime (IR)	3	0.0090
Orthogonal contrasts		
IR-30% vs. IR-45%	1	0.0043
IR-45% vs. IR-60%	1	0.7463
IR-15% vs. (IR-30% and IR-45%)	1	0.0793
Error	63	
Total	71	

Fig. 5. Number of needle primordial formed in the terminal buds of white spruce seedlings (1+0) grown in air-slit containers under four different irrigation regimes (IR-15%, IR-30%, IR-45%, and IR-60%, v/v) at the end of their first growing season (October 30, 1998). Means with the same letter are not significantly different at $p \leq 0.05$ (n = 18).



over the last 180 min of the experiment was similar for all of the irrigation regimes, indicating that without artificial rehydration, there may not have been a significant effect of irrigation regime on cuticular transpiration rate.

The wax covering the epicuticular surface of leaves and needles helps to protect them from desiccation and other adverse environmental conditions. The cylindrical hollow wax crystalloids found on the epicuticular needle surface of the seedlings grown in this study are described in Stowe (2001). The density of wax covering the needle surface appeared to be inversely related to the water content of the substrate in which the seedlings were grown. No consistent relationship between the amount of epicuticular wax and the rate of cuticular transpiration was found, however. Water loss through the cuticle is governed by the chemical composition on the physical structure of the waxes embedded in the cuticular matrix (Schönherr and Reiderer 1989; Kerstiens 1996).

Bud characteristics

To prepare for dormancy at the end of the growing season, a seedling diverts its energy from height growth to diameter growth, root growth, and bud development. The environmental conditions that have the greatest influence on the rate of bud development are temperature, photoperiod, seedling nutrient status, and substrate water content (Colombo 1997; Landis et al. 1999). The mean daily air temperatures in the tunnel during the period of bud development were above 25°C, the level necessary for optimum bud development (Pollard and Logan 1976). All of our seedlings were grown under the same conditions of natural daylength and temperature. Furthermore, the fertility of the substrate and the tissues were maintained at the same level for all of the irrigation regimes. Thus, the observed differences in the rate of bud development were due to substrate water content. Low substrate water contents are commonly used to induce bud development in containerized seedlings grown under conditions of natural daylength (Landis et al. 1989, 1999). Since the most rapid phase of needle primordia initiation is at the beginning of bud morphogenesis, it is during this period of time, starting about 12–14 weeks after seeding, that substrate water content is the most important (Pollard and Logan 1977). Although we did not dissect buds throughout the period of bud development to assess the degree of primordia development, we did find that the seedlings grown in substrates with the lowest water contents (IR-15%) completed bud development before the seedlings from the other three irrigation regimes (Table 6, Fig. 4).

All of the seedlings in our study completed bud development before being moved out of the tunnel during the last week of October 1998 (Fig. 4). Folk and Grossnickle (1997) cautioned against lifting and shipping interior spruce seedlings before the end of the exponential phase of primordia development, normally 5 weeks after bud initiation. Seedlings that have completed bud development before being moved outside are much more frost tolerant and are better able to withstand the stresses associated with overwintering (Koppenaal and Colombo 1988; Templeton et al. 1993; Colombo 1997).

IR-30% provided the best substrate water content for primordia development. On average, the terminal buds of the seedlings grown under this regime contained 232 primordia. This is comparable with the results obtained with the extended culture of first-year black spruce seedlings in Ontario under heated greenhouse conditions (Colombo 1997). Contrary to other studies (Colombo et al. 1989; Colombo 1997), we did not find a relationship between the length of the hardening interval and the number of primordia formed. This may have been due to the difference in species as well as the difference in environmental conditions between greenhouse and tunnel production.

Conclusions

Time domain reflectometry (MP-917) can be used on an operational scale in forest nurseries for irrigation management and monitoring substrate water content. It enables the user to sample large volumes of substrate quickly and nondestructively. The MP-917 is easy to use and provides precise, reproducible results. The irrigation regime under which air-slit containerized white spruce seedlings (1+0) were grown during their first growing season had an effect on the growth and physiology of the seedlings. Seedlings grown with a low substrate water content (IR-15%, v/v) were smaller and completed bud development earlier in the season than seedlings grown in substrates with higher substrate water contents. No significant difference was found in the height, root collar diameter, and dry mass of the shoots or roots of the seedlings grown under IR-30%, IR-45%, and IR-60%, but the formation of needle primordia was enhanced under IR-30%. The rate of cuticular transpiration was highest in seedlings grown under IR-45%. Irrigation regime had few significant effects on the water-relation variables of the seedlings during their first two growing seasons.

These results indicate that substrate water content in air-slit containers (IPL 25-350A) should be maintained at 30% (v/v) during the rapid growing phase of the first growing season of white spruce (1+0) seedlings. Decreasing the substrate moisture content to 15–20% (v/v) for a brief period approximately 12–14 weeks after seeding will initiate bud development and hardening. This will not only improve the frost tolerance of the seedlings but will also reduce winter drying effects in forest nurseries. This strategy will encourage bud development without compromising seedling growth. Because less water is necessary to maintain this irrigation strategy, leaching of fertilizers into the water table can be minimized, and the cost of fertilization and irrigation practices can be reduced.

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