

Interior spruce seedlings compared with emblings produced from somatic embryogenesis. III. Physiological response and morphological development on a reforestation site

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Received May 10, 1993

Accepted December 21, 1993

GROSSNICKLE, S.C., and MAJOR, J.E. 1994. Interior spruce seedlings compared with emblings produced from somatic embryogenesis. III. Physiological response and morphological development on a reforestation site. *Can. J. For. Res.* 24: 1397–1407.

Interior spruce (*Picea glauca* (Moench) Voss \times *Picea engelmannii* Parry) seedlings and emblings produced through somatic embryogenesis tissue culture were planted on a reforestation site in the central interior of British Columbia. Gas exchange and water relations patterns were monitored over the first growing season and morphological development was monitored over two growing seasons. During shoot elongation, osmotic potential at saturation (Ψ_{sat}) and turgor loss point (Ψ_{tlp}) of seedlings and emblings increased, while their maximum bulk modulus of elasticity (ϵ_{max}) and total turgor (Ψ_{Ptotal}) decreased, resulting in utilized turgor (Ψ_{Putil}) exceeding 100%. From bud set in late July through October 8, Ψ_{sat} and Ψ_{tlp} decreased, Ψ_{max} and ϵ_{Ptotal} increased, with Ψ_{Putil} between 55 and 70% for both seedlings and emblings. There were few seasonal differences in shoot water relations parameters of seedlings and emblings. One-year-old and current-year needle conductance (g_{wv}) decreased as vapour pressure deficit (VPD) increased in a similar manner for both seedlings and emblings. Response surface models for net photosynthesis (P_n) of current-year needles showed P_n to increase as photosynthetically active radiation (PAR) increased to around 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and thereafter remained stable, but as VPD increased P_n decreased at all PAR levels. Emblings, compared with seedlings, had 15% greater P_n under optimal field site atmospheric conditions (i.e., VPD < 3.0 kPa and PAR > 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). There was a linear increase in P_n as g_{wv} increased in both seedlings and emblings, though 1-year-old needles had higher P_n for a given level of g_{wv} than current-year needles. Height and diameter growth across the first and second growing seasons were similar for seedlings and emblings. Seedlings and emblings had similar total root weight after two growing seasons. After the first winter on the field site, survival was 87% for seedlings and 91% for emblings, while emblings had a lower proportion of their foliage damaged by winter conditions. Results indicate that seedlings and emblings were largely comparable in their field performance over two growing seasons on a reforestation site.

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Nous avons planté des semis et des plants issus d'embryogénèse somatique d'épinette de l'intérieur (*Picea glauca* (Moench) Voss \times *Picea engelmannii* Parry) sur un site de reboisement dans le centre de la Colombie-Britannique. Les patrons d'échange gazeux et de relations hydriques ont été suivis au cours de la première saison de croissance et le développement morphologique a été suivi au cours des deux premières saisons de croissance. Au cours de la période d'élongation des tiges, les potentiels osmotiques à saturation (Ψ_{sat}) et au point de perte de turgescence (Ψ_{tlp}) des deux types de plants se sont accrus alors que le modulus d'élasticité volumétrique maximal (ϵ_{max}) et le potentiel de pression de turgescence total (Ψ_{Ptotal}) ont décliné, résultant en une turgescence utilisée (Ψ_{Putil}) de plus de 100%. De la mise en place des bourgeons, en fin juillet, jusqu'au 8 octobre, Ψ_{sat} et Ψ_{tlp} ont décliné alors que ϵ_{max} , Ψ_{Ptotal} et Ψ_{Putil} se sont accrus de 55 à 70% chez les deux types de plants. Il n'y a eu que peu de différences de relations hydriques des tiges entre les semis et les plants d'origine somatique. La conductance (g_{wv}) des aiguilles de l'année et des aiguilles âgées de un an a décliné de façon similaire chez les deux types de plants en réponse à un accroissement du déficit de pression de vapeur (VPD). L'analyse des surfaces de réponse de la photosynthèse nette (P_n) des aiguilles de l'année montre un accroissement de P_n jusqu'à un rayonnement photosynthétiquement actif (PAR) de 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ et un plafonnement par la suite, mais une baisse de P_n à tous les niveaux de PAR suite à un accroissement de VPD. Le P_n des plants d'origine somatique était de 15% supérieur à celui des semis en conditions de terrain optimales (VPD < 3,0 kPa et PAR > 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). L'accroissement de P_n était relié linéairement à celui de g_{wv} chez les deux types de plants; pour une valeur de g_{wv} donnée, le P_n des aiguilles de un an était plus élevé que celui des aiguilles de l'année. Les croissances en hauteur et en diamètre des deux premières saisons de croissance étaient similaires chez les semis et chez les plants d'origine somatique. Les masses racinaires des deux types de plants étaient similaires après ces deux saisons. Après le premier hiver au champ, les taux de survie étaient respectivement de 87% et de 91% chez les semis et les plants d'origine somatique; le pourcentage de dommage foliaire hivernal était moins élevé chez les plants d'origine somatique. Les résultats indiquent des performances au champ comparables des semis et des plants d'origine somatique après deux saisons de croissance sur un site de reboisement.

[Traduit par la rédaction]

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Introduction

Somatic embryogenesis is an asexual propagation method involving the recapitulation of normal processes of embryo development using tissue culture procedures. This method has been successfully applied to interior spruce (*Picea glauca* (Moench) Voss \times *Picea engelmannii* Parry) to produce plant propagules called emblings (Roberts et al. 1990; Cyr et al. 1991). It is now possible to mass propagate interior spruce emblings through somatic embryogenesis tissue culture protocols (Webster et al. 1990).

Forestry organizations are interested in knowing whether spruce seedlings and emblings have comparable field performance on reforestation sites. However, there is no published information on the field performance of conifers produced through somatic embryogenesis (Tautorus et al. 1991). The only reported field trials are on tissue culture derived plants produced through organogenesis, or micropropagation (reviewed by Gupta et al. 1991). If emblings have good performance capability within forest regeneration operations, then somatic embryogenesis can be an effective technology for mass clonal propagation in reforestation programs.

Seedlings and emblings planted on reforestation sites can be exposed to a wide array of environmental conditions. Growing season conditions, including alterations in heat exchange processes and site water relations, will influence plant water relations and gas exchange processes (Miller 1983). Low temperatures can reduce a plant's capability to survive winter conditions on reforestation sites (Sakai and Larcher 1987). Comparative information on the physiological response and subsequent morphological development of newly planted interior spruce seedlings and emblings to reforestation site conditions will improve our understanding of their establishment capability.

In the first paper of this series, interior spruce seedlings and emblings were monitored during nursery development, fall acclimation, and frozen storage (Grossnickle et al. 1994). Though emblings were smaller than seedlings, all other measurements indicated that seedlings and emblings were in the desired physiological state when lifted and placed in frozen storage. In the second paper of this series, a stock quality assessment procedure was conducted on seedlings and emblings just prior to field planting (Grossnickle and Major 1994). Seedlings had better field performance potential than emblings under optimal environmental conditions, while seedlings and emblings had similar field performance potential under low-temperature and drought conditions. This paper examines the physiological and growth responses of interior spruce seedlings and emblings over two growing seasons on a reforestation site in the central interior of British Columbia.

Materials and methods

Plant material

Seed used to produce seedlings and embryogenic cultures came from interior spruce, a natural hybrid of white spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*Picea engelmannii* Parry) from the interior of British Columbia. Seedlings and emblings came from wild population collections of seed (open-pollinated families 81, 103, and 118 were original wild population collections from trees within a 15-km river valley) from a progeny trial located in the north-central interior of British Columbia (53°N, 122°W). Results from the 10-year progeny trial and final nursery shoot height measurements on

seedlings and emblings from the individual families showed a 5–7% difference in shoot growth among these families, with no family consistently having greater or less shoot growth at all measurement periods. Details on shoot growth data, rankings, and numbers of plants produced per open-pollinated family are found in Grossnickle et al. (1994; Table 1). Specific information on the progeny trial is described in Kiss and Yeh (1988).

Identical individual genotypes of seedlings and emblings were not tested in this research program. This was due to the inherent difficulty in producing an adequate number of emblings from certain clones within a family (Webster et al. 1990). It is recognized that families were unequally represented in the embling population, which can potentially result in apparent embling–seedling differences simply due to differential genotype sampling. Although identical individual genotypes of seedlings and emblings were not tested in this research program, small differences in shoot growth, which is indirectly related to shoot growth patterns (i.e., similar adaptability), and the close proximity of the original seed collection sites indicates that these families were of comparable genetic origin. In addition, continuous morphological and physiological testing of repeated random samples were taken from the larger sample population over this portion of the research program. This ensured a greater probability of balanced genetic representation for results between seedlings and emblings. Owing to the morphological similarity of test population families, as well as the extensive population sampling approach, this differential genotypic sampling problem was considered minor.

Seed used for producing 1050 seedlings came from the same open-pollinated families used for embling production. A total of 968 emblings were produced from nine interior spruce clones of the three open-pollinated families. For seedlings or emblings, seed or young emblings were randomly planted across the styrofoam blocks. This approach ensured that all testing of seedlings and emblings, throughout the entire research program, came from randomly selected plants of all three open-pollinated families.

Specifics on nursery culture and greenhouse experimental design are described in Grossnickle et al. (1994), with a brief description presented below. Emblings were produced through somatic embryogenesis protocols described in Webster et al. (1990) and briefly in Grossnickle et al. (1994). Emblings were produced in the laboratory during the 1989–1990 winter and transferred to the nursery in early April 1990. Seed was sown in mid-February 1990, and both seedlings and emblings were grown in 313B styroblocks® (Beaver Plastics Ltd.) at Pelton Reforestation Ltd. Maple Ridge, British Columbia (49°18'N) from early April until mid-August in a greenhouse under optimal conditions to ensure rapid development. In mid-August, the nursery cultural regime was modified to ensure acclimation to fall conditions. Seedlings and emblings were lifted and placed in frozen storage (–2°C) on December 3, 1990, until just before planting in mid-June 1991.

Field site conditions

The field site was located at the University of British Columbia's Alex Fraser research forest, on the interior plateau of British Columbia, Canada (52°28'N, 122°41'W, elevation 1020 m). The site was clear-cut logged in the mid 1980s and site prepared with blade scarification in October 1990. Only sparse low lying vegetation was on the site during the 1991 and 1992 growing seasons. Soil on the site had a 10–20 cm organic layer overlying a clay to clay-loam down to 50 cm. The biogeoclimatic zone is defined as interior cedar–hemlock; moist cool (ICH_{E2}) site (Klinka et al. 1984).

Seedlings and emblings were planted June 10 and 11, 1991, in two blocks, in 10 randomly selected rows per block with 10 plants planted in a 1 \times 1 m spacing per row, for a total of 200 seedlings and 200 emblings.

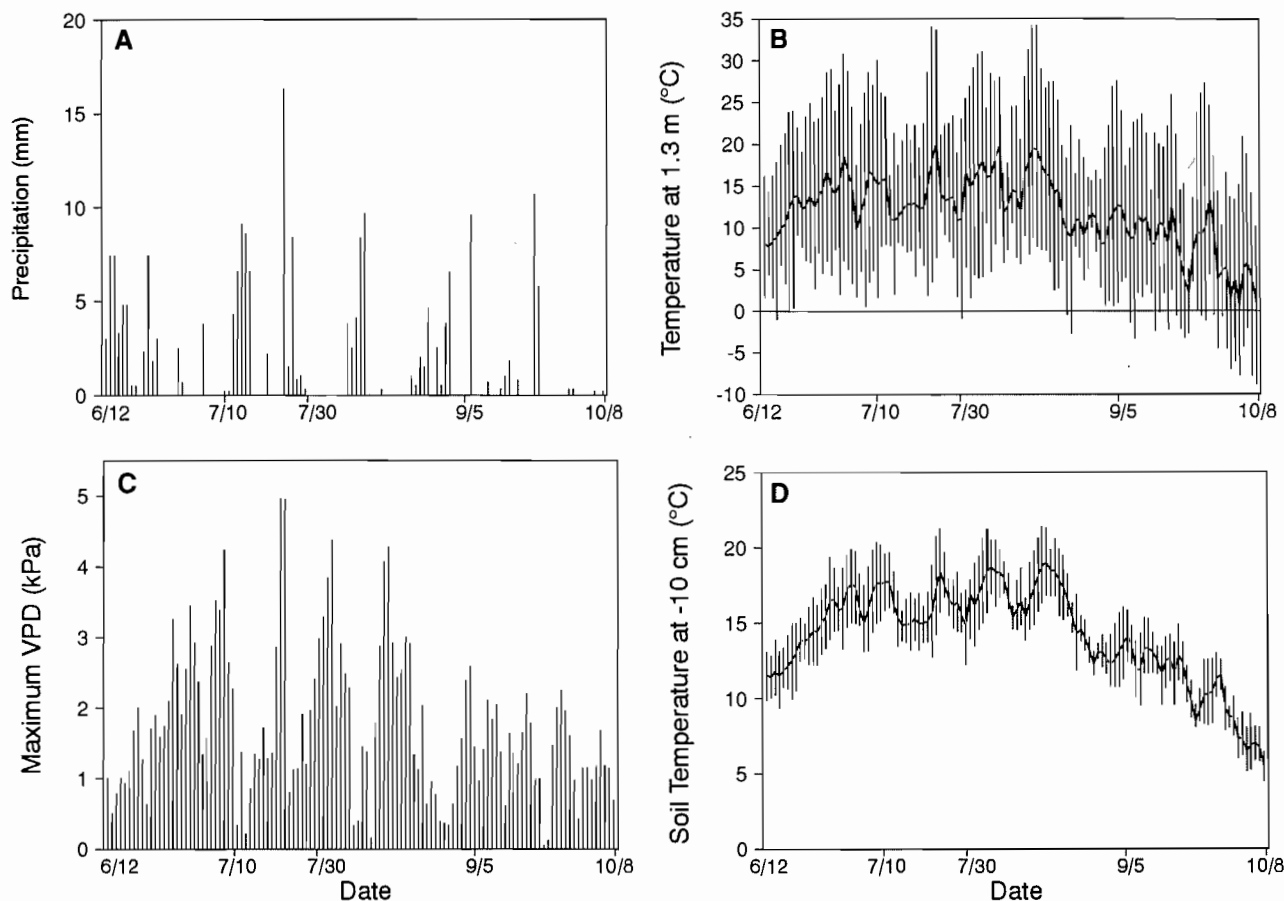


FIG. 1. Reforestation site environmental conditions for (A) precipitation, (B) air temperature at 1.3 m, (C) daily maximum vapour pressure deficit (VPD), and (D) soil temperature at -10 cm during the 1991 growing season. Temperature data are represented by the daily minimum and maximum values (vertical lines) and daily averages connected across the season by a line. Dates on the x -axis indicate days when physiological measurements were taken.

Measurement of site environmental conditions

Field site environmental data were recorded from June 12, 1991, through September 15, 1992, with a data logger (21x Campbell Scientific Inc.). Environmental variables recorded were air temperature at 1.3 m above the soil surface and at mid-plant height (10 cm), soil temperature (-10 cm), relative humidity at plant height, photosynthetically active radiation (PAR), and precipitation. All readings, except precipitation, were collected on a 10-s basis. Air temperature at 1.3 m and soil temperature at -10 cm were averaged hourly, and are reported as mean, maximum, and minimum daily values during the 1991 growing season. Vapour pressure deficit (VPD) was determined from air temperature and relative humidity at plant height, with an assumed 100% relative humidity inside the stomatal antechamber. Daily maximum VPD is reported throughout the 1991 growing season. Daily precipitation was measured at a nearby weather installation and reported on a daily basis.

Measurement of physiological data

Shoot water potential (Ψ) was measured on individual branch tips using a pressure chamber (Soil Moisture Corp. Model 3005) according to Ritchie and Hinckley (1975). Net photosynthesis (P_n) and needle conductance (g_{wv}) were measured with a LI-6200 (LI-COR Inc.) gas exchange system and a 250 mL (LI-6200-13) sample chamber. Both VPD and PAR measurements associated with each reading were taken simultaneously by the gas-exchange system.

Predawn (Ψ_{pd}) and the assumed daily minimum (Ψ_{min}) shoot water potentials were measured on six seedlings and six emblings,

randomly selected, during each data collection period from June through October 1991 (June 12, July 10 and 30, September 5, and October 8). Measurement of Ψ_{pd} was determined at 07:00 on plants darkened overnight with vented aluminum cones and Ψ_{min} was determined at 13:00. A measurement of Ψ_{pd} was not collected on October 8 due to overnight and morning freezing temperatures.

During each data-collection period, six seedlings and six emblings, randomly selected, were excavated and transferred in 4-L pots to the laboratory for pressure-volume analysis (completed within 4 days). Plants in the 4-L pots were rehydrated in a tub containing 4 cm of water, sealed in a large plastic bag, and placed in the dark in a controlled-environment room (22°C air temperature). After 14 h in the dark, lateral shoots were removed from the potted plants and saturated weight measured. Pressure-volume curves were determined by measurements of shoot mass and water potential at periodic intervals, while, between measurements, shoots transpired in the controlled environment room (22°C air temperature, 50% relative humidity, and $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) using standard procedures (Hinckley et al. 1980; Grossnickle 1989). Dry weights of shoots were measured after oven drying at 65°C for 48 h. Shoots consisted of 1-year-old needles for pressure-volume analysis on June 12, 1-year-old and current-year needles on July 10, and current-year needles on July 30, September 5, and October 8.

Gas-exchange data were collected from June through October during the 1991 growing season on the same days, and on the same six seedlings and six emblings used for pressure-chamber measurements described above. After Ψ_{pd} measurements were taken at 07:00, gas exchange was measured starting at approxi-

TABLE 1. Predawn (Ψ_{pd}) and minimum (Ψ_{min}) shoot water potentials (MPa), and total turgor (Ψ_{Ptotal} ; reforestation)

	June 12				July 10			
	Ψ_{pd}	Ψ_{min}	Ψ_{Ptotal}	Ψ_{Putil}	Ψ_{pd}	Ψ_{min}	Ψ_{Ptotal}	Ψ_{Putil}
Seedling	$-0.44 \pm 0.08a^*$	$-1.18 \pm 0.08a$	$12.5 \pm 1.0b$	72	$-0.61 \pm 0.04a$	$-1.23 \pm 0.04a$	$4.9 \pm 0.2a$	>100
Embling	$-0.50 \pm 0.09a$	$-1.13 \pm 0.11a$	$14.6 \pm 0.6a$	58	$-0.63 \pm 0.03a$	$-1.24 \pm 0.05a$	$5.3 \pm 0.2a$	>100

NOTE: Values are means \pm SE.*A difference in letter between seedlings and emblings indicates a significant difference at $p = 0.05$ as determined by a t -test.

†Data not reported owing to freezing temperatures.

mately 08:00 and every 2–2.5 h thereafter until approximately 19:00. Gas-exchange samples consisted of 1-year-old needles on June 12 and July 10, and current-year needles on July 30, September 5, and October 8. After each day of data collection, samples were clipped, needle surface area was indirectly determined with an area meter (LI-3100, LI-COR Inc.), then multiplied by four to estimate total needle surface area, and gas-exchange measurements were then recalculated with estimated surface areas (Larsen and Kershaw 1991). Though interior spruce needles can be more rhomboid than square in cross section, the above approach gave representative needle area values that were comparable among samples.

On July 30 and September 5, additional gas-exchange data were collected over the summer range of PAR and VPD conditions. Shade racks at varying levels of opaqueness provided a full range of PAR conditions. Shade racks were placed over an additional group of randomly selected plants (nine seedlings and nine emblings) in the evening before measurements were taken and kept in this environment throughout the entire sample day. These additional gas-exchange measurements were collected from 08:00 to 15:00.

Measurement of morphological data

Three or four randomly selected seedlings or emblings in each row were planted in buried cylindrical (25 cm diameter and 30 cm length) porous felt bags specifically for morphological measurements. This facilitated removal of 25 seedlings and 25 emblings at 1, 4, and 16 months after planting. Morphological parameters measured were: (i) shoot height; (ii) new height growth; (iii) root collar diameter; (iv) new, (v) old, and (vi) total shoot dry weight; root dry weight (vii) in container plug, (viii) in soil, and (ix) in total. From the morphological data, the following parameters were calculated: (i) plant water balance ratio (total shoot dry weight/(root-collar diameter \times root dry weight)), and (ii) soil-root to root plug ratio. Incremental height and diameter growth during the first growing season (1991) was measured on the same days as physiological measurements described above. Mortality in the entire field population was determined on October 10, 1991, June 2, 1992, and September 15, 1992. On June 2, 1992, the entire field population was assessed for 1st-year winter injury by determining the percentage having their terminal bud alive (TB_a) and estimating the percentage of needles that had turned brown for each plant.

Data evaluation and analysis

Gas exchange response patterns of seedlings and emblings were determined for 1-year-old (combined data from June 12 and July 10) and current-year (combined data from July 30 and September 5) needles during the 1991 growing season. Gas-exchange response on October 8, 1991, was examined independently owing to overnight and morning freezing temperatures.

Boundary line analysis was used to determine maximum P_n and g_{wv} response to VPD. Field gas exchange measurements are simultaneously influenced by an array of environmental factors. Thus, when P_n or g_{wv} is plotted against another single independent environmental variable, a scatter diagram results. Webb

(1972) credited the scatter of points below the boundary line to errors in measurement, variability of biological data, and interaction with other controlling or limiting factors. The premise of boundary-line analysis is that a scatter diagram's upper limit of gas-exchange measurements in response to an independent environmental variable, known to limit P_n or g_{wv} , indicates the maximum gas exchange response to that particular environmental variable when other conditions are not limiting (Jarvis 1976). Validity of this premise depends on sufficient gas exchange data to describe the upper limit and the assumption of no measurement errors for points which form the upper boundary.

For the response of g_{wv} to VPD, measurements were systematically taken from the upper region of a scatter diagram. The range of VPD measured was partitioned into 0.25 kPa segments with the greatest two to five g_{wv} measurements from that segment included in the maximum response data set (i.e., 35–50% of the entire data set). For P_n , any measurements that were taken when PAR was $<160 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ were removed from the data set. This ensured that very low PAR levels were not limiting P_n response. Maximum response data sets for P_n in relation to VPD or g_{wv} included 75–80% of the entire data set. This resulted in maximum gas exchange response data sets for statistical analysis (Webb 1972). This procedure reduced the tendency for individual data points to force the curve upward causing an overestimation of the response line (Chambers et al. 1985). Response models were developed using linear or nonlinear regression analysis (Kleinbaum et al. 1988). Model selection was determined by including significant ($p \leq 0.05$) variables that contributed to the highest r^2 . Further details on this boundary line analysis procedure are found in Grossnickle and Arnott (1992).

In the field, PAR and VPD continually change in an interdependent fashion. Thus, the relationship of current-year needles, P_n to both PAR and VPD was examined using response surface models. Multivariate linear least squares regression analysis on the entire data set for seedlings or emblings ($n = 150$) determined their P_n response to both PAR and VPD. Model selection was determined as described above.

Pressure-volume curves defined the osmotic potential at saturation (Ψ_{sat}) and turgor loss point (Ψ_{tlp}), maximum bulk modulus of elasticity (ϵ_{max}), symplastic fraction (SF), and relative water content at turgor loss point (RWC_{tlp}) (Schulte and Hinckley 1985). Höfler diagrams were used to determine total turgor (Ψ_{Ptotal}) and utilized turgor (Ψ_{Putil}) where turgor is defined as the appropriate area beneath the turgor pressure to RWC curve (Roberts and Knoerr 1977; Grossnickle 1988; Colombo and Teng 1992). Total turgor is the turgor integral from RWC at full saturation to RWC_{tlp} (Roberts and Knoerr 1977). Both Ψ_{pd} and Ψ_{min} were located on the total water potential line of the Höfler diagrams to determine the shoot RWC at which Ψ_{pd} and Ψ_{min} occurred. Available turgor is the integral of turgor from RWC at Ψ_{pd} to RWC_{tlp}, divided by Ψ_{Ptotal} and multiplied by 100. Utilized turgor is the integral of turgor from RWC at Ψ_{pd} to RWC at Ψ_{min} , divided by available turgor and multiplied by 100 (Grossnickle 1988).

A t -test ($p = 0.05$) determined the significant differences between shoot water relations parameters, and Ψ_{pd} and Ψ_{min} of

MPa·%) and utilized turgor pressure (Ψ_{util} ; %) for interior spruce seedlings and emblings over one growing season on a site

July 30				September 5				October 8			
Ψ_{pd}	Ψ_{min}	Ψ_{Total}	Ψ_{util}	Ψ_{pd}	Ψ_{min}	Ψ_{Total}	Ψ_{util}	Ψ_{pd}	Ψ_{min}	Ψ_{Total}	Ψ_{util}
$-0.76 \pm 0.10a$	$-1.13 \pm 0.04a$	$7.7 \pm 0.2a$	67	$-0.48 \pm 0.04a$	$-1.20 \pm 0.07a$	$12.7 \pm 0.5a$	54	$-0.76 \pm 0.10a$	$-1.45 \pm 0.08a$	$14.2 \pm 0.2a$	$-0.76 \pm 0.10a$
$-0.59 \pm 0.08a$	$-1.06 \pm 0.12a$	$8.1 \pm 0.4a$	71	$-0.54 \pm 0.04a$	$-1.35 \pm 0.07a$	$10.4 \pm 1.0b$	69	$-0.59 \pm 0.08a$	$-1.31 \pm 0.14a$	$14.9 \pm 0.6a$	$-0.59 \pm 0.08a$

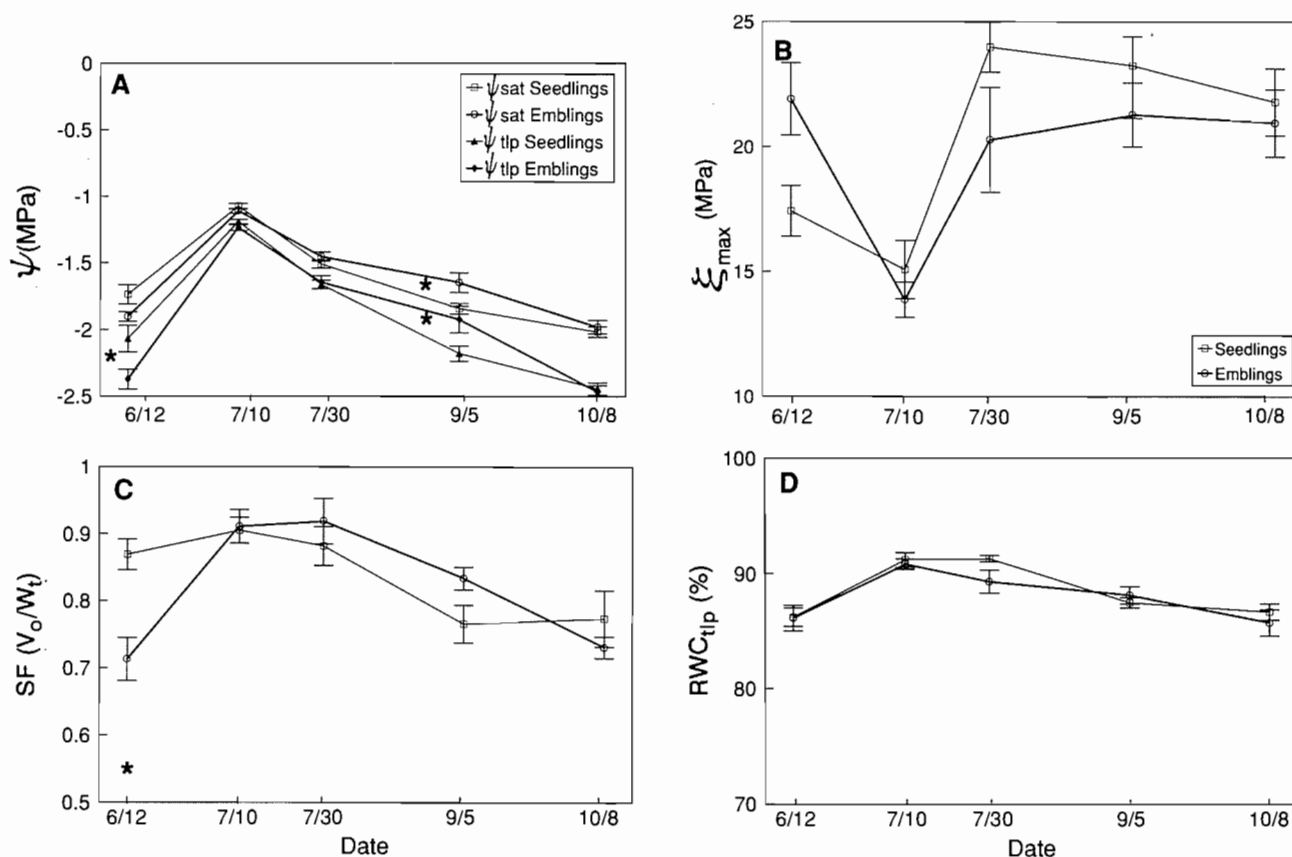


FIG. 2. Seasonal patterns (mean \pm SE) of interior spruce seedlings and emblings: (A) saturated (Ψ_{sat}) and turgor loss point (Ψ_{tlp}) osmotic potentials, (B) maximum bulk modulus of elasticity (ϵ_{max}), (C) symplastic fraction (SF), and (D) relative water content at turgor loss point (RWC_{tlp}) during the first growing season on a reforestation site. Significant difference between seedlings and emblings determined by a *t*-test ($p = 0.05$).

seedlings and emblings at each data-collection period (Steel and Torrie 1980). Differences between seedlings and emblings for each morphological parameter measured at 1, 4, and 16 months after planting were determined with a *t*-test ($p = 0.05$).

Results and discussion

Field site environmental conditions

During the 1991 growing season, precipitation was frequent, with no extended rainfall-free periods (Fig. 1A). From June through August, minimum air temperature occasionally dropped below 0°C , while after September 1, minimum air temperatures were regularly below 0°C (Fig. 1B). Mean air temperature was above 10°C for most of the growing season. Maximum VPD peaked in late July and then declined throughout the remainder of the growing season (Fig. 1C). Low daily maximum VPD coincided with periods of precipitation. Soil temperature was between 10 and 20°C from

June through mid-September and between 5 and 10°C thereafter (Fig. 1D).

Shoot water relations

Seedlings and emblings had similar seasonal Ψ_{pd} values, which ranged from -0.44 to -0.76 MPa and -0.50 to -0.63 MPa, respectively (Table 1). These high Ψ_{pd} values reflect frequent seasonal precipitation. Seasonal Ψ_{min} values ranged from -1.13 to -1.45 MPa for seedlings and -1.06 to -1.35 MPa for emblings. These relatively high Ψ_{min} values reflect the moderate air temperatures (Fig. 1B) and maximum VPD (Fig. 1C) noted during the growing season.

Seasonal changes in shoot water relations parameters corresponded with shoot growth and field site environmental patterns. Emblings, compared with seedlings, had lower Ψ_{tlp} (Fig. 2A) and SF (Fig. 2C), and a higher ϵ_{max} (Fig. 2B) on June 12. The lower SF along with a higher dry weight frac-

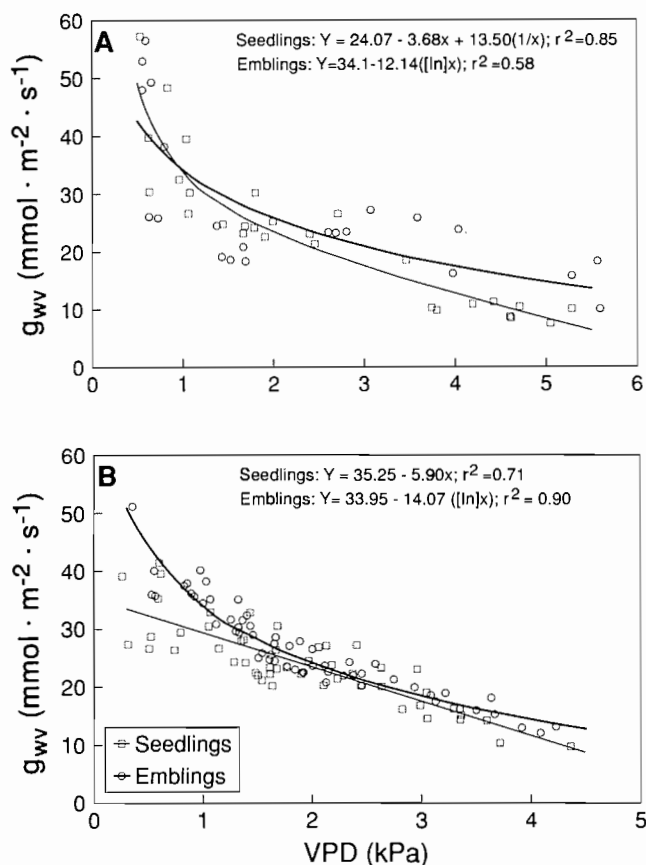


FIG. 3. Needle conductance (g_{wv}) in response to vapour pressure deficit (VPD) for interior spruce seedlings and emblings (A) 1-year-old needles and (B) current-year needles during the first growing season on a reforestation site.

tion (DWF) of emblings (Grossnickle and Major 1994) contributed to their lower Ψ_{tlp} in the absence of differences in Ψ_{sat} . Higher DWF with an accompanying decrease in symplastic volume causes passive osmotic adjustment through the concentration of a similar content of existing solutes (Doi et al. 1986). Emblings also had greater Ψ_{Ptotal} than seedlings (Table 1). The parameter Ψ_{Ptotal} is considered a more comprehensive indicator of turgor maintenance capacity for white spruce than either osmotic potential or cell elasticity considered separately (Grossnickle 1988; Colombo and Teng 1992).

During the period of shoot elongation (July 10), seedlings and emblings had a similar increase in Ψ_{sat} , Ψ_{tlp} , SF, and RWC_{tlp} , and a decrease in ϵ_{max} (Fig. 2). This increase in Ψ_{sat} and Ψ_{tlp} immediately following spring bud break is typical for white spruce (Grossnickle 1989; Colombo and Teng 1992). Low ϵ_{max} during shoot elongation indicates tissue with highly elastic properties, while high SF values are due to increases in cell osmotic volume, without accompanying increases in cell dry weight. These changes in shoot water relation parameters, during the time plants went from a dormant to an active growing state, caused Ψ_{Ptotal} to decrease by 61% for seedlings and 64% for emblings (Table 1) and this commonly occurs during bud break and shoot elongation in white spruce (Grossnickle 1988; Colombo and Teng 1992). As a result of this decrease in Ψ_{Ptotal} , Ψ_{Putil} exceeded 100% (i.e., $\Psi_{Putil} \geq 100\%$ indicates turgor loss) on July 10, even though Ψ_{min} was only -1.23 and -1.24 MPa for seedlings and emblings, respectively (Table 1). Field-planted white

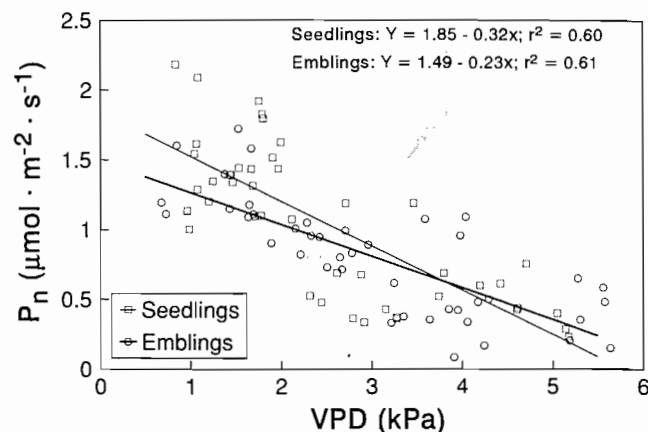


FIG. 4. Net photosynthesis (P_n) in response to vapour pressure deficit (VPD) for 1-year-old needles of interior spruce seedlings and emblings during the first growing season on a reforestation site.

spruce seedlings can have Ψ_{Putil} exceed 100% during shoot elongation (Grossnickle 1988). These seasonal shifts in shoot water relations parameters during shoot elongation makes interior spruce seedlings and emblings susceptible to drought stress just after planting.

Seedlings and emblings had a similar decrease in Ψ_{sat} , Ψ_{tlp} , SF, and RWC_{tlp} , and an increase in ϵ_{max} from July 10 throughout the rest of the growing season (Fig. 2). This change in shoot water relations parameters coincided with the cessation of height growth in July (Fig. 7A) and the steady decline in site temperatures during August and September (Fig. 1). This is a typical growing season pattern for Ψ_{sat} and Ψ_{tlp} in white spruce (Grossnickle 1988, 1989; Colombo and Teng 1992). High ϵ_{max} after bud set is indicative of mature tissue characterized by rigid cell walls (Cheung et al. 1975). A similar late growing season decline in SF and a high RWC_{tlp} , which only declined slightly after bud set, has been found in white spruce (Grossnickle 1989). As a result of these changes in shoot water relations parameters, Ψ_{Ptotal} increased throughout the remainder of the growing season and Ψ_{Putil} was between 54 and 67% for seedlings and between 69 and 71% for emblings (i.e., $\Psi_{Putil} < 100\%$ indicates turgor maintenance). A similar increase in Ψ_{Ptotal} after bud set occurs in white spruce (Grossnickle 1988; Colombo and Teng 1992).

Gas exchange measurements

For seedlings and emblings, g_{wv} of 1-year-old needles had a concave decrease as VPD increased (Fig. 3A). Current-year needles of emblings had a curvilinear decrease in g_{wv} , while g_{wv} of seedlings declined in a linear fashion with increasing VPD (Fig. 3B). This curvilinear to linear decrease in g_{wv} with increasing VPD occurs in white (Goldstein et al. 1985; Grossnickle and Blake 1987) and Engelmann (Kaufmann 1976, 1982; Grossnickle and Reid 1985) spruce.

For seedlings and emblings, P_n decreased as VPD increased for 1-year-old needles (Fig. 4). Sitka spruce (*Picea sitchensis* (Bong. Carr.) also has a similar decrease in P_n with increasing VPD (Sanford and Jarvis 1986). For VPD conditions < 2.5 kPa, seedlings had slightly higher P_n than emblings. Seedlings had greater P_n than emblings just prior to field planting under controlled-environment testing in optimum (22°C) and low (7.5°C) root temperature conditions (Grossnickle and Major 1994). Most of these higher field

Seedlings

Emblings

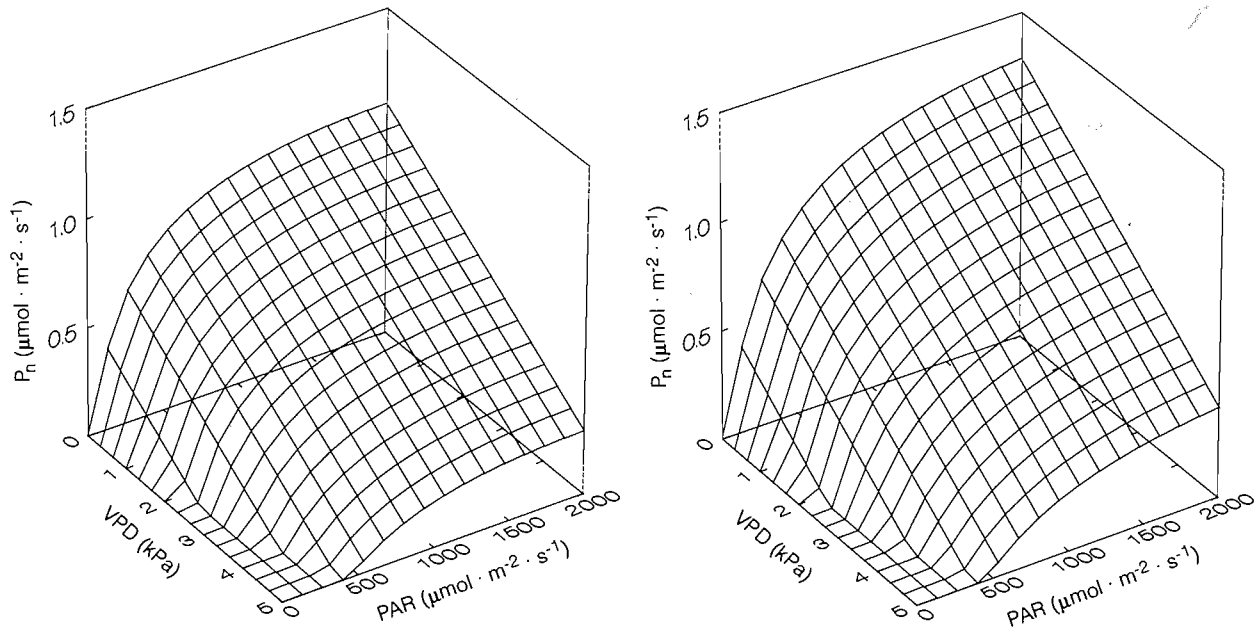


FIG. 5. Net photosynthesis (P_n) in response to photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) for current-year needles of interior spruce seedlings and emblings during the first growing season on a reforestation site. The regression model for seedlings is: $P_n = -1.550 - 0.00019(\text{PAR}) + 2.657(1/\text{PAR}) + 0.394([\ln]\text{PAR}) - 0.155(\text{VPD})$; $r^2 = 0.70$. The regression model for emblings is: $P_n = -1.904 - 0.00017(\text{PAR}) + 5.551(1/\text{PAR}) + 0.466([\ln]\text{PAR}) - 0.175(\text{VPD})$; $r^2 = 0.78$.

P_n readings for seedlings were recorded just after planting on June 12, 1991. By July 10, 1991, no difference in P_n was found between seedlings and emblings.

Response-surface models of P_n to PAR and VPD for current-year needles of seedlings and emblings showed P_n to increase to PAR levels of around $1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and then become stable at a given value of VPD (Fig. 5). However, as VPD increased P_n decreased, with P_n at 5.0 kPa approximately 30% of values at 0.5 kPa. As VPD increased, greater PAR levels were required to meet the photosynthetic compensation point. This dynamic response of P_n to both PAR and VPD occurs in other conifers (e.g., yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach); Grossnickle and Russell 1991). Emblings, compared with seedlings, had 15% greater P_n under optimal field site atmospheric conditions (i.e., $\text{VPD} < 3.0 \text{ kPa}$ and $\text{PAR} > 1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

For seedlings and emblings, P_n of 1-year-old (Fig. 6A) and current-year (Fig. 6B) needles increased in a linear fashion as g_{wv} increased. A linear response between P_n and g_{wv} for Engelmann spruce was thought to be merely correlative, with nonstomatal factors limiting P_n (DeLucia 1986). Nevertheless, stomata maintain a variable balance between CO_2 uptake while restricting plant water loss (Schulze and Hall 1982), and the P_n to g_{wv} relationship is important in assessing gas-exchange efficiency. For a given increase in g_{wv} , P_n was up to 30% greater in 1-year-old compared with current-year needles of seedlings and emblings. Measurement of these higher P_n values in 1-year-old needles occurred during shoot elongation of seedlings and emblings. Higher P_n measurements during shoot growth have been attributed to a greater sink demand for currently produced carbohydrates (Maier and Teskey 1992).

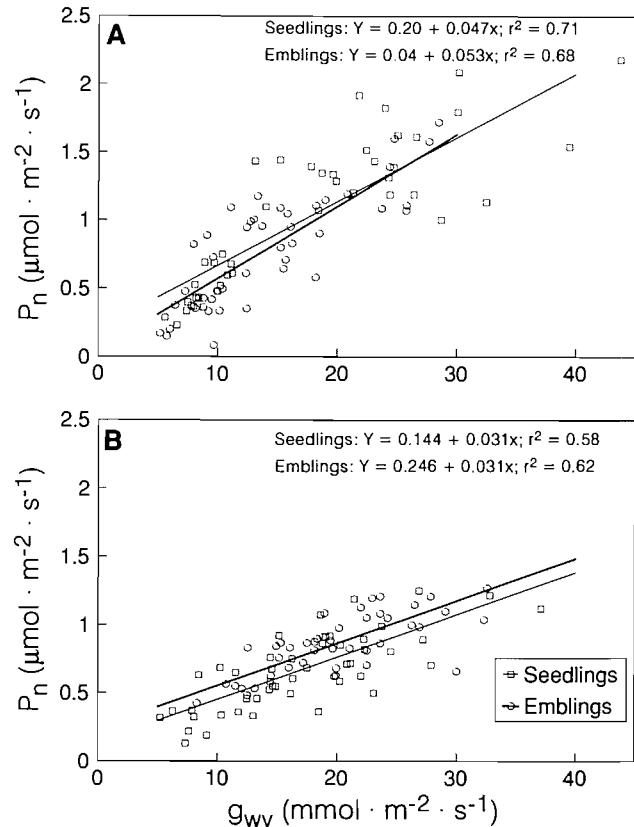


FIG. 6. Net photosynthesis (P_n) in response to needle conductance (g_{wv}) for interior spruce seedlings and emblings: (A) 1-year-old needles and (B) current-year needles during the first growing season on a reforestation site.

TABLE 2. Aboveground morphology and biomass of interior spruce seedlings and emblings at 1, 4, and 16 months after planting on a reforestation site

Treatment	Height (cm)	New height growth (cm)	Diameter (mm)	New shoot dry wt. (g)	Old shoot dry wt. (g)	Total shoot dry wt. (g)
1 month after planting (July 10, 1991)						
Seedlings	25.8±0.9a	5.1±0.4a	4.5±0.1a	0.88±0.05a	2.77±0.16a	3.64±0.16a
Emblings	17.3±0.6b	4.5±0.3a	3.8±0.1b	0.61±0.05b	1.64±0.15b	2.24±0.18b
4 months after planting (October 9, 1991)						
Seedlings	28.0±1.1a	6.9±0.6a	5.4±0.1a	2.44±0.14a	3.13±0.19a	5.58±0.25a
Emblings	18.7±0.8b	5.7±0.7a	5.1±0.2a	1.96±0.12b	1.94±0.08b	3.89±0.17b
16 months after planting (September 15, 1992)						
Seedlings	30.3±1.1a	3.1±0.4a	6.5±0.2a	2.17±0.24a	6.37±0.49a	8.54±0.57a
Emblings	23.3±1.0b	3.6±0.4a	6.1±0.2a	2.34±0.22a	4.64±0.27b	6.98±0.40b

NOTE: A difference in letter between seedlings and emblings indicates a significant difference at $p = 0.05$ as determined by t -test.

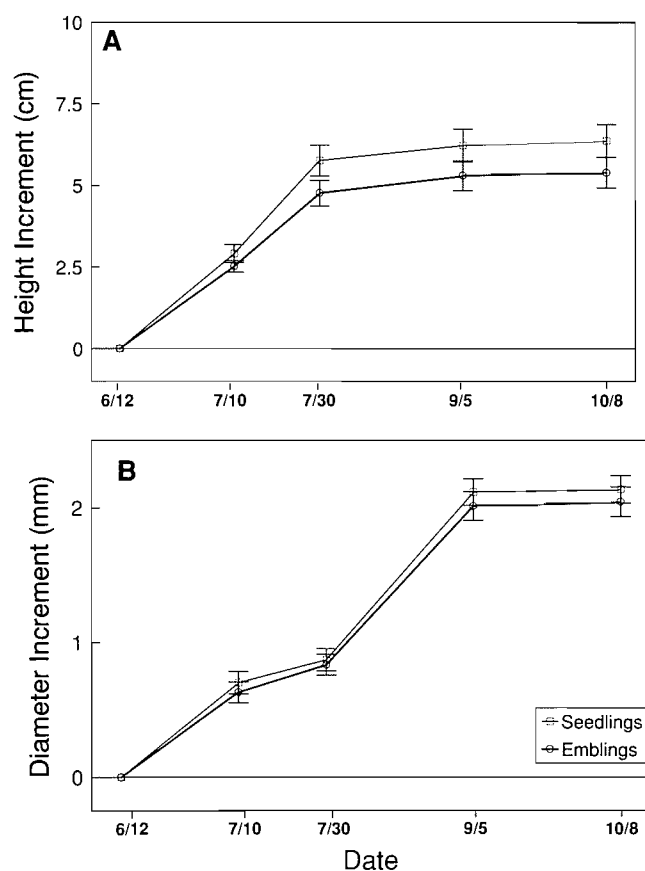


FIG. 7. Incremental height (A) and diameter (B) growth (mean \pm SE) of interior spruce seedlings and emblings over the first growing season on a reforestation site. There were no significant difference between seedlings and emblings on any measurement date as determined by a t -test ($p = 0.05$).

Early October consistently had nighttime freezing air temperatures, with October 8, 1991, having a minimum air temperature of -8.0°C (Fig. 1B). Seedlings and emblings had their subsequent g_{wv} response reduced to 32 and 20%, respectively, of predicted values for similar VPD conditions (from Fig. 3B). Engelmann spruce also has reduced g_{wv} after an exposure to continual below freezing minimum air temperatures in the fall (Smith et al. 1984). After exposure to nighttime freezing conditions, P_n was reduced to 73% in

seedlings and to 38% in emblings of predicted values for similar VPD and PAR conditions (from Fig. 5). A reduction in P_n occurs in Engelmann spruce after exposure to below-freezing minimum air temperatures (DeLucia and Smith 1987). The gas-exchange capability of emblings was more susceptible to fall freezing temperatures than seedlings.

Morphological development

During the first growing season, height growth was comparable for seedlings and emblings (Fig. 7A). Shoot extension of Engelmann and white spruce is primarily due to an elongation of preformed stem units contained in the previous season's terminal resting bud (Burdett et al. 1984). Seedlings and emblings had a comparable number of needle primordia in their terminal buds, measured just prior to field planting (Grossnickle and Major 1994). They also had similar height growth during the second growing season, though at a reduced rate compared with the first growing season (Table 2). Reduced 2nd-year height growth, or planting check, commonly occurs in interior spruce (Vyse 1981; Burdett et al. 1984) and has been attributed to environmental stress limiting the physiological response and subsequent morphological development of newly planted seedlings (Burdett et al. 1984; Grossnickle and Blake 1987; Grossnickle 1988). Seedlings and emblings had a comparable rate of diameter growth over the first (Fig. 7B) and second (Table 2) growing seasons.

One, 4 and 16 months after planting, seedlings had greater height and total shoot dry weights than emblings (Table 2). At the end of the second growing season, seedlings had greater height than emblings (30.3–23.3 cm, respectively), though they had similar incremental height growth over both growing seasons. Seedlings had greater new shoot dry weight during the first growing season. However, seedlings and emblings had comparable new shoot dry weight during the second growing season. Greater biomass accumulation during the first growing season combined with the larger shoot system at time of planting (Grossnickle and Major 1994) resulted in seedlings having a greater total shoot weight than emblings, 8.54–6.98 g, respectively, after 2 years in the field.

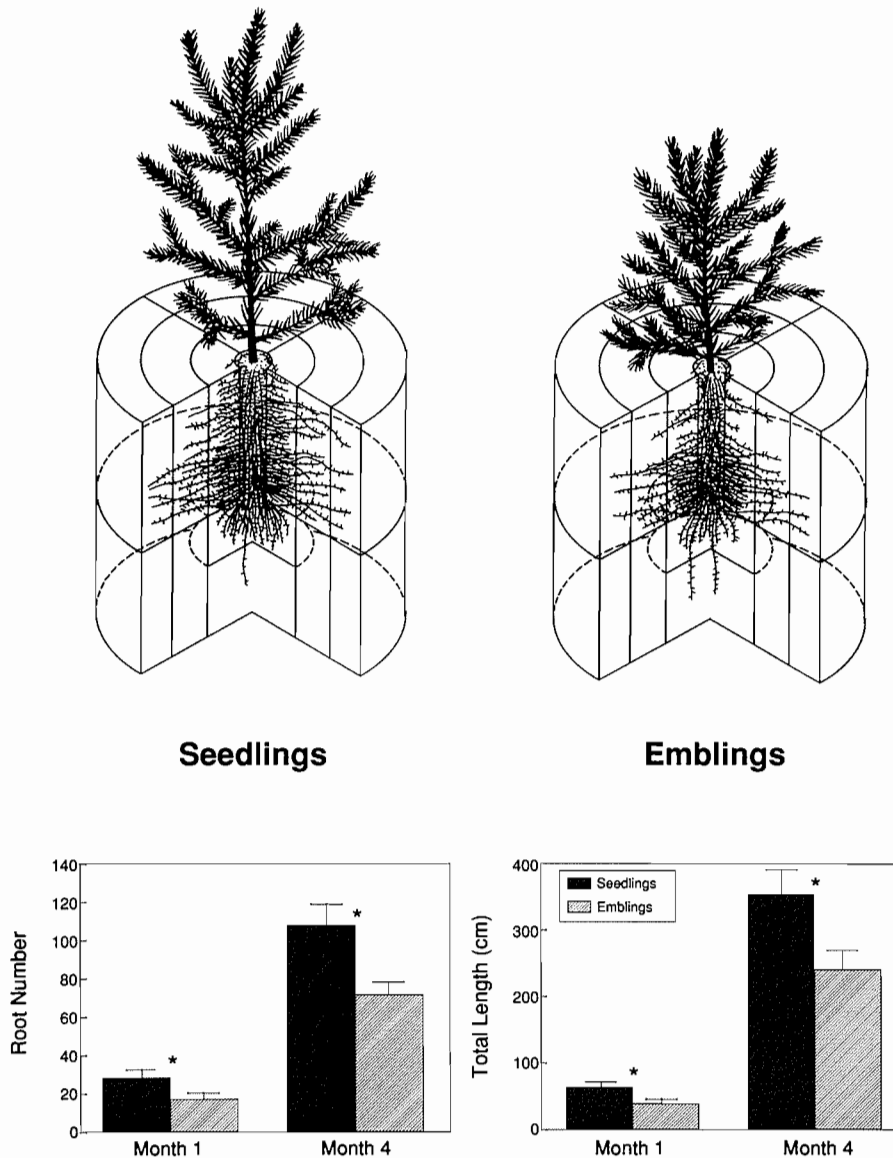
Seedlings had greater root development than emblings as measured by soil-root dry weight (Table 3), new root length, and root number (Fig. 8) after 1 and 4 months in the field. This reflects the greater initial root system size, which

TABLE 3. Belowground biomass and ratios of interior spruce seedlings and emblings at 1, 4, and 16 months after planting on a reforestation site

Treatment	Root plug dry wt. (g)	Soil-root dry wt. (g)	Total root dry wt. (g)	Soil-root to root plug ratio	Water balance ratio*
1 month after planting (July 10, 1991)					
Seedlings	1.23±0.07a [†]	0.036±0.005a	1.27±0.07a	0.033±0.006a	6.72±0.37a
Emblings	0.81±0.08b	0.019±0.004b	0.86±0.08b	0.024±0.004a	7.99±0.65a
4 months after planting (October 9, 1991)					
Seedlings	1.72±0.08a	0.78±0.05a	2.50±0.08a	0.484±0.045a	4.19±0.19a
Emblings	1.18±0.07b	0.55±0.09b	1.73±0.09b	0.489±0.048a	4.67±0.23a
16 months after planting (September 15, 1992)					
Seedlings	2.10±0.10a	1.11±0.08a	3.22±0.15a	0.540±0.027a	4.15±0.19a
Emblings	2.30±0.17a	0.75±0.10b	3.05±0.26a	0.315±0.028b	4.25±0.32a

NOTE: Values are means ± SE.

*Water balance ratio = shoot dry weight/(total root dry wt. × diameter (cm)).

[†]A difference in letter between seedlings and emblings indicates a significant difference at $p = 0.05$ as determined by *t*-test.FIG. 8. Diagrammatic representation ($n = 25$) of interior spruce seedling and embling morphological development after one growing season on a reforestation site. Number of roots and total root length (mean + SE) in the soil for seedlings and emblings at 1 and 4 months after planting. Statistical approach described in Fig. 2.

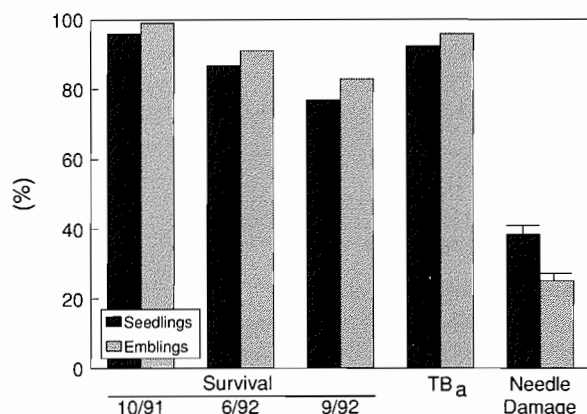


FIG. 9. Percent survival of interior spruce seedlings and emblings at the end of the first growing season (October 1991) after the first winter (June 1992) and at the end of the second growing season (September 1992) on a reforestation site. Percent terminal buds alive (TB_a) and needle damage for interior spruce seedlings and emblings after the first winter (June 1992) on a reforestation site.

enhanced root growth capacity, of seedlings measured just prior to field planting (Grossnickle and Major 1994). Studies have found that greater initial root mass is related to enhanced new root growth (Johnson et al. 1988; Williams et al. 1988). Seedlings and emblings had similar soil-root to root plug ratios during the first growing season (Table 3). Putting root development on this comparative root size scale indicates that seedlings and emblings had a similar capability to grow new roots.

Seedlings had greater soil-root dry weight than emblings after the second growing seasons in the field, though total root dry weights were comparable (Table 3). Emblings had a large increase in root plug dry weight, which caused them to have a lower soil-root to root plug ratio, compared with seedlings.

Though seedlings were larger than emblings, they had similar plant water balance ratios during the first (Table 3 and represented in Fig. 8) and second (Table 3) field seasons. Similar morphological balance is reflected in their comparable water relations and gas exchange patterns over the first growing season. Previous work has shown seedlings of different shoot sizes, but with comparable seedling water balance ratios, will have similar reforestation site water relations and gas exchange patterns (Grossnickle et al. 1991).

Survival percentage of seedlings and emblings steadily declined over two growing seasons to 77 and 83%, respectively (Fig. 9). After the first winter, seedlings and emblings had a comparable percentage of their populations with live terminal buds, while emblings had a lower percentage of their needles damaged. This degree of winter injury is a common occurrence for spruce planted in the British Columbia interior (L.J. Herring, personal communication), and is due to either a lack of freezing tolerance and (or) winter desiccation (Sakai and Larcher 1987). The factor, or factors, causing the winter injury to seedlings and emblings were not determined in this study.

Conclusions

Emblings produced from somatic embryogenesis tissue culture can successfully become established on a reforestation site. Emblings were largely comparable to seedlings in their summer seasonal water relations patterns and gas-exchange

responses to site environmental conditions. Emblings were comparable to, if not superior to, seedlings in resistance to winter injury. Seedlings and emblings had comparable incremental height and diameter growth over two growing seasons, though the larger initial size of seedlings resulted in greater total shoot development after 2 years. The seedlings' greater initial root growth was related to their root systems being initially larger at planting. However, seedlings and emblings had the same amount of root biomass after two growing seasons. The performance of emblings over two growing seasons on a reforestation site indicates they have the desired performance traits needed for use in forest regeneration programs.

Acknowledgments

Support for this research came from the National Research Council of Canada (CA949-9-0016), British Columbia Ministry of Forests (Project No. 4.1), and Science Council of British Columbia (Contract No. 241 (SA-1)). The authors thank Dr. Tom Hinckley and Dr. Bill Parker for helpful comments during initial manuscript development.

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