Interior spruce seedlings compared with emblings produced from somatic embryogenesis. I. Nursery development, fall acclimation, and over-winter storage

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Interior spruce (*Picea glauca* (Moench) Voss × *Picea engelmannii* Parry) seedlings and emblings (plants produced via somatic embryogenesis tissue culture) were grown as container 1+0 plants. Seedling and embling morphological development was monitored during the growing season. Needle freezing tolerance, days to terminal bud break (DBB_t), root growth capacity (RGC), and shoot dry weight fraction (DWF) were monitored during the fall and in frozen storage. Emblings had slower height, diameter, and root growth rates during the initial 2.5 months in the nursery. Thereafter, seedlings and emblings had equal height growth rate, while emblings had greater diameter and root growth rates. At the end of the growing season, seedlings and emblings, respectively, had 23.8 and 14.2 cm shoot height, 4.0 and 3.4 mm diameter, and 0.81 and 0.80 g root dry weight. During the fall, DBB_t of both seedlings and emblings decreased, with emblings having a more rapid decrease. Both seedlings and emblings showed a similar increase in freezing tolerance. Emblings had a greater increase in DWF. During the fall, RGC decreased then increased, with seedlings displaying a greater increase than emblings. While in frozen storage, seedlings and emblings maintained a low DBB_t, and a high RGC and DWF. Freezing tolerance decreased while in frozen storage, with the loss more pronounced among seedlings. A degree growth stage model describes the first year cycle of development for seedlings and emblings. Results indicate that seedlings and emblings have slightly different patterns of first year growth and fall acclimation. However, both seedlings and emblings were at the end of rest when lifted for frozen storage.

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Nous avons produit des plants 1+0 en récipients d'épinette de l'intérieur (*Picea glauca* (Moench) Voss × *Picea engelmannii* Parry) à partir de semis et de plantules produits par embryogénèse somatique. Nous avons effectué un suivi du développement morphologique des semis et des plants d'origine somatique au cours de la saison de croissance. À l'automne et durant l'entreposage au froid, nous avons suivi la tolérance des aiguilles au gel, le nombre de jours nécessaires au débourrement terminal, la capacité de croissance racinaire et la fraction de masse sèche dans la cime. Au cours des premiers 2,5 mois en pépinière, les taux de croissance en hauteur, en diamètre et en masse racinaire étaient plus faibles chez les plants d'origine somatique que chez les semis. Après cette période, les taux de croissance en hauteur étaient égaux chez les deux types de plants, alors que les taux de croissance en diamètre et en masse racinaire étaient plus élevés chez les plants d'origine somatique. À la fin de la saison de croissance, les semis et les plants d'origine somatique avaient une hauteur de 23,8 et 14,2 cm, un diamètre de 4,0 et de 3,4 mm et une masse sèche racinaire de 0,81 et de 0,80 g respectivement. Au cours de l'automne, le nombre de jours nécessaires au débourrement terminal a diminué chez les deux types de plants, la diminution étant plus prononcée chez les plants d'origine somatique. L'accroissement de la tolérance au gel a été similaire chez les deux types de plants. Pendant cette même période, la capacité de croissance racinaire a d'abord baissé puis s'est accrue, les semis montrant un accroissement plus important que les plants d'origine somatique. Pendant l'entreposage au froid, les semis et les plants d'origine somatique ont maintenu un nombre de jours au débourrement bas, ainsi qu'une capacité de croissance racinaire et une fraction de masse sèche dans la cime élevées. La tolérance au gel a aussi diminué pendant l'entreposage, avec une diminution plus forte chez les semis. Un modèle d'étapes de croissance en degrés est utilisé pour décrire le premier cycle annuel de développement et d'acclimatation automnale des semis et des plants d'origine somatique. Les résultats indiquent de légères différences dans les patrons entre les semis et les plants d'origine somatique. Cependant, les semis et les plants d'origine somatique étaient tous deux à la fin de leur période de repos lorsque transférés pour l'entreposage au froid.

[Traduit par la rédaction]

Introduction

Somatic embryogenesis is a method of asexual propagation that involves recapitulating the normal process of seed embryo development using tissue culture (Tautorus et al. 1991). This procedure has been successfully applied to interior spruce (*Picea glauca* (Moench) Voss × *Picea engelmannii* Parry). Somatic embryos are derived from excised seed embryos that are placed on the proper medium to produce a culture composed of many proembryos (i.e., early stage somatic embryos), similar in appearance to zygotic embryos soon after fertilization (Hakman and von Arnold 1985; Webb et al. 1989). Each culture can produce essentially an unlimited number of proembryos; each proembryo being a clone of the original explant. To produce plants, cultures are placed on a different medium where proembryos stop proliferating and proceed through more advanced stages of

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TABLE 1. Identification of plant material used for interior spruce seedling and embling comparison studies

Stock type	Open-pollinated family[a]	Ranking[b]	Clone	Number of plants
Seedlings	81	43		350
	103	34		200
	118	64		500
Total				1050
Emblings	81	43	W27	37
			W66	160
	103	34	W14	79
			W42	97
			W63	138
			W54	74
	118	64	W38	180
			W46	110
			W70	93
Total				968

[a]The seed used to produce this plant material came from selected open-pollinated trees located in north-central British Columbia.

[b]Ranking is based on height growth over 10 years in a progeny trial of 173 parent trees. Tree heights that determined the 10-year rankings were 157.84, 160.69, and 150.91 cm for open-pollinated families 81, 103, and 118, respectively (G. Kiss, unpublished information).

embryogenesis, resulting in the formation of cotyledonary embryos similar to a mature seed (Roberts et al. 1990*a*; Flinn et al. 1991). Somatic embryos are germinated in test tubes to produce plants (emblings) which resemble young seedlings (Roberts et al. 1990*b*; Cyr et al. 1991). Emblings are transferred from test tubes to styrofoam blocks, acclimatized to ex vitro conditions and placed in the nursery (Webster et al. 1990).

Forestry organizations are particularly interested in finding out whether emblings are comparable with seedlings during nursery development and field performance on a reforestation site. However, there is no published information on the performance of conifers produced through somatic embryogenesis protocols during all phases of forest regeneration (Tautorus 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.

The objective of this research program was to determine whether interior spruce emblings produced through somatic embryogenesis are comparable with seedlings during all phases of forest regeneration. Results from this research program are presented as a series of papers examining nursery development, stock quality assessment before planting, and performance on a reforestation site. This first paper compares growth and development of seedlings with emblings during the nursery growing season, and physiological response during the fall and in frozen storage.

Materials and methods

Source of 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. Open-pollinated seed (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) was used for producing seedlings and emblings. Specific information on the progeny trial is described in Kiss and Yeh (1988), with details on rankings and numbers of plants produced per open-pollinated family found in Table 1. At planting of the progeny trial, seedlings from families 81, 103, and 118 were 16.22, 15.52, and 16.65 cm in shoot height, respectively (G. Kiss, unpublished information). The progeny trial showed a 7% difference in shoot height, with an inconsistent ranking, between these three families at both planting and after 10 years in the field (10-year height data in Table 1).

A total of nine clones made up the embling test population (Table 1). An uneven number of emblings were produced per clone due to the inherent difficulty in producing emblings from certain clones (Webster et al. 1990). Emblings produced from nine clones, were randomly planted across the styrofoam blocks. For seedlings, seed from all three seed lots 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. Final nursery shoot heights for the individual families (mean ± SE; $n = 24$; measured October 31, 1990) showed no family differences for seedlings (23.86 ± 1.06, 22.90 ± 0.98, and 22.84 ± 1.16 for families 81, 103, and 118, respectively) or emblings (13.28 ± 0.63, 13.95 ± 0.70, and 13.32 ± 0.84 cm for families 81, 103, and 118, respectively).

It is recognized that families are unequally represented in the embling population, which can potentially result in apparent embling–seedling differences simply owing 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 original seed collection sites indicate that these families were of comparable genetic origin. In addition, continuous morphological, phenological, 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 repeated population sampling approach, this differential genotypic sampling problem was considered minor.

This research program had a companion part which examined interior spruce plant material produced from seed collected in the East Kootenay region of the southeastern interior of British

Columbia. Both seed sources showed similar results for seedling and embling comparisons during all phases of testing, thus only plant material from the north-central interior of British Columbia (Table 1) are reported in this series of papers.

Production of spruce emblings

Excised immature embryos were placed onto basal media containing appropriate plant growth regulators (Webb et al. 1989). Cultures were maintained in the dark and subcultured every 2 weeks. To promote proembryo development, cultures were placed on basal medium containing 3.4% sucrose and appropriate levels of growth regulators (Webster et al. 1990). Late cotyledonary embryos were removed from cultures and placed into a high relative humidity (HRH) treatment for 3 weeks (Roberts et al. 1990*b*). For germination, embryos were removed from the HRH treatment and placed, cotyledons up, into shell vials on half-strength basal media and 2% sucrose. Five weeks after germination, emblings from the different clones were transferred to 313B (65 mL per cavity) styroblocks® (Beaver Plastics Ltd.) containing a mixture of peat and sawdust (60:40 v:v) with a 180-day release fertilizer (16:10:10, N–P–K) mixed into the growing medium (0.13 kg/m^3) and acclimatized at 65% relative humidity and 16-h photoperiod at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 1 week (Webster et al. 1990). After an additional 3 weeks acclimatization at 65% relative humidity and 19-h photoperiod at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, styrofoam blocks containing a total of 968 emblings were transported to the nursery and grown with seedlings.

Nursery culture

Interior spruce seed was sown (1050 cavities) in mid-February 1990 in 313B styroblocks® with the same growing medium used for emblings. Emblings were germinated in mid-February and transferred from the controlled environment to the nursery in early April 1990. Seedlings and emblings were grown at Pelton Reforestation Ltd., Maple Ridge, B.C. (49°18′N), in a greenhouse maintained at a minimum day:night temperature of 16°C and in natural light supplemented with high pressure sodium vapour lamps (6 $\mu mol \cdot m^{-2} \cdot s^{-1}$) maintained at a minimum photoperiod of 16 h, to prevent bud set, until August 15, 1990, for seedlings and August 20 for emblings. Plants were watered and fertilized, as required, with a 100 ppm (from April through August) or 70 ppm (September and October) N (14.5% ammonium, 85.5% nitrate) continuous feed fertilization regime containing macro- and micro-nutrients (P, 31.3 ppm; K, 101.1 ppm; Ca, 61.2 ppm; Mg, 20.0 ppm; S, 26.0 ppm; Fe, 2.0 ppm; Cu, 0.61 ppm; Mn, 0.3 ppm; Zn, 0.3 ppm; B, 0.51 ppm; Mo, 0.0027 ppm).

From August 16–20, 1990, seedlings were exposed to an 8-h photoperiod to stop height growth. Seedlings were then placed in an outdoor compound under seasonal temperature and light conditions. Emblings received no short-day treatment, but lights producing the extended photoperiod were turned off August 20, 1990. Emblings remained in the greenhouse under seasonal photoperiods for an additional week before being placed in the outdoor compound on August 27, 1990. These nursery cultural procedures resulted in height growth cessation and bud set in seedlings and emblings within 1 week of each other.

Both seedlings and emblings were kept outdoors until plants were lifted for frozen storage on December 3, 1990. Seedlings or emblings were extracted from styrofoam blocks, randomly placed in groups of 10–24, depending on their end use, and their roots wrapped with plastic film. They were then placed into plastic-lined paper bags inside wax-coated cardboard boxes and stored at −2°C until removed for physiological testing during frozen storage, stock quality testing in mid-May 1991, or field planting in mid-June 1991.

The nursery study was a completely randomized design with styrofoam blocks of both seedlings and emblings randomly located across three greenhouse benches. During the growing season, styrofoam blocks were randomly rotated every 2.5 weeks.

Nursery morphological development

Height and diameter measurements were collected on 24 seedlings and 24 emblings every 2.5 weeks from April 4, 1990, to December 3, 1990. Plants measured were randomly selected on April 4, 1990, tagged, and then remeasured across the growing season.

Shoot and root dry weight were measured on 20 seedlings or emblings every 5 weeks from April 4, 1990, to October 9, 1990. Plants measured were randomly selected from the entire sample population, excluding plants tagged for height and diameter measurements.

Response during the fall and in frozen storage

At 2-week intervals during the fall until lifting for frozen storage (September 12, 1990, to November 27, 1990) and at 6-week intervals during frozen storage (December 18, 1990, to May 22, 1991), 10 seedlings and 10 emblings were randomly selected from the nursery population and brought back to the laboratory for testing. At each testing period, days to terminal bud break, freezing tolerance, dry weight fraction, and root growth capacity were measured on the sample population (n = 10), though freezing tolerance was measured on only six of 10 plants. Samples for freezing tolerance and dry weight fraction were collected from lateral shoots on the lower one third of the stem. This sampling procedure retained shoot integrity for days to terminal bud break and root growth capacity measurements taken on the same plants.

Root systems were washed free of growing medium, plants were tagged for identification and then randomly placed in a darkened aerated hydroponic system in a growth room with a controlled environment having air and water temperatures at 22°C, 50% relative humidity, and a 16-h photoperiod at 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$. From September 1 through December 3, 1990, while seedlings and emblings were in the outdoor compound, air temperature at plant height was recorded to determine the accumulated number of hours ≤5°C (i.e., chilling sum).

Days to terminal bud break

Bud dormancy status was determined by bringing plants into a forcing environment (Ritchie 1984; Lavender 1991). Seedlings and emblings were brought into the above described controlled environment and plants were checked thrice weekly to determine the number of days required to break the terminal bud (DBB_t). If plants had not broken bud after 14 days, fertilizer (10:10:10, N–P–K) was added to the hydroponic system at 0.125 g/L. Terminal buds were considered to be broken when bud scales parted and needles extended at least one mm. Mean DBB_t values were reported as ≥100 for September collection periods because at least 50% of seedlings and emblings measured during this period had not broken bud when measurements were discontinued at 120 days.

Freezing tolerance

Freezing tolerance was determined using the freeze-induced electrolyte leakage (FIEL) procedure modified from Burr et al. (1990). In the morning after plants were placed in the growth room, needle tissue was removed from a branch on the bottom third of the stem and used for FIEL measurements. Needles from each plant were cut at both ends into 0.5 cm lengths, washed in deionized water and transferred, in random groups of 24, to glass culture tubes containing 0.5 mL deionized water. One tube from each plant was stoppered and placed in ice water as a control at 1°C. Four tubes from each plant were placed in an ethanol bath at −2°C, cooled by a refrigeration system (Forma Scientific MC-8-80). Water in all tubes in the ethanol bath was nucleated simultaneously with ice crystals after 0.5 h, and tubes were stoppered. The ethanol bath was then cooled at a rate of 5°C/h.

Four temperatures were selected to bracket the anticipated 50% tissue electrolyte leakage value. When tubes for each plant were removed at selected temperatures, contents were allowed to thaw in ice water. After the contents of all tubes had thawed,

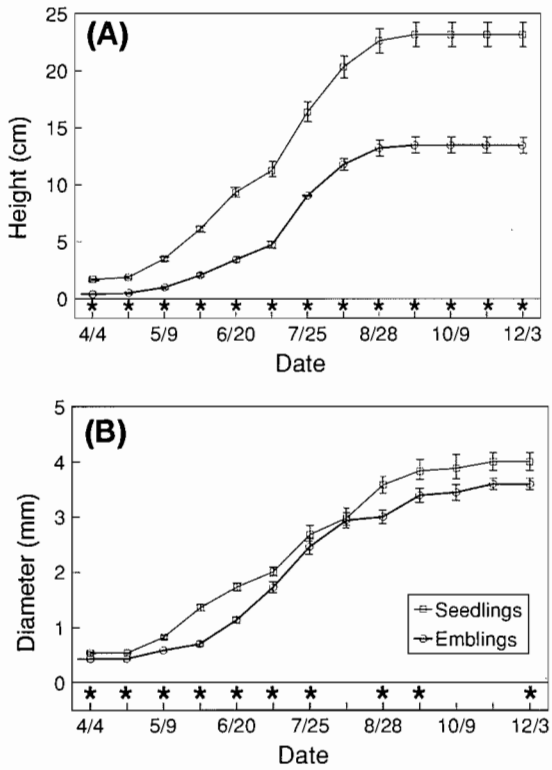

FIG. 1. Shoot height (A) and root collar diameter (B) (mean ± SE) of interior spruce seedlings and emblings during the nursery growing season. *Significant difference between seedlings and emblings on that measurement date as determined by a *t*-test ($p = 0.05$).

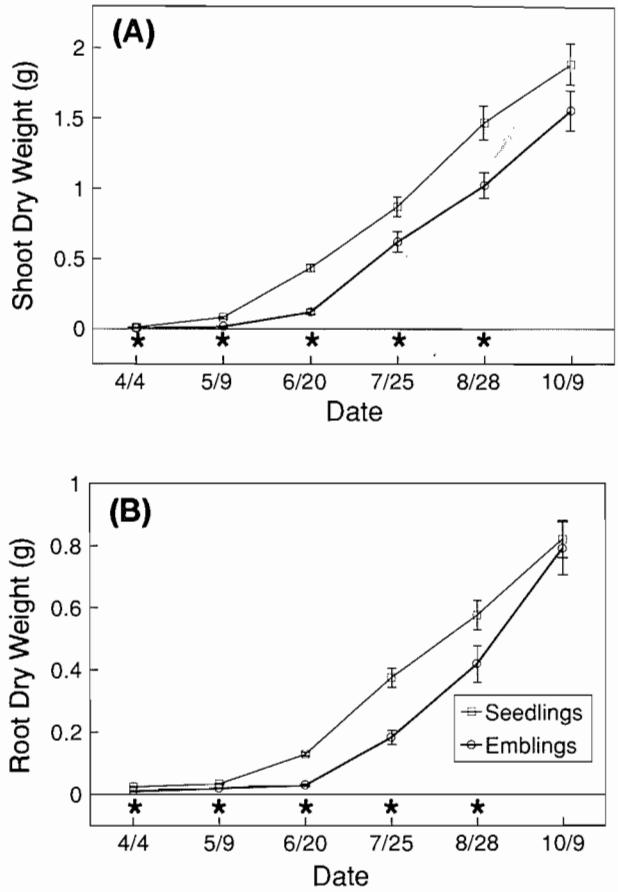

FIG. 2. Shoot (A) and root (B) dry weight (mean ± SE) of interior spruce seedlings and emblings during the nursery growing season. Statistical approach described in Fig. 1.

5.5 mL of deionized water was added to each tube. Tubes were then stoppered and placed on a 100 rpm shaker at 24°C for 20–24 h. Conductivity of the solution in each tube was measured after incubation. Tubes were then placed in a 90°C water bath for 15 min to induce maximum tissue injury and conductivity was remeasured after an additional 20 h on a 100 rpm shaker at 24°C.

Measured FIEL values were interpreted as an index of injury (II) based on procedures of Dexter et al. (1930) and Flint et al. (1967) with modifications made by Burr et al. (1990). Test results were reported as percent II calculated by the following formula:

$$[1] \quad \text{II} = 1 - \frac{1 - \left(\frac{T_1}{T_2}\right)}{1 - \left(\frac{C_1}{C_2}\right)} \times 100$$

where T_1 and T_2 are the conductivities of treatment tubes after freezing and after boiling, respectively, and C_1 and C_2 are the conductivities of control tubes before and after boiling, respectively.

For each plant in each measurement period, a linear regression model was determined from a data set consisting of II at each measurement temperature. Temperatures at which 50% foliage electrolyte leakage occurred (i.e., LT_{50}) were calculated from the linear regression model for each plant.

Root growth capacity

Seedlings and emblings were grown with roots in darkened aerated hydroponic system in the above described controlled environment. Root growth capacity (RGC) was determined by counting the number of new white roots ≥0.5 cm in length after 14 days (Ritchie 1985).

Dry weight fraction

Dry weight fraction (DWF) was determined from a branch on the bottom third of the stem. After the plants had rehydrated over the first night in the growth room, a 6 cm branch was removed just prior to the lights being turned on and weighed for determination of saturated weight. Branch dry weight was measured after oven drying at 65°C for 48 h. The DWF was determined as grams dry weight (DW) per gram saturated weight (SW) for each branch (Grossnickle 1989).

Statistical analysis

Differences in morphological development between seedlings and emblings were determined by a *t*-test ($p = 0.05$). For fall and overwinter frozen storage data collection, controlled environment measurements used a completely random experimental design with an equal number of replicates for seedlings and emblings. A *t*-test ($p = 0.05$) was used to determine differences between seedlings and emblings for all parameters measured at each data collection period. During the fall and prior to frozen storage, the relationship between LT_{50} and DWF for seedlings and emblings was determined using linear regression (Steel and Torrie 1980).

First-year degree growth stage model

A degree growth stage model illustrates the dynamic nature of morphological and physiological changes taking place in plants throughout their annual cycle in response to seasonal environmental conditions (Fuchigami et al. 1982; Fuchigami and Nee 1987). The degree growth stage model concept has been used by Burr (1990), and Ritchie and Tanaka (1990) to define the theoretical seasonal cycle of conifer seedlings.

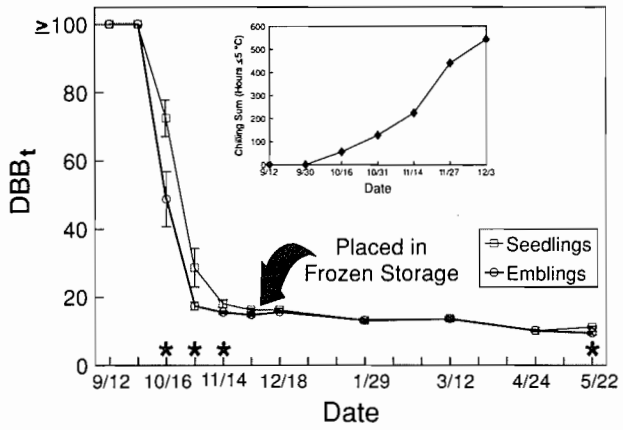

FIG. 3. Days to terminal budbreak (DBB_t) (mean ± SE) of interior spruce seedlings and emblings during the fall in the nursery and after being placed in frozen storage. Inset figure shows the chilling sum described as the accumulated hours ≤5.0°C. Statistical approach described in Fig.1.

A degree growth stage model of interior spruce seedlings and emblings summarizes their growth and development during the first growing season, fall acclimation and frozen storage in the nursery. This model represents the annual cycle as a sine wave from 0 to 360° with major degree growth stage (°GS) points estimated on the curve as: 5°GS, start of growth for both seedlings and emblings in the nursery; 45°GS, considered the end of the juvenile growth phase; 90°GS, maturity induction; 180°GS, vegetative maturity; 270°GS, maximum rest; 315°GS, end of rest. In this model, bud break on the reforestation site is theoretically defined as 360°GS. Parameters used to define the major degree growth stage points for seedlings and emblings are described in the discussion of results.

Results and discussion

Nursery morphological development

Shoot height increased at a greater rate in seedlings compared with emblings from April 20 to July 4; with seedlings being approximately twice as tall as emblings at the end of this time period (Fig. 1A). From July 4 through August 14, all plants had a high rate of shoot elongation. From mid-August through mid-September, height growth slowed, then stopped and bud set occurred in all plants. By the end of the growing season, seedlings were 70% taller than emblings.

Root-collar diameter of seedlings increased at a steady rate from April 20 until August 14, while embling diameter growth started slowly and then increased rapidly; the result being that seedlings and emblings had similar diameters on August 14 (Fig. 1B). In late August, seedlings, compared with emblings, had a rapid increase in diameter growth. From September through October, all plants had a small, but continual, increase in diameter growth. No diameter growth occurred in November.

The first recorded increase in shoot dry weight was on May 9 for seedlings and on June 20 for emblings (Fig. 2A). Thereafter, shoot dry weight increased until October 9 at a similar rate for both seedlings and emblings, though seedlings continually had a greater shoot dry weight.

Seedlings and emblings fit the "idealized" shoot height growth curve, which has a juvenile stage of slow growth just after seed germination, or for emblings after being brought from the controlled environment, an exponential phase of rapid growth during spring–summer, and a senescence phase of growth cessation and bud development during late summer (Sinnott 1960).

However, emblings' spring shoot and diameter growth lagged when in transition between the juvenile and exponential growth phases. Sunny and unseasonably warm conditions prevailed just after emblings were brought from the laboratory to the nursery. These conditions probably caused moisture stress in emblings and delayed spring growth because plants are especially sensitive to moisture stress during the spring portion of the exponential growth phase (McDonald 1984). Tissue culture plantlets can have problems adapting to ex vitro conditions because of their inability to balance water uptake with water loss (Conner and Thomas 1981; Sutter 1988; Drew et al. 1992). Emblings were initially acclimatized in a growth room under fairly high relative humidity (65%) and a low light level (6 $\mu mol \cdot m^{-2} \cdot s^{-1}$). When emblings are transferred to the nursery, they probably require further acclimation by limiting exposure to stressful environmental conditions. Standard procedures used in rooting cuttings (e.g., shade with misting or fog) would probably improve embling acclimatization. Previous work has not shown emblings to have delayed growth just after being brought from the laboratory to the nursery under nonstressful environmental conditions (Webster et al. 1990).

Root dry weight of seedlings increased after May 9 and after June 20 for emblings (Fig. 2B). From June 20 to August 28, root dry weight of seedlings and emblings increased at a similar rate. In September, root dry weight of emblings increased more rapidly than seedlings, resulting in similar root dry weights by October 9. This rapid root growth at the end of the growing season coincided with a slowing and then cessation of shoot elongation (Fig. 1). The potential for root growth is high in late summer and early fall when shoot elongation declines (Ritchie and Dunlap 1980; Ritchie and Tanaka 1990). Greater root growth for emblings, compared with seedlings, during September could result from the cavity size of 313B styroblock® containers (i.e., 65 mL/cavity) restricting further seedling root development.

Response during the fall and in frozen storage

Days to terminal bud break

Days to terminal bud break were ≥100 during September and decreased rapidly during October to below 20 days by November 14, for both seedlings and emblings (Fig. 3). Emblings had a more rapid decrease in DBB_t during October and early November, with both seedlings and emblings at a similar DBB_t on November 27. A rapid decline in DBB_t after bud set is a typical pattern found in spruce seedlings (e.g., interior, Ritchie et al. 1985; Engelmann, Burr et al. 1989; white, Grossnickle 1989; Sitka (*Picea sitchensis* (Bong.) Carr.), Cannell et al. 1990). This rapid decrease in DBB_t for both seedlings and emblings coincided with the accumulation of chilling hours (≤5°C) during October and early November, though only 224 chilling h occurred during this period. The cumulative hours of chilling required to overcome dormancy are generally between 675 and 1000 h for mature white spruce (Nienstaedt 1966). Handling seedlings in a way that differs from normal field summer seasonal patterns (e.g., alteration of photoperiod, water, and nutrient availability) can alter the DBB_t response to environmental cues during the dormant period (Lavender 1991). Seedlings and emblings were grown under optimum nursery cultural

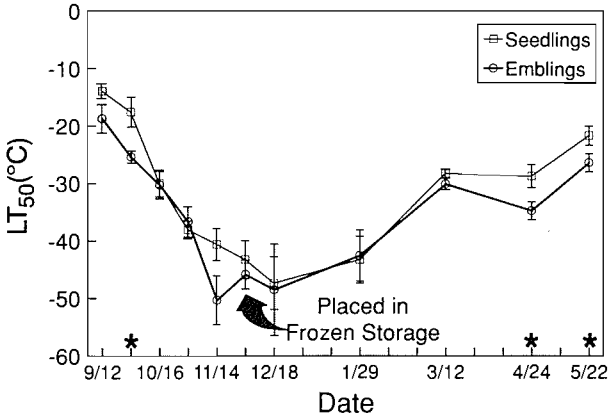

FIG. 4. Freezing tolerance (LT_{50}) (mean ± SE) of interior spruce seedlings and emblings during the fall in the nursery and after being placed in frozen storage. Statistical approach described in Fig. 1.

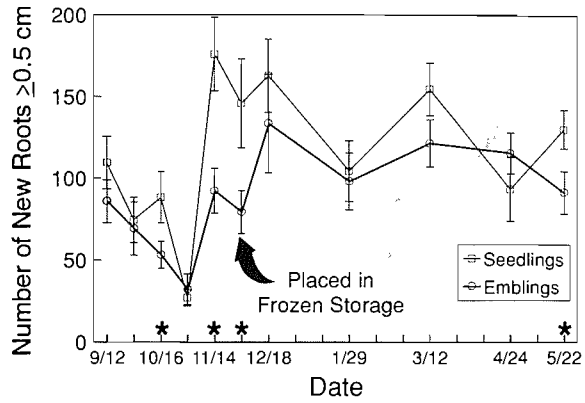

FIG. 5. Number of new roots ≥0.5 cm grown during root growth capacity testing (mean ± SE) for interior spruce seedlings and emblings during the fall in the nursery and after being placed in frozen storage. Statistical approach described in Fig. 1.

conditions to enhance shoot growth (i.e., extended photoperiod, and an optimal water and nutrient regime) during the 1st year indeterminant growth phase. As a result, their DBB_t pattern does not reflect the DBB_t pattern of mature spruce.

After plants were placed in frozen storage, DBB_t decreased slightly to 11 and 9 DBB_t by May 22, 1991, for seedlings and emblings, respectively (Fig. 3). Only a slight change in the dormancy level of interior spruce seedlings occurs during frozen storage when they are fully released from dormancy at lifting (Ritchie et al. 1985; Camm and Harper 1991).

Freezing tolerance

During the fall, LT_{50} decreased rapidly in both seedlings and emblings, with LT_{50} being −43 and −46°C for seedlings and emblings, respectively, just prior to lifting for frozen storage (Fig. 4). This rapid decline in LT_{50} after bud set in the fall also occurs in interior spruce (Simpson 1990) and other spruce species (e.g., Engelmann, Burr et al. 1989; black (*Picea mariana* (Mill.) B.S.P.), Colombo et al. 1989; Sitka, Cannell et al. 1990; white, Bigras and D'Aoust 1992).

While in frozen storage, seedlings and emblings lost 49 and 43%, respectively, of their freezing tolerance developed during the fall (Fig. 4). Seedlings, compared with emblings, had higher LT_{50} temperatures during April and May. This partial loss of freezing tolerance during storage is reported for frozen-stored bareroot interior spruce (Ritchie et al. 1985) and Sitka spruce cold stored at 0.5°C (Cannell et al. 1990). The loss of normal seasonal light and temperature cues and a decrease in carbohydrate reserves have been hypothesized as reasons for a decrease in freezing tolerance during storage (Ritchie 1986).

Root growth capacity

Root growth capacity declined for both seedlings and emblings from September 12 to October 30 (Fig. 5). On November 14, seedlings had a very rapid increase in RGC, which remained high through December 18. For emblings, RGC increased steadily from October 30 until just after placement in frozen storage on December 18. Interior spruce seedlings have increased root growth in late fall (Ritchie et al. 1985), and this increased root growth in spruce occurs after bud set and a period of exposure to low temperatures (Burr et al. 1989; Cannell et al. 1990; McKay and Mason 1991).

For both seedlings and emblings, RGC remained fairly stable throughout frozen storage (Fig. 5). The only exception was a decline in embling RGC after 5 months of frozen storage (May 22). This contrasts with other work showing a decline in RGC with 6 month frozen storage of interior (Ritchie et al. 1985) and white (Camm and Harper 1991) spruce.

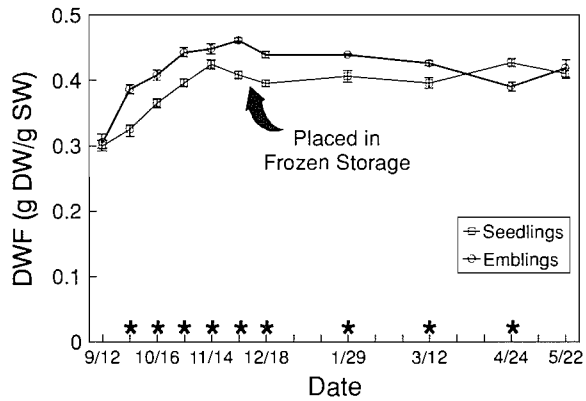

FIG. 6. Dry weight fraction (DWF) of interior spruce seedlings and emblings during the fall in the nursery and after being placed in frozen storage (mean ± SE). Statistical approach described in Fig. 1.

Dry weight fraction

Shoot DWF increased during the fall from 0.30 to 0.41 g DW / g SW for seedlings and from 0.30 to 0.46 g DW / g SW for emblings (Fig. 6). The DWF of white spruce normally increases during the fall (Grossnickle 1989). Increased DWF results from continued cell development and maturation with an accompanying decrease in symplastic volume (Doi et al. 1986). The DWF of both seedlings and emblings remained stable throughout frozen storage. Emblings had greater DWF than seedlings during every measurement period, except just after bud set (September 12) and during the last 2 months of frozen storage (April and May).

During the fall, LT_{50} for both seedlings and emblings decreased in a linear fashion as DWF increased (seedlings: $LT_{50} = 58.32 - 241.42(DWF)$, $r^2 = 0.78$; emblings: $LT_{50} = 47.37 - 203.01(DWF)$, $r^2 = 0.69$) with emblings having a greater DWF at any given LT_{50} value. Foliage water content influences freezing tolerance via concentration of cell solutes (Sakai and Larcher 1987). During fall acclimation, increased DWF is an indirect measure of increased freez-

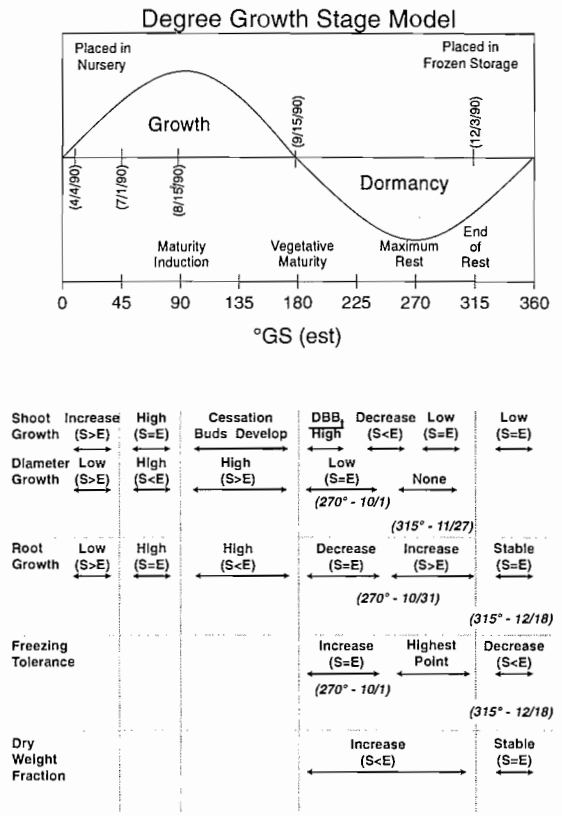

FIG. 7. Degree growth stage (GS) model for interior spruce seedlings (S) and emblings (E) during their first year of development in the nursery. The degree GS points identified on the *X*-axis, or in parentheses, are estimates (est) of different developmental phases. DBB_t, days to terminal budbreak.

ing tolerance (Ritchie 1984) and this relationship occurs in black spruce (Colombo 1990) and other conifers (Timmis and Worrall 1975; Grossnickle 1992).

First-year degree growth stage model

Interior spruce seedlings and emblings had differing growth strategies for the 0–180°GS growth portion of the model. During the juvenile growth phase (0–45°GS), seedlings initially had greater height, diameter, and root growth than emblings, though root growth was low in all plants (Fig. 7). During the exponential phase of rapid growth (45–90°GS), the rate of height and root growth were similar between seedlings and emblings, while emblings had greater diameter growth.

Maturity induction (90°GS) is a point where plants first become responsive to daylength, which promotes the development of vegetative maturity (Fuchigami et al. 1982). Seedlings and emblings were exposed to an artificially extended photoperiod until August 15th to prevent maturity induction. Once seedlings were exposed to a short-day treatment and emblings were exposed to natural daylength, their shoot elongation slowed and terminal buds started to develop (Fig. 7). However, diameter growth (seedlings > emblings) and root growth (seedlings < emblings) continued at a high rate during this time period.

The fall acclimation of spruce seedlings can be altered by photoperiod regimes that cause growth cessation, the development of dormancy, and freezing tolerance (D'Aoust and Hubac 1986; Colombo et al. 1989; Bigras and D'Aoust 1992). Even though seedlings and emblings were exposed to different photoperiod regimes, their shoot elongation stopped and buds developed during the same time period. This is because the emblings' exposure to natural day length (approximately 13 h) resulted in a similar response to that in seedlings exposed to an 8-h photoperiod. With conifers, the end of shoot elongation and development of overwinter buds is an indication of vegetative maturity (Burr 1990) and is considered the first stage of fall acclimation to low temperatures (Weiser 1970; Levitt 1980). Thus, even though seedlings and emblings were exposed to different photoperiod regimes, they were probably in a similar state of vegetative maturity (180°GS) by mid-September (Fig. 7).

As dormancy intensifies from vegetative maturity at 180°GS to maximum rest at 270°GS, theoretically DBB_t is supposed to increase (Fuchigami et al. 1982; Burr 1990), RGC declines to a low level (Burr 1990; Ritchie and Tanaka 1990), and there is a small (Burr 1990) or large (Fuchigami et al. 1982) increase in freezing tolerance. Interior spruce seedlings and emblings did not completely conform to this expected pattern, even though they responded in a similar manner. During the 180–270°GS portion of the model, seedlings and emblings had no measured increase in DBB_t and only a small period of high DBB_t from September 12 to October 1, RGC continually declined from September 12 until October 31, and freezing tolerance began increasing at a rapid rate starting on October 1 (Fig. 7). In addition, diameter was still increasing slightly while DWF was increasing at a greater rate in emblings compared with seedlings. The combination of optimum nursery cultural conditions, used to enhance and continue growth beyond the time when maturity induction naturally occurs, and the 1st year indeterminant growth pattern inherent in interior spruce probably altered early dormancy development patterns. As a result, measured parameters for interior spruce seedlings and emblings did not conform with the expected 180–270°GS pattern.

Maximum rest (270°GS) until the end of rest (315°GS) is a period characterized by a total absence of shoot growth, DBB_t decreases to a low level, freezing tolerance increases rapidly and reaches the maximum level (Fuchigami et al. 1982; Burr 1990), and RGC increases (Burr 1990; Ritchie and Tanaka 1990). From early October through mid-December, all measured parameters for seedlings and emblings started to conform with the expected patterns (Fig. 7). They responded in the following manner: (*i*) a cessation of diameter growth; (*ii*) DBB_t decreased to a low level; (*iii*) RGC increased to the highest seasonal level (seedlings > emblings); (*iv*) freezing tolerance increased rapidly in late October and early November, and reached the maximum level by December; and (*v*) DWF was at the highest seasonal level (seedlings < emblings). Thus, interior spruce seedlings and emblings were at or near the end of rest (315°GS) when lifted and placed in frozen storage. This is considered the best time to lift and store seedlings to ensure the highest stress resistance, freezing tolerance, and RGC for improved growth and survival on a reforestation site (Burr 1990; Ritchie and Tanaka 1990).

While in frozen storage (315–360°GS), seedlings and emblings maintained a low DBB_t and a high RGC and DWF (Fig. 7). Only freezing tolerance decreased while in frozen storage, with seedlings having a larger loss than emblings. During frozen storage, seedlings and emblings maintained the physiological condition desired for successful establishment of interior spruce (Simpson 1990).

The next paper in this series (Grossnickle and Major 1994) describes stock quality assessment of interior spruce seedlings and emblings after removal from frozen storage in May 1991, prior to planting on a reforestation site in June 1991.

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