Integration of Somatic Embryogenesis into Operational Forestry: Comparison of Interior Spruce Emblings and Seedlings during Production of 1+0 Stock[1]

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Abstract - Somatic embryos from 15 different clones representing 4 open-pollinated families were germinated in vitro concomitantly with the germination of genetically related seed in the nursery. Emblings (plants produced via somatic embryogenesis) were transferred to styroblocks five weeks following germination, acclimatized to ex vitro conditions under high humidity and low light and then transferred to the nursery where they were grown alongside control seedlings. Seedlings and emblings were tested under the British Columbia Ministry of Forests operational stock quality testing standards. Both seedlings and emblings reached the desired level of frost hardiness before lifting for frozen storage. Post-storage testing showed seedlings and emblings met or exceeded all morphological stock specifications and had high root growth capacity values.

INTRODUCTION

Somatic embryogenesis is a method of asexual propagation which involves recapitulating the normal process of seed embryo development using tissue culture. For spruce species, somatic embryos are derived from the seed embryo (Hakman and von Arnold, 1985; Webb et al. 1989). Cultures are initiated by excising the embryo from seed, placing it on the proper medium where it produces a culture composed of many early stage somatic embryos (proembryos) reminiscent of how zygotic embryos appear soon after fertilization. Each culture can produce essentially an unlimited number of proembryos; each proembryo is a clone of the original explant. In order to produce plants, cultures are placed on a different medium where proembryos stop proliferating. They then proceed through more advanced stages of embryogenesis resulting in the formation of cotyledonary embryos that are similar to those of a mature seed (Roberts et al. 1990a; Flinn et al. 1991). Somatic embryos are germinated in test tubes to produce plants (emblings) which resemble young seedlings (Roberts et al. 1990b, Cyr et al. 1991). Emblings are transferred from test tubes to styroblocks, acclimatized to ex vitro conditions and placed in the nursery (Webster et al. 1990).

It is now possible to propagate spruce through somatic embryogenesis and forestry organizations including the British Columbia Ministry of Forests and Forestry Canada are evaluating the technology for mass clonal propagation. Somatic embryogenesis is still more expensive than seed or cuttings and the costs must be reduced through bulk handling and automation before the process can be used operationally. Biotechnologists, tree breeders and foresters are particularly interested to find out if emblings are equivalent to seedlings and if they can be grown in the nursery to reach operational seedling specifications. This report compares growth and development of emblings with seedlings during their first year in the nursery and assesses stock quality before and after frozen storage.

Stock quality tests used for operational forest regeneration programs in British Columbia are designed to improve production and handling practices in forest nurseries and to give some estimate of performance potential just prior to field planting. These stock quality assessment procedures: 1) define seedlings minimum, maximum and target morphological specifications for a plantable seedling, 2) determine when to lift for frozen

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storage, and 3) measure performance potential with rootgrowth capacity testing just prior to field planting (Simpson 1990). The objective was to determine the performance of seedlings and emblings during nursery development based on operational stock quality testing criteria established by the British Columbia Ministry of Forests for British Columbia forest nurseries.

MATERIALS AND METHODS

Production of Spruce Emblings

Embryogenic cultures were initiated by placing excised immature embryos from open-pollinated families of interior spruce (Picea glauca engelmannii complex; families included Prince George 81, 103, 118) onto basal medium containing the appropriate plant growth regulators (Webb et al. 1989). Cultures were maintained in the dark and subcultured every two weeks. To promote proembryo development, cultures were placed onto basal media containing 3.4% sucrose and appropriate levels of growth regulators (Webster et al. 1990). Late cotyledonary embryos were removed from the cultures and placed into a high relative humidity (HRH) treatment for three weeks (Roberts et al 1990b). For germination, embryos were removed from the HRH treatment and placed, cotyledons up, into shell vials on 1/2 strength basal medium and 2% sucrose. Five weeks after germination the emblings were transferred to styroblocks and acclimatized under high humidity and low light for 1 week (Webster et al. 1990). After an additional 3 weeks of acclimatization under low humidity and high light, styroblocks were transported to the nursery and grown alongside control seedlings.

Nursery culture

Interior spruce seed, from a mixture of Prince George open pollinated families 81, 103, 118 (British Columbia Ministry of Forests), was sown (900 cavities) in mid February 1990 in 313B styroblocks (Beaver Plastics Ltd.) in a mixture of peat and sawdust (60/40 v/v) with a 180 day release fertilizer (16-10-10) mixed into the growing media (1 kg/.13 cu m). Emblings (approximately 800) were germinated in mid February and transferred from the laboratory to the nursery in early April 1990. Seedlings and emblings were grown at Peltons Reforestation, Maple Ridge, B.C., 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 mol m^{-2} s^{-1}) maintained at a minimum photoperiod of 16h, to prevent budset, until August 15, 1990 for the seedlings and August 20 for the emblings. Plants were watered and fertilized, as required, with a 100 ppm (from April through August) or 70 ppm (September and October) nitrogen (14.5% Ammonium, 85.5% Nitrate) continuous feed fertilization regime containing a complete macro- and micro-nutrient package (P 31.3 ppm, K 101.1 ppm, Ca 61.2 ppm, Mg 20 ppm, S 26 ppm, Fe 2 ppm, Cu .61 ppm, Mn .3 ppm, Zn .3 ppm, B .51 ppm, Mo .0027 ppm).

From August 16th to 20th, seedlings were exposed to a short-day treatment, to stop height growth, by reducing the photoperiod to 8 hours. Seedlings were then placed in an outdoor compound under seasonal temperature and light conditions. Emblings received no short-day treatment, to allow additional height growth to occur, but lights producing the extended photoperiod were turned off August 20th. Emblings remained in the greenhouse under seasonal photoperiods for an additional three weeks before being placed in the outdoor compound on September 10th. Both seedlings and emblings were kept outdoors until plants were lifted for frozen storage on December 3, 1990. Seedlings or emblings were extracted from all styroblocks, randomly placed in groups of 10 to 24 depending on their end use, wrapped with mylar film, and placed into plastic-lined paper bags inside cardboard boxes, at -2°C until just before field planting in mid June 1991.

Morphological parameters

Plant height and diameter were measured on 24 seedlings or emblings every two and one-half weeks from April 20, 1990 to October 1, 1990. Diameter was also measured on October 31, 1990. Plants measured were randomly selected at the beginning of April, tagged and then re-measured across the growing season.

In mid May, 1991 seedlings or emblings (n=24) were removed from frozen storage and measured for shoot height, root collar diameter, shoot dry weight, root dry weight and shoot to root dry weight ratio.

Frost hardiness

Seedlings and emblings index of injury at -18°C (II @ -18°C) was determined biweekly from mid September, 1990 until just before lifting for frozen storage. The freeze induced electrolyte leakage procedure was used to determine II @ -18°C (Burr et al. 1990). Six seedlings or emblings were randomly selected from the nursery population at each sampling period. Needle tissue from the bottom third of the shoot was used for frost hardiness measurements.

Root growth capacity

In mid May 1991, seedlings or emblings (n=30) were removed from frozen storage, root systems were washed free of growing medium and placed in a darkened aerated hydroponic system (Grossnickle et al. 1991). Plants were grown for 14 days in a growth room with air and root temperature at 22°C, 50% relative humidity, and a 16h photoperiod at 650 mol m^{-2} s^{-1}. Root growth capacity was determined by counting the number of new white roots greater than 0.5 cm in length.

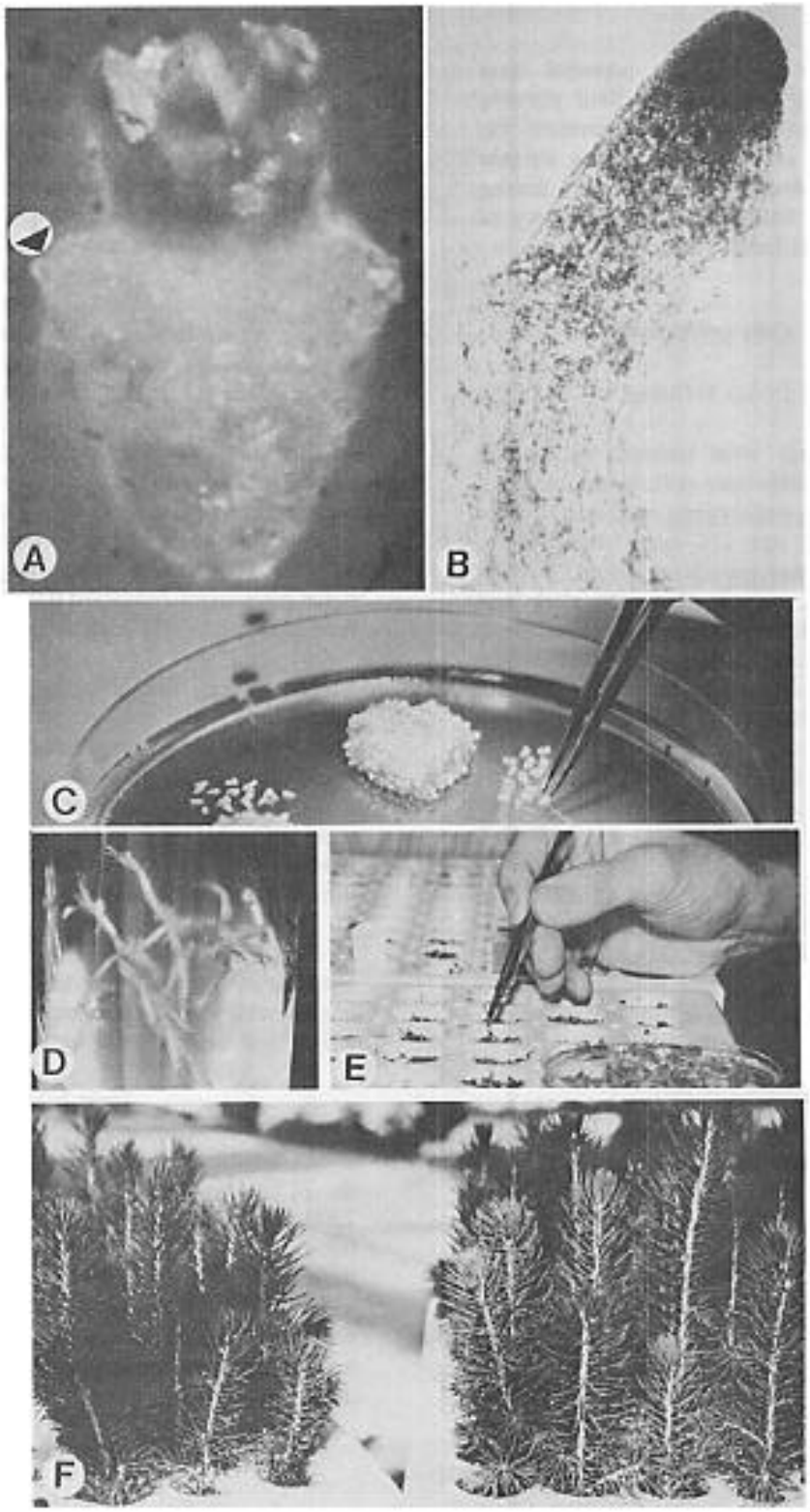

Figure 1. Propagation of interior spruce via somatic embryogenesis. An excised seed embryo forming embryogenic callus (A), arrow points to the callus, and (B) a proembryo removed from the callus and stained with acetocarmine. Cotyledonary embryos from the cultures following maturation (C). Emblings following one week of germination (D) and transfer of five week old emblings to styroblocks (E). Emblings (left) and seedlings (right) after one nursery growing season (F).

Statistical analysis

The nursery study was a completely randomized design with styroblocks of both seedlings and emblings randomly located across three greenhouse benches. During the growing season, styroblocks were randomly rotated every two and one-half weeks. All tests used a completely random experimental design and an equal number of replicates for seedlings and emblings. A t-test was used to determine differences between seedlings and emblings for spring morphological measurements and root growth capacity testing.

RESULTS

Production of Spruce Emblings

Embryogenic cultures were initiated from seed embryo explants on December 14, 1987 and maintained by subculturing every two weeks. Somatic embryo maturation was initiated during the fall of 1989, when the culture collection was 2.5 years old and 16 of 72 original genotypes (each genotype is a culture line originating from a single explant) were capable of producing mature embryos at a high frequency (data not shown). Utilizing 9 calli per genotype (144 total calli) resulted in the production of about ca. 2000 late cotyledonary embryos by early January of 1990 (Fig. 1 and 2). Only embryos exhibiting normal development patterns (1379 or 69% of original population) were placed on germination medium in late January following 3 weeks of high relative humidity (HRH) treatment. After a 10 day germination period, only embryos showing root emergence (1034 or 52% of original population) were allowed to continue development for an additional 4 weeks in shell vials. Plantable emblings (5 weeks following germination) were defined as those having a root, non-swollen hypocotyl and epicotyl development (920 or 46% of original population). Emblings placed in styroblocks showed a high survival rating during the controlled environment and nursery acclimatization phases (800 or 40% of original population). Twenty-two weeks were required from the start of embling production until the four week nursery stage.

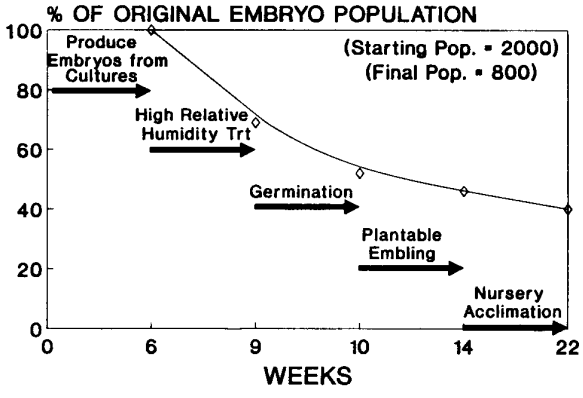

Figure 2. Duration and efficiency for different steps in the production of interior spruce emblings.

Spring-summer growth pattern

Shoot height increased at a greater rate in seedlings compared to emblings from April 20th to July 4th; with seedlings being approximately twice as tall as emblings at the end of this time period (Fig. 3). From July 4th through August 14th all plants had a similar rapid growth pattern. On August 15th seedlings were placed in a short-day treatment to slow height growth, while this treatment was not applied to emblings in an attempt to allow additional height growth during late summer. After mid August, height growth had slowed in all plants, with budset occurring in the early part of September. By the end of the growing season seedlings were 70% taller than emblings.

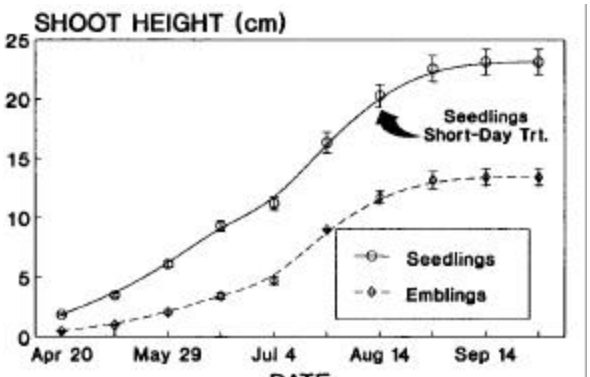

Figure 3. Shoot height (Mean ± SE) of interior spruce seedlings and emblings during the nursery growing season.

Root collar diameter of seedlings increased at a steady rate from April 20th until July 4th, while emblings diameter growth started slowly and then increased rapidly; the result being seedlings and emblings had similar diameters on July 4th (Fig. 4). From July 4th through August 14th, all plants had similar diameter growth. In late August, seedlings, compared to emblings, had a rapid increase in diameter growth. During September and October, all plants had small, but continual, increase in diameter growth.

Fall acclimation

Frost hardiness testing showed seedlings index of injury @ -18°C (II @ -18°C) decreased from a high of 69% on September 12th to 9% on October 30th, while emblings II @ -18°C decreased from a high of 54% to 9% over the same time period (Fig. 5). From October 30th until November 27th, II @ -18°C was around 9% for all plants.

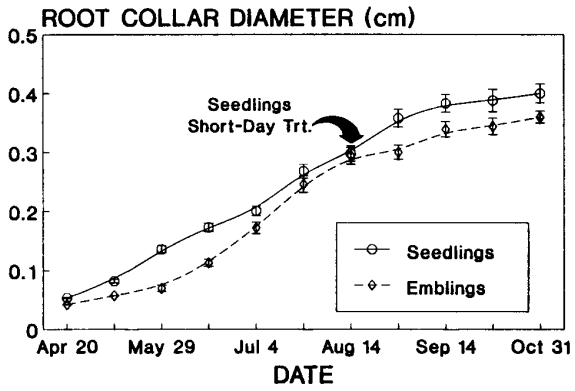

Figure 4. Root collar diameter (Mean ± SE) of interior spruce seedlings and emblings during the nursery growing season.

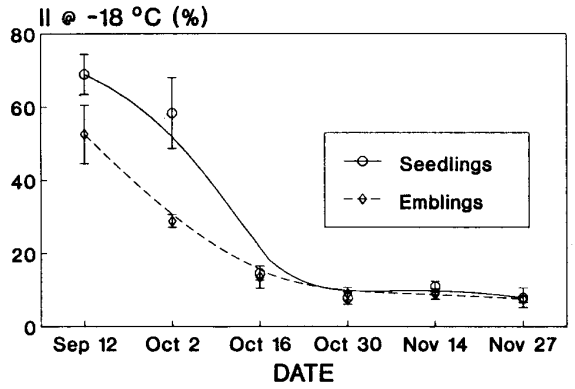

Figure 5. Frost hardiness as determined by index of injury at -18°C (II @ -18°C; Mean ± SE) for interior spruce seedlings and emblings during nursery fall acclimation.

Post-storage stock quality assessment

Morphological parameters

Seedlings, compared to emblings, had greater shoot height, shoot dry weight and root dry weight (Table 1). Seedlings and emblings had a similar root collar diameter and shoot to root dry weight ratio.

Root growth capacity

Seedlings, compared to emblings, had a larger number of roots greater than 0.5 cm in length, though both seedlings and emblings had high root development capability (i.e.>80 new roots) (Fig. 6). All plants had broken bud by the end of the two week test period.

Table 1. Morphology parameters (Mean ± SE) of interior spruce seedlings and emblings after removal from frozen storage.

	Shoot Height (cm)	Root Collar Diameter (cm)	Shoot Dry Weigth (g)	Root Dry Weigth (g)	Shoot to Root (g/g)
Seedlings	22.67a*	0.32a	2.56a	0.92a	2.92a
	±1.0	±0.01	±0.20	±0.06	±0.2
Emblings	13.18b	0.30a	1.71b	0.72b	2.67a
	±0.7	±0.01	±0.12	±0.07	±0.1

*A difference in the letter for a morphological variable between seedlings and emblings indicates a significant difference at p+0.05 as determined by a t-test.

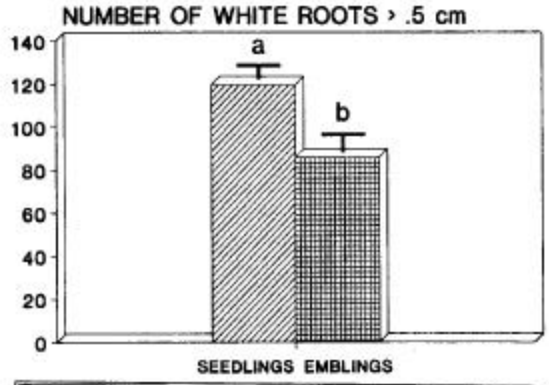

Figure 6. Root growth capacity (Mean + SE) of interior spruce seedlings and emblings after six months in frozen storage. A difference in letter indicates a significant difference at p=0.05 as determined by a t-test.

DISCUSSION

Embling production requires approximately 22 weeks from the time cultures begin maturation to nursery establishment. These steps include the biological processes equivalent to zygotic seed development, germination and initial growth required for seedling production. Starting with the maintenance cultures, about 9 weeks are required to produce a “germinable” somatic embryo which is about 1/2 the time required for normal seed development. From the time that spruce emblings are germinated, about 12 weeks are required for nursery establishment, which is about twice

the time required for a seedling to germinate and begin primary needle development. The increased time required for emblings to develop, compared to seedlings, reflects a less vigorous germination and possible transplant shock caused by the transfer process to styroblocks. Present production practices result in 40% embling survival from the cotyledonary embryo stage to 4 weeks after placement in the nursery, with latter development stages having greater survival (Fig. 2). Since these steps are labour intensive, it's important to maximize their efficiency. By shortening the time required between embling germination and nursery establishment (by improving germination and transplanting procedures), and by decreasing the labour costs (through bulk handling and automation), it should be possible to produce emblings for operational use.

Seedlings and emblings, for the most part, fit the reported "idealized" shoot height growth curve which has log (i.e. slow growth just after seed germination), linear (i.e. rapid growth during spring-summer) and senescence (i.e. slow growth during late summer) phases (McDonald 1984). However, emblings, compared to seedlings, spring shoot measurements indicated a lag in initial height growth when plants were in transition between the log and linear growth phases. McDonald (1984) states that plants are especially sensitive to moisture stress during the early part of the spring linear growth phase. 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 the linear growth phase. Emblings initial inability to cope with stressful nursery environmental conditions indicates that improved acclimation during transition between laboratory and nursery will be critical for better shoot growth. Research is ongoing at the present time to improve embling acclimatization to nursery conditions.

Both seedlings and emblings showed linear shoot growth through early summer. Seedlings required a short-day treatment to trigger the senescence in late summer. Seasonal photoperiods, after August 20th, were short enough to cause a cessation of shoot growth in emblings produced from a Prince George seed source.

Root collar diameter seasonal growth patterns were, for the most part, comparable between seedlings and emblings. There were two periods when seasonal growth patterns were different. First, embling diameter growth was slowed during the laboratory to nursery acclimation phase early in the growing season. Emblings diameter growth was greater than seedlings during the month of June which resulted in comparable diameters by early July. Second, right after the application of the short-day treatment, seedlings had greater diameter growth. Diameter growth continued into the fall and only started to slow as seasonal temperatures declined.

Frost hardiness of seedlings and emblings increased (i.e. declining II @ -18°C) rapidly over a four week period from September 12th to October 16th. Other studies with interior spruce (Simpson 1990), white and black spruce (Colombo et al. 1982) and Engelmann spruce (Burr et al. 1990) seedlings have also shown a rapid increase in frost hardiness early in the fall acclimation period.

Interestingly, emblings had greater frost hardiness during early fall. Increased frost hardiness in late-summer and fall results after growth cessation and the initiation of dormancy (Burr 1990). In late summer, emblings' shoot growth slowed and bud initiation occurred in response to natural daylength conditions, while seedlings required a short-day treatment to stop shoot growth. Thus, emblings might have initiated dormancy earlier in the season which allowed for a faster development of frost hardiness.

By October 30th, II @ -18°C had decreased to around 9% and remained at this level until all plants were lifted for frozen storage in early December. Part of an operational stock quality testing program for British Columbia forest nurseries requires that plants destined for overwinter storage at -2°C must attain a foliage mortality of less than 25 % at -18°C before they can be lifted and placed in frozen storage (Simpson 1990). Both seedlings and emblings were well below the recommended maximum 25 % foliage injury level when lifted for frozen storage.

Both seedlings and emblings met height specifications (12 to 25 cm with a 17 cm target), with seedlings 5.7 cm above target and emblings 3.2 cm below target (British Columbia Ministry of Forests, Silviculture Branch 1989 Stock Specifications). Seedlings surpassed and emblings equaled the target (3.0 mm) root collar diameter. Both seedlings and emblings surpassed the target (.7 g) root dry weight. Based on required stock morphological specifications, seedlings and emblings met or surpassed all standards required for a plantable seedling.

Seedlings, compared to emblings, had larger shoot and root systems, though they had comparable shoot to root ratios. In newly planted seedlings, a low shoot to root ratio is important to ensure survival by avoiding the development of high water deficits when absorption lags behind transpiration (Kramer and Kozlowski 1979, and Thompson 1985). Root to shoot ratios for both seedlings and emblings are at values shown to provide good drought avoidance capability in western hemlock (Grossnickle et al. 1991).

Seedlings planted in British Columbia are required to have acceptable root growth capacity levels when tested just prior to field planting (Simpson 1990). Root growth capacity testing provides a good measure of general seedling health and vigor (Ritchie 1985, Burdett 1987). Based on root growth capacity test results, both seedlings and emblings would be classified as healthy, vigorous and having the capability to grow roots. Other studies with

interior spruce seedlings having similar root growth capability showed a better than 90% first year field survival (Burdett et al. 1983, Burdett 1987, Simpson 1990). Furthermore, first year field shoot elongation of interior spruce seedlings increases with greater root growth capacity (Simpson 1990). Thus, seedlings and emblings have root growth capability indicating the potential for good survival and growth in the field.

All seedlings and emblings had broken bud during the two week root growth capacity test. This indicated that seedlings and emblings were in the post-dormancy phase and ready to resume rapid shoot development under favorable environmental conditions (Burr 1990).

CONCLUSIONS

Interior spruce 1+0 containerized seedlings and emblings were tested with operational stock quality testing criteria developed for British Columbia forest nurseries. During fall acclimation, both seedlings and emblings were well below the *25* % maximum foliage injury level for frost hardiness testing at -18°C required by fall lifting for frozen storage. Post-storage morphological grading showed seedlings were above and emblings were below target shoot height, but emblings shoot height was within the acceptable range. Seedlings and emblings root collar diameter and root dry weight equaled or exceeded target standards. Post-storage root growth capacity testing showed seedlings and emblings had high root growth capability which indicated they were healthy vigorous plants. Post-storage tested seedlings and emblings were in the post-dormancy phase and ready for rapid resumption of shoot growth under favorable environmental conditions.

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